
Evaluation of the OptiMAL[®] test for diagnosis of malaria in Venezuela.

Noraida Zerpa¹, Rosalba Pabón², Albina Wide³, Magda Gavidia⁴, Mehudi Medina⁴, José Luis Cáceres⁵, Jacinta Capaldo², Meghan Baker⁶ and Oscar Noya^{2,3}.

¹Fundación Instituto de Estudios Avanzados (IDEA), Centro de Biociencias, Sartenejas,

²Laboratorio para Estudios sobre Malaria-IAESP "Arnoldo Gabaldon",

³Cátedra de Parasitología, Escuela de Medicina "Luis Razetti", Facultad de Medicina, Universidad Central de Venezuela. Caracas, ⁴Ministerio de Salud, Dirección General de Salud Ambiental ⁵Facultad de Ciencias de la Salud, Universidad de Carabobo, Maracay. Venezuela. ⁶Massachusetts General Hospital and Brigham and Women's Hospital, Department of Infectious Diseases, Boston, Massachusetts, USA.

Key words: OptiMAL[®], *Plasmodium falciparum*, *Plasmodium vivax*, parasitaemia.

Abstract. We evaluated the OptiMAL[®] rapid dipstick test by comparing it with the conventional standard thick-blood film method, for the detection of malaria in two groups of individuals from different Venezuelan endemic areas. One of the groups consisted of individuals with malaria-like symptoms (n = 113) and the other of asymptomatic individuals (n = 89). The classical microscopy analysis of these populations determined that 67.5% were infected with *P. vivax*, 31.3% with *P. falciparum*, and 1.2% with mixed infections. The OptiMAL[®] test showed 96.4% sensitivity, 100% specificity, 100% positive predictive value, 97.5% negative predictive value and optimal concordance ($kappa = 0.97$), capable of detecting any malaria infection in the evaluated population. However, these parameters were lower when the parasitaemia was ≤ 300 parasites/ μ L. Freezing of the samples did not affect the sensitivity and specificity of the test. We concluded that this rapid malaria test is sensitive and specific for rapid diagnosis of malaria in the field and it is a complement to conventional microscopy.

Evaluación de la prueba OptiMAL® para el diagnóstico de la malaria en Venezuela.

Invest Clin 2008; 49(1): 93 - 101

Palabras clave: OptiMAL®, *Plasmodium falciparum*, *Plasmodium vivax*, parasitemia.

Resumen. Se evaluó el test rápido OptiMAL® comparándolo con el examen convencional de observación de muestras sanguíneas para la detección de la malaria en dos grupos de individuos procedentes de diferentes áreas endémicas de Venezuela. Uno de los grupos (n = 113) con síntomas sugestivos de malaria, y otro representado por individuos asintomáticos (n = 89). El examen microscópico de las muestras de sangre de estas poblaciones determinó que 67,5% estaban infectados con *P.vivax*, 31,3% con *P. falciparum* y 1,2% con infecciones mixtas. La prueba OptiMAL® mostró 96,4% de sensibilidad, 100% de especificidad, con valor predictivo positivo de 100% y valor predictivo negativo de 97,5%. La detección de cualquier infección malárica en la población total presentó una concordancia óptima ($kappa = 0,97$). Sin embargo, estos parámetros fueron más bajos cuando la parasitemia era ≤ 300 parásitos/ μ L. El congelamiento de las muestras no afectó la sensibilidad y especificidad de la prueba. Nosotros concluimos que esta prueba rápida de malaria es sensible y específica para el diagnóstico rápido de la malaria en el campo y puede complementar a la microscopia convencional.

Received: 30-01-2007. *Accepted:* 03-07-2007.

INTRODUCTION

Malaria causes more than three hundred million acute infections and it kills at least one million people every year. Ninety percent of deaths due to malaria take place in Sub-Saharan Africa and most of them in children under the age of five (1). There are around 45,000 cases of malaria per year in Venezuela, with the highest prevalence in Bolivar and Sucre states. The classical chosen method for detection of the malaria parasite is the examination of Giemsa-stained thick and thin blood films. This method allows to estimate parasitaemia, to distinguish between parasite growth stages and to identify the four different *Plasmodium* species. However, it has certain limitations, the need for trained staff

