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Evaluation of a rapid immunochromatographic card test for *Plasmodium falciparum* in Ibadan, Nigeria

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Summary

This short report describes the results of a rapid, simple and cost effective immunodiagnostic test for malaria in Ibadan, Nigeria. A total of 77% patients presenting at the children outpatient clinic, University College Hospital with malaria symptoms were screened for malaria parasites by microscopy using Giemsa stain and by the immunochromatographic card test. The immunodiagnostic test had a sensitivity of 93.1% and a specificity of 95.8%, making a good alternative for malaria diagnosis especially in rural areas without electricity, where microscopy is not possible, and a decision is to be made on when to start treatment.

Keywords: *Plasmodium falciparum*, malaria, diagnosis

Résumé

Le court rapport deovit les resultats d'un lest unmunodiaquortique rapide, simple et onereux pour le paludisme a Ibadan, au Nigeria . 77 patients selectionuns en consultation externe de pediatue, an cetre Hospitalier Universitavie avec des symptoms de paludisive out ete terte microscopie pour le parasite due paludisme en untilsant la coloration de Giemsa et par le tert de la carte immuno choromatograhique. Le tert immounodiagnostiqu avait une sensitivete de 93.1% et une specite de 95.8% fairoant de liu une bonne alternative pour le diagnotique du paludisme, specialement dans les zones rutuales sans elecbucite, ou la miolosocopies n'est pas possibile, et une decision doit ete prise pour commenler le trautement.

Introduction

A rapid and accurate diagnosis of Plasmodium falciparum infection is extremely important for the initiation of effective therapy. Microscopical examination of Giemsa-stained thick blood films is the gold standard currently in use for the diagnosis of malaria [1]. The detection of parasites in blood smears although efficient is not however available at primary health care level in most areas of Nigeria.

The ICT Malaria Pf™ test (ICT Diagnostics, Sydney, Australia) is an in vitro immunodiagnostic method for detecting circulating histidine-rich protein-2 (PfHRP-2) in whole blood first published by Garcia et al [2]. The HRP-2 is a water-soluble antigen expressed by trophozoites and immature gametocytes of P. falciparum. Field studies conducted at a hospital in Solomon Islands demonstrated that the test had a sensitivity of 100% and a specificity of 96.2% when compared to Giemsa-stained slides. Another malaria diagnostic kit, ParaSight-F dipstick (PSF) (Beckton Dickinson, USA), has been reported to have a sensitivity of 61% - 96% and a variable specificity of 81% - 98% [3,4]. Apart from the lower sensitivity and specificity of Para Sight-F when compared with the immunochromatographic test, the shorter time need to obtain a definitive result and the practical advantage of using only a single reagent both make the ICT™ preferable to ParaSight-F [5].

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The present study was conducted in the paediatric outpatient clinic of the University College Hospital, Ibadan in southwestern Nigeria. Patients presenting with fever (38 °C and above), and other symptoms indicative of malaria, were included in the study after consent was obtained from them or in the case of children, from their parent or guardian. Approximately 60% of the subjects had taken antimalarial drugs a day to 3 weeks prior to the hospital visit. Two capillary tubes provided with the immunodiagnostic test kit were used to collect blood by finger pricking: one for the test and the other for thick and thin films. The procedure described for the test was faithfully followed. Blood collected with the capillary tubes after finger pricking was applied to a sample pad on the immunodiagnostic test card. Several drops of reagent provided in the test kit were placed above and below the pad. The blood runs up the full length of the test strip, and then the card was closed. The blood cleared