Rev Saúde Pública 2003;37(3):390-2
www.fsp.usp.br/rsp

Performance of an immunochromatography test for *vivax* malaria in the Amazon region, Brazil

Desempenho de um teste de imunocromatografia para malária por *P. vivax* na Amazônia

Alberto Ferreira Figueiredo Filho[a,*], Maria Cristina Figueredo[a], José Maria Nascimento[a], Vanja Suely Pachiano Calvosa[a], Marinete Marins Póvoa[a] and Ricardo Luiz Dantas Machado[a,b]

[a]*Serviço de Parasitologia do Instituto Evandro Chagas. Belém, PA, Brazil.* [b]*Departamento de Doenças Dermatológicas, Infecciosas e Parasitárias da Faculdade de Medicina de São José do Rio Preto. São Jose do Rio Preto, SP, Brazil*

Keywords
Malaria, *vivax,* diagnosis. *Plasmodium vivax*. Cromatography.

Abstract
The study was carried out to evaluate the diagnostic performance of the ICT malaria Pf/Pv^{TM} test for *vivax* malaria diagnosis in Belém, Amazon region, Brazil. The results of blood malaria parasites examination using an immunochromatography test were compared with thick blood film (TBF) examination. It was also evaluated the performance of this test storaged at three different temperatures (25°C, 30°C, and 37°C) for 24 hours before use. Overall sensitivity of ICT Pf/Pv^{TM} was 61.8% with a specificity of 100%, positive and negative predictive value of 100% and 71.8%, respectively and accuracy of 80.6%. The test sensitivity was independent of the parasite density. This test needs to be further reviewed in order to have better performance for *P. vivax* malaria diagnosis.

Descritores
Malária vivax*, diagnóstico.* Plasmodium vivax. *Cromatografia.*

Resumo
Avaliação do teste ICT malaria Pf/Pv^{TM} para o diagnóstico da malária por P. vivax *em Belém, Estado do Pará. Foram comparados os resultados do teste imunocromatográfico com a gota espessa (GE) e avaliados o comportamento desse teste, estocado a três temperaturas distintas (25^{0}C/ 30^{0}C/ 37^{0}C), 24 horas antes de seu uso. A sensibilidade do ICT malaria Pf/Pv^{TM} foi de 61,8% com especificidade de 100%, valores preditivo positivo e negativo de 100% e 71,8%, respectivamente, e acurácia de 80,67%. A sensibilidade desse teste foi independente da densidade parasitária. Este teste necessita de reavaliação para melhorar o seu comportamento no diagnóstico da malária por* P. vivax.

Malaria is one of the most prevalent infectious diseases in tropical areas world-wide and is one of the main causes of human morbidity in the Amazon region, north of Brazil. In this area, *Plasmodium vivax* is the most common human malaria parasite, causing more than 80.2% of all cases reported in 2000.

The rapid and accurate diagnosis of malaria is essential for reducing its morbidity and mortality as well as for its control. The diagnosis of malaria has been traditionally based on microscopic examination of thick and thin blood films. Although simple and cheap, this procedure is however labor-intensive, time consuming and dependent upon training and expert knowledge of morphologic differentiation of plasmodial species.[3] Alternative immunochromatography tests have been developed, particularly for *Plasmodium falciparum* that showed to be effective, quick and easy to use.[1] *P. vivax* malaria blood samples from Turkey were analyzed by ICT-Malaria Pv^{TM} test (it detects *P.*

Correspondence to:
Ricardo Luiz Dantas Machado
Departamento de Doenças Dermatológicas, Infecciosas e Parasitárias
Av. Brigadeiro Faria Lima, 5416
15090-000 São José do Rio Preto, SP, Brazil
E-mail: ricardomachado@famerp.br

*Aluno bolsista da Faculdade de Medicina da Universidade Federal do Pará.

Received on 9/9/2002. Reviewed on 15/2/2003. Approved on 12/3/2003.

vivax histidine-rich protein (HRP)-2 antigen) and showed 85.7% sensitivity, 100% specificity, and positive and negative predictive values of 100% and 87.1%, respectively.[2] It has been suggested that this test is simple, rapid and reliable, allowing diagnosis where traditionally diagnostic tests are not available (rural and remote areas). Recently, it came out the ICT Malaria Pf/Pv™ (ICT-Malaria Pf/Pv, AMRAD, Australia), a commercial *in-vitro* immunochromatography test for the detection and speciation of both *P. falciparum* and *P. vivax* malaria parasites. The aim of this study is to evaluate the diagnostic performance of the ICT Pf/Pv™ test for diagnosis of *vivax* malaria in Belém, Amazon region, Brazil.

Subjects seen at the Evandro Chagas Institute, Belém, from January to March 2000 with symptoms and signs suggestive of malaria and later had a positive slide test for *P. vivax* malaria were randomly tested using ICT Pf/Pv™. Informed consent was obtained and symptoms details were registered. There were 20 asymptomatic individuals in the negative control groups, and the same number of *P. falciparum* infected individuals with positive thick blood film (TBF) was selected. *P. vivax* malaria patients had been treated with chloroquine 25 mg/kg of body weight divided in three days (10 mg/kg in day 1 and 7.5 mg/kg in days 2 and 3) plus primaquine 0.25 mg/kg of body weight for 14 days starting five days after the *vivax* malaria diagnosis.