and specialized equipments, which have stimulated the development of new diagnostic methods suitable for field conditions. Other non-microscopical diagnostic techniques have been developed. These methods include different PCR-based techniques like PCR and RT-PCR. The detection limits of these methods (2.5 to 10 parasites/ μ L) are similar to those provided by light microscopy, but in general, they are technically difficult to perform and their use is restricted to reference centers (2-6). Other techniques for the laboratory diagnosis of malaria have been developed to be used in endemic and non-endemic areas such as the immunodiagnostic methods. These are based on antibody capture of circulating antigens from *Plasmodium* spp, demonstrating them to be fast and reliable. Bear-

ing in consideration the need to develop and to adapt reliable and simple techniques to be used in the field, we considered that the simplest and more suitable test for field use is the immunochromatographic strip test. There are three methods based on the capture of histidine-rich protein (HRP2): Two test use Ig G monoclonal antibody, the ParaSight[™]-F (Beckton and Dickinson) (7) and Now[®] ICT[™] Malaria Pf/Pv (Binax, Inc., Portland, ME), which capture *Plasmodium* aldolase, in addition to the HRP2 protein (8) and, PATH Falciparum Malaria IC test (9), that uses Ig M monoclonal antibody, which binds to the HRP2 antigen.

The OptiMAL[®] Rapid Malaria test (Flow Inc, Portland, Org) is an immunochromatographic assay that uses monoclonal antibody-coated strips to detect parasite lactate dehydrogenase (pLDH) in blood (10, 11). There is no cross-reaction with human LDH (12). This test can be performed in less than 20 minutes and does not require highly trained personnel. An advantage of the OptiMAL[®] test is that it detects only live parasites, thereby providing a sensitive mean of monitoring treatment (13, 14). Prompt treatments depend on immediate and accurate malaria diagnosis. Our objective was to evaluate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPP) of the OptiMAL[®] rapid test in comparison with standard microscopy for diagnosis of malaria caused by *P. falciparum* and *P. vivax* in two regions from Venezuela.

MATERIALS AND METHODS

Study areas and populations

This study was conducted from January to May 2000 in endemic areas from Bolivar and Sucre states, Venezuela. Two sets of patients were used, one of them (n = 113) was referred from the diagnostic center "Dr. Francesco Vitanza", Tumeremo,

Bolivar state, with malaria-like symptoms. The other group consisted of asymptomatic individuals (n = 89), residents from Yaguaraparo, Sucre state, chosen by random active seeking in a cross sectional study. They correspond to individuals with no symptoms for at least 30 days and who had not taken any anti-malarial drugs, as demonstrated by thin layer chromatography of urine samples. Bolivar state is a well known endemic area for malaria in Venezuela (9,252 cases for year 2000), being *P. vivax* the most prevalent species (72.4%) at the time of the study, followed by *P. falciparum* (27.6%). Sucre state is also an endemic area (12,255 cases for year 2000) being *P. vivax* the prevalent species. Eligible subjects were more than 20 years old and provided consent for inclusion in this study. This research was approved and conducted under the supervision and support of the Venezuelan Ministry of Health and Social Development (MSDS).

Sample collections

Whole blood samples were taken, ear-tagged for thick and thin blood preparations and collected into EDTA-coated capillary tubes for the OptiMAL[®] test which was performed immediately. The thick and thin smears were processed within 30 minutes of sample collection. A limited number of samples were taken by venopuncture from 48 patients who agreed to donate 5 mL of blood, which were frozen in liquid nitrogen for further testing at the laboratory.

Malaria diagnosis with thin and thick blood smears

Slides were stained with freshly prepared 10% (v/v) Giemsa solution for 15 minutes, examined for the presence of parasites and blindly compared against the results from the rapid diagnostic test. Slides were re-examined in the same way by two independent expert readers in the labora-

tory. Parasitaemia was measured by counting the number of asexual parasites against 500 leukocytes on the thick blood film assuming an average of 8000 white blood cells (WBC)/ μL (15). Smears were considered negative if no parasites were found in 500 fields ($1000 \times$ magnification) on a thick blood film.

Malaria diagnosis with OptiMAL

The OptiMAL[®] test (DiaMed SA, 1785, Cressier sur Morat, Switzerland under license from Flow, Inc, Portland, OR 97201, USA) was performed following the manufacturer's instructions. Briefly, one drop of whole blood was mixed with two drops of lysis buffer A, which disrupts the red blood cells and releases the pLDH. The specimens were then allowed to migrate to the top of the pLDH strip. After eight minutes, the strips were placed in washing buffer B, which separated the hemoglobin from the strip. The test results were read immediately. Negative control samples were taken from an individual who had not been exposed to malaria for three years, and included within each batch tested. The assay has an internal control band at the top, a pan-*Plasmodium*-specific band in the middle (which also recognizes the pLDH isoform from *P. vivax*), and a *P. falciparum*-specific band at the bottom. The test was completed in 10-15 minutes. All dipstick tests were performed blindly with respect to results from other diagnostic tests.

Data analysis

Sensitivity was calculated as true positives/(true positives + false negatives) and specificity as true negatives/(true negatives + false positives). Sensitivity and specificity were determined at different levels of parasitaemia. Positive and negative predictive values were calculated based on the prevalence of *Plasmodium* spp (or malaria parasite) during the study. The (*kappa*) sta-

tistic method was used to measure agreement between the tests. This represents the proportion of agreement between observation and chance: a value of 1 indicates perfect agreement, and 0 indicates agreement than would be expected to occur on the basis of chance alone (16).

The statistical analysis were conducted with SPSS 7.5 for Windows and Epi-Info 6.04

RESULTS

A total of 202 individuals were investigated for malaria parasites by microscopy in Giemsa-stained blood smears, and 83 (41.1%) were found to be positive (78 symptomatic and 5 asymptomatic). From these, 56 (67.5%) were infected with *P. vivax*, 26 (31.3%) with *P. falciparum* and 1 (1.2%) mixed infection (Table I). On the other hand, the OptiMAL[®] test identified malaria infection in 80 (39.6%) individuals. From these, 56 (70.0%) had *P. vivax* infection and 23 (28.8%) had *P. falciparum* infection. Microscopy identified 3 cases of *P. falciparum* and one mixed (1.2%) infection that the OptiMAL[®] test could not discriminate (Table II). Two independent expert microscopists identified the same number of positive and negative blood films for malaria (data not shown).

Based on microscopy results, the OptiMAL[®] test showed 96.4% sensitivity (S), and 100% specificity (E). The Positive Predictive Value (PPV) was 100 % and the Negative Predictive Value (NPV) was 97.5%, with optimal concordance (*kappa* = 0.97) for detection of any malaria infection in the total population. No false-positive results were observed. Results for *P. falciparum* infections were: sensitivity 88.5%, specificity 100%, PPV 100%, and NPV 98.3%, with optimal concordance (*k* = 0.93). Meanwhile, results for *P. vivax* infections were: 100% sensitivity, specificity, PPV and NPV and

TABLE I
SUMMARY OF FINDINGS IN SUSPECTED MALARIA INDIVIDUALS (SYMPTOMATIC) AND ASYMPTOMATIC FOR MICROSCOPY AND THE OptiMAL[®] TEST AT THE HEALTH SERVICE IN ENDEMIC AREAS AND CENTRAL LABORATORY

	Microscopy		OptiMAL [®]
	Health service in endemic area	Central Laboratory	Health service in endemic area
Symptomatics			
<i>P. vivax</i>	54	51	51
<i>P. falciparum</i>	27	26	23
Mixed infections	2	1	ND
Negatives	30	35	38
Positive	83/113	78/113	75^a/113
Negative	30/113	35/113	38/113
Asymptomatics			
<i>P. vivax</i>	5	5	5
<i>P. falciparum</i>	0	0	0
Mixed infections	0	0	0
Negatives	84	84	84
Positive	5/89	5/89	5/89
Negative	84/89	84/89	84/89
TOTAL	202	202	202

^aInclude one mixed infection not discriminated of *P. falciparum* infection. ND: Not discriminated.

TABLE II
COMPARATIVE PERFORMANCE OF THE OptiMAL[®] TEST IN SUSPECTED MALARIA PATIENTS AND ASYMPTOMATIC INDIVIDUALS AT HEALTH SERVICE IN ENDEMIC AREAS AND CENTRAL LABORATORY WITH MICROSCOPY

OptiMAL [®]	Microscopy at Health Service in endemic area			Microscopy Central Laboratory*		
	Positive	Negative	Total	Positive	Negative	Total
Positive	80	0	80	80 ^a	0	80
Negative	8	114	122	3	119	122
TOTAL	88	114	202	83	119	202