The results of blood obtained through finger-prick and immediate evaluation using ICT Malaria Pf/Pv™ test (as by the manufacturer's instructions) on day 0 and days 1, 2, 3 and 4 after treatment start were compared with standard microscopy examination of malaria parasites in TBF. Fifteen samples were also evaluated using ICT Malaria Pf/Pv™ test storaged at three different temperatures (25°C, 30°C and 37°C) for 24 hours before use. TBF were examined by independent experienced microscopists who were unaware of the results, following the World Health Organization[5] recommended procedures.

In order to compare the performance of ICT Pf/Pv™ test, 30 *vivax* malaria patients before (day 0) and after chloroquine treatment (day 1 to 4) were included in the study. On day 0, 30 samples of *P. vivax* patients showed parasite density between 200 and 7,750 infected red blood cells/mm^3, while on day 1 the parasitemia had ranged from 10 to 1,020 infected red blood cells/mm^3. Three samples were negative. On day 2 15 samples had parasite density between 10 and 300 infected red blood cells/mm^3, and 15 samples were negative. The parasitemia in day 3 was detected only in four samples and ranged from 10 to 40 infected red blood cells/mm^3. In day 4 all 30 samples were negative. The ICT Pf/Pv™ test detected *P. vivax* in 28 samples in day 0, while in day 1 it was detected in sixteen and in day 2 only in four samples. None *P. vivax* sample was detected using ICT Pf/Pv™ test in day 3. Both TBF and ICT Pf/Pv™ test were negative in day 4. The ICT Pf/Pv™ was negative in all 20 samples (control group) with no parasites in the microscopy examination. The ICT Pf/Pv™ test detected all 20 *P. falciparum* samples that were seen in the microscopy examination.

Table - Performance of the ICT Malaria Pf/Pv™ test in diagnosing *Plasmodium vivax* in Belém, Brazil, compared to thick blood film (TBF).

ICT Pf/Pv™ test	Microscopy (TBF) Positive	Negative	Total
Positive	47	0	47
Negative	29	74	103
Total	76	74	150

Sensitivity: 61,84%; specificity: 100%, positive predictive value: 100%; negative predictive value: 71,84% and accuracy: 80,66%.

A total of 150 paired samples were analyzed. The test performance was assessed in terms of its sensitivity and specificity. Overall sensitivity of ICT Pf/Pv™ for *vivax* malaria diagnosis was 61.8%, specificity of 100%, positive and negative predictive values of 100% and 71.8%, respectively and accuracy of 80.7% (Table). The results of ICT Pf/Pv™ were variable for the three different temperatures tested. It was observed that the color of positive results (pink line) losses its intensity with increasing temperatures.

The great area of land and water, and the uncontrolled occupation of the Brazilian Amazon region, associated with the lack of personnel to carry out rapid diagnosis, all contribute to the elevated number of malaria cases in this region.

The Quantitative Buffy Coat® and serological tests are useful but have limitations for the diagnosis of malaria infections and alternative methods are needed to overcome these problems. Molecular techniques are sensitive, accurate and specific[4] but expensive and require expert knowledge. Complementary and alternative diagnosis methods not dependent on microscopy are commercially available.

Although the ICT Pf/Pv™ test is known for its ease of use even for personnel not familiar with the test format, it has showed variable results in *P. vivax* detection and low sensitivity in the present study. The study results suggest that differences in the sensitivity of ICT Pf/Pv™ test for *vivax* malaria infections were independent of *P. vivax* density in blood sam-

ples. Storage temperature is a crucial factor for the efficiency of the ICT Pf/Pv™ test, especially in the north of Brazil where temperatures can reach more than 30°C (maximum storage temperature recommended by the kit).

Although the cost of ICT Pf/Pv™ (US$ 1.50) is higher than microscopy technique (US$ 0.70), this method is simple and rapid, allowing diagnosis where specialized laboratory and personnel are not available. This test can play a useful role in assessing *P. vivax* malaria diagnosis in developing countries, but it needs to be further reviewed for a better performance. The TBF is still the best indicator of *P. vivax* malaria in infected patients.

The study protocol was reviewed and approved by the Research Board of the Evandro Chagas Institute.

ACKNOWLEDMENTS

To all patients enrolled in this study and staff of the Malaria Laboratory at the Evandro Chagas Institute for their technical assistance.

REFERENCES

1. Abul Faiz MA, Rashid R, Palit R, Rahman MR, Bin Yunus E, Hussain A et al. ParaSight™- F test results in cerebral malaria patients before and after treatment in Chittagong Medical College Hospital, Bangladesh. *Trans R Soc Trop Med Hyg* 2000;94:56-7.

2. Araz E, Tanyhksel M, Ardic N, Tabuk C. Performance of a commercial immunochromatografic test for the diagnosis of *vivax* malaria in Turkey. *Trans R Soc Trop Med Hyg* 2000;94:55-6.

3. Chiodini PL. Non-microscopic methods for diagnosis of malaria. *Lancet* 1998;351:80-1.

4. Machado RLD, Garret DO, Adagu IS, Warhurst DC, Póvoa MM. Simplified diagnosis of malaria infection: GFM/PCR/ELISA a simplified nucleic acid amplification technique by PCR/ELISA. *Rev Inst Med Trop S Paulo* 1998;40:333-4.

5. World Health Organization. *Basic malaria microscop*. Geneva; 1991. Part I p.17-68.