^aMixed infections are included. * Sensitivity: 96.4%. Specificity: 100%. PPV: 100%. PNV: 97.5%. *k*: 0.97.

perfect concordance ($k = 1$). In symptomatic patients, the sensitivity was 96.1%, specificity 100%, PPV 100% NPV 92.1% and optimal concordance ($kappa = 0.93$) (Table II). However, in the asymptomatic individuals, the same indicators were 100% with perfect concordance ($k = 1$). The Opti-

MAL[®] and microscopy results for sensitivity, specificity, PPV and NPV at various levels of parasitaemia are shown in Table III. The OptiMAL[®] test did not detect the lowest level of parasitaemia (64, 73 and 200 parasites/ μ L) for *P. falciparum* infections, the minimum level of parasitaemia de-

TABLE III
PARASITE LEVELS DETECTED BY THIN-THICK SMEAR AND THE OptiMAL® TEST

Parasites/ μ L	N° of positives by thick and thin smears	N° of Positives by OptiMAL®	Sensitivity %	Specificity %	PPV %	NPV %
100	2	0	0.0	100	100	99.0
101-200	1	0	0.0	100	100	99.5
201-300	6	5	83.0	100	100	99.4
301-400	2	2	100	100	100	100
401-500	2	2	100	100	100	100
>500 (640-41,339)	70	70	100	100	100	100
Total	83	79	95.0	100	100	96.7

tected was 240 parasites/ μ L. In order to evaluate the effect of freezing on the OptiMAL® test, blood samples were frozen and stored for 30 days. The OptiMAL® test results were compared with fresh samples and did not show differences for both conditions: sensitivity (97.7%), specificity (100%), PPV (100%) and NPV (75.0%), with optimal concordance ($k = 0.86$).

DISCUSSION

This study compares the performance of the OptiMAL® test with standard microscopy in patients with suspected malaria and asymptomatic individuals from different regions of Venezuela. The OptiMAL® assay demonstrated 100% sensitivity for *P. vivax* and 88.5% for *P. falciparum* infections in the evaluated populations. The estimated prevalence of malaria infection, evaluated by this method, was 39.6%. The sensitivity of the method decreases when the infection was under 300 parasites/ μ L. Studies conducted in Pakistan by Iqbal et al., (17) found that OptiMAL® had a sensitivity, PPV and PNV values considerably lower for *P. vivax* infections than for *P. falciparum* infections. In our study, the concordance between the OptiMAL® test and microscopy

for detecting *P. vivax* infections in asymptomatic individuals was excellent. The reason for this result is that these individuals had high parasitaemia (280-5,640 parasites/ μ L). Similar results have been found in other studies conducted in our laboratory (unpublished data). However, Coleman et al. (18) reported the combination of poor assay specificity and low sensitivity in asymptomatic individuals with parasite densities <500/ μ L with the same method. Incorporation of more sensitive tests for detecting low parasite densities, like PCR, could support the results obtained with the OptiMAL® method, as shown previously (19, 20).

Studies from Brazil have reported good sensitivity when parasitaemia was greater than 250 parasites/ μ L for *P. vivax* and greater than 400 parasites/ μ L for *P. falciparum* (21). Lower sensitivity (88.2-92.0%) for detecting infections with 500-1000 parasites/ μ L, and even lower sensitivity for detecting infections with less than 500 parasites/ μ L, has been reported in studies conducted in Gambia (22), Indonesia (23), Colombia (24), Peru (25), and Mexico (26). Differences in the sensitivity observed in these studies can be explained by the following: 1. expertise of the micros-

copist to detect and to differentiate between infections; 2. differences in the methods to estimate parasite density and 3. immunity due to previous exposure. Since circulating malaria antigens could be complexed to antibodies, they might not be available for immunocapture by the two antibodies present in the diagnostic strip. However, we did not obtain evidence from sample populations to suggest that pre-existing anti-pLDH antibodies interfered with OptiMAL in detecting malaria. It is important to note that sample freezing did not affect the performance of the OptiMAL® test. This result provides evidence of pLDH stability as a target for malaria diagnosis.

Microscopy identified 3 cases of *P. falciparum* and one mixed infections that the OptiMAL® test could not discriminate it. It is possible that low levels of parasitaemia in those patients (< 200 parasites/ μ L) correspond to low pLDH blood concentrations, which are probably within the sensitivity limits of the test, resulting in failure to capture the antigen by the monoclonal antibody present in the dipstick. Another reason could be the retention of parasites in the endothelium of capillary vessels (27) or the presence of pLDH as immune complexes avoiding its capture by MABs.

The OptiMAL® test was very sensitive for detecting *P. vivax* in populations with co-endemic *P. falciparum* and *P. vivax* infections. However, in mixed infections, this method did not discriminate *P. falciparum* because of cross-reactivity by the pan-specific band. The inability to distinguish mixed *P. falciparum* infections with the OptiMAL® dipstick test limits its clinical use, especially in areas where drug resistance is prevalent. Therefore, the sensitivity of the test depended more on the density of parasites than on the species present.

The concordance found between the OptiMAL® test and microscopy for detect-

ing *P. vivax* infections in asymptomatic conditions was excellent. However, in other studies, the OptiMAL® showed markedly less sensitivity than microscopy for detecting infections in asymptomatic people (17). These results suggest that the immunochromatographic test may be a useful epidemiological tool for early detection and treatment of malaria, particularly in areas where *P. vivax* gametocytes appear at the onset of the infection and the risk of transmission appears earlier than in *P. falciparum* infections. Regarding to symptomatic patients, the OptiMAL® test provides an easier, rapid, objective and applicable alternative for most remote endemic areas lacking specialized laboratory facilities and personnel (17), allowing early treatment and avoiding complications. This is particularly true in *P. falciparum*-infected patients, which can be negative by microscopy due to the parasites being kept at the vascular endothelium (27). We are aware that, although this test should not replace conventional microscopy, it is a valuable tool as a complementary diagnosis for malaria in the field and at the laboratory.

ACKNOWLEDGMENTS

We thanks the Ministry of Health and Social Development MSDS, and the Rural Endemic Services from Bolivar and Sucre states for allowing access and given support to this study. We also thanks, the Francesco Vitanza Research Center from Tumeremo, Bolivar State, where some assays were carried out. Partial support for travel expenses was provided by DiaMed SA, Cressier sur Morat, Switzerland. The presentation of these results in national and international meetings was sponsored by FONACIT.

REFERENCES

1. WHO/TDR Malaria Database (<http://www.who.edu.au/MalDB-www/intro.html>).

2. **Snounou G, Bourne T, Jarra W, Viriyakosol S, Wood JC, Brown KN.** Assessment of parasite population dynamics in mixed infections of rodent plasmodia. *Parasitology* 1993; 105:363-374.
3. **Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN.** Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol Biochem Parasitol* 1993; 58:283-292.
4. **Ndao M, Bandyayera E, Kokoskyn E, Gyorkos TW, Mac-Leen JD, Ward BJ.** Comparison of blood smear antigen detection and Nested PCR methods for screening refugees from regions where malaria is endemic after a malaria outbreak in Quebec, Canada. *J Clin Microbiol* 2004; 42:2694-2700.
5. **Siribal S, Nakasiri S, Louareesuwai S, Chavalishewinkoon-Pelmirti P.** Identification of human malaria parasites and detection of mixed infection in Thai patients by nested PCR. *South Asian J Trop Med Public Health* 2004; 35:5-9.
6. **Malhotra I, Dent A, Mungui P, Muchiri E, King CL.** Real-time quantitative PCR for determining the burden of *Plasmodium falciparum* parasites during pregnancy and infancy. *J Clin Microbiol* 2005; 43:3630-3635.
7. **Shiff C, Premji Z, Minjas JN.** The rapid manual ParaSight-F test. A New diagnostic tool for *Plasmodium falciparum* infections. *Trans R Soc Trop Med Hyg* 1993; 87:646-648.
8. **Moody A.** Rapid diagnostic test for malaria parasites. *Clin Microbiol Rev* 2002; 15:66-78.
9. **Mills CD, Burgess DC, Taylor HJ, Kain C.** Evaluation of a rapid and inexpensive dipstick assay for the diagnosis of *Plasmodium falciparum* malaria. *Bull World Health Organ* 1999; 77:553-559.
10. **Piper R, Vander-Jagt DL, Holbrook JJ, Makler M.** Malaria lactate dehydrogenase target for diagnosis and drug development. *Ann Trop Med Parasitol* 1996; 90:433.
11. **Piper R, Lebras J, Wentworth L, Hunt-Cooke A, Houze S, Chiodini P, Makler M.** Immunocapture diagnostic assays for malaria using *Plasmodium* lactate dehydrogenase (pLDH). *Am J Trop Med Hyg* 1999; 60:109-118.
12. **Makler MT, Hindrichs DJ.** Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitaemia. *Am J Trop Med Hyg* 1993; 48 (2):205-210.
13. **Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baum MK, Ager AL.** Evaluation of the OptiMAL Test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* Malaria. *J Clin Microbiol* 1998; 36:203-206.
14. **Vanderjagt TA, Ikeh FI, Ujah OA, Belmonte J, Glew RH, Vanderjagt DJ.** Short communication: Comparison of the OptiMAL rapid test and microscopy for detection of malaria in pregnant women in Nigeria. *Trop Med Int Health* 2005; 10 (1):39-41.
15. **Warhurst DC, Williams JE.** Laboratory diagnosis of malaria. *J Clin Path* 1996; 49:533-538.
16. **Fleiss JL.** The measurement of interrater agreement. In: *Statistical methods for rates and proportions*, 2nd ed. New York, John Wiley & Sons; 1981, p 212-236.
17. **Iqbal J, Muneer A, Khalid N, Ahmed MA.** Performance of the OptiMal test for malaria diagnosis among suspected malaria patients at the rural health centers. *Am J Trop Med Hyg* 2003; 68(5): 624-628.
18. **Coleman RE, Maneechar N, Ponlawat A, Kumpitak C, Rachapaew N, Miller RS, Sattabongkot J.** Short report: Failure of the OptiMAL[®] rapid Malaria test as a tool for the detection of asymptomatic malaria in an area of Thailand endemic for *Plasmodium falciparum* and *P. vivax*. *Am J Trop Med Hyg* 2002; 67(6):563-565.
19. **Wide A, Pabón R, De Abreu N, Pérez H, Noya O.** Detección de portadores asintomáticos de *Plasmodium vivax* en el municipio Cagigal estado Sucre, Venezuela. *Parasitol Latinoamer* 2005; 60(1-2):225.
20. **Rodolfo H, De Donato M, Mora R, Gonzalez I, Contreras CM.** Comparison of the diagnosis of malaria by microscopy, immunochromatography and PCR in en-

- demic area of Venezuela. *Brazilian J Med and Biol Res* 2007; 40:535-543.
21. **De Sousa-Rodrigues R, Fugikaha E, Lorenzetti A, Tomé-Alves R, Cavasini C, Baptista Rossi A, Pachiano-Calvosa V, D'Almeida-Couto A, Dantas-Machado R.** Evaluation of an immunochromatographic test for malaria diagnosis under different storage conditions. *Rev Soc Bras Med Trop* 2005; 38:94-195.
 22. **Hunt-Cooke A, Chiodini PL, Doherty T, Moody AH, Ries JP.** Comparison of a parasite lactate dehydrogenase-based immunochromatographic. Antigen detection assay (OptiMAL) with microscopy for the detection of malaria parasite in human blood samples. *Am J Trop Med Hyg* 1999; 60: 173-176.
 23. **Fryauff DJ, Purnomo S, Sutamihardja MA, Elyazar IR, Krisin IS, Subianto B, Marwoto H.** Performance of the OptiMAL® assays for detection and identification of malaria infection in asymptomatic residents of Irian Jaya, Indonesia. *Am J Trop Med Hyg* 2000; 63:139-145.
 24. **Ferro BE, González IJ, Carvajal de F, Palma GI, Sarabia NG.** Performance of OptiMAL® in the diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* infections in a malaria Referral Center in Colombia. *Mem Inst Oswaldo Cruz* 2002; 97:731-735.
 25. **Soto-Tarazona A, Solari-Zerpa L, Mendoza-Requena D, Llanos-Cuentas A, Magill A.** Evaluation of the rapid diagnostic test OptiMAL for diagnosis of malaria due to *Plasmodium vivax*. *Braz J Infect Dis* 2004; 8:151-155.
 26. **González-Cerón I, Rodríguez MH, Betanzos AF, Abadía A.** Efficacy of a rapid test to diagnose *Plasmodium vivax* in symptomatic patients of Chiapas, México. *Salud Pública Mex* 2005; 47:282-287.
 27. **Simalut K, Phu NH, Whitty C, Turner GD, Louwrier K, Mai NT, Simpson JA, Hien TT, White NJ.** A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. *Am J Pathol* 1999; 155:395-410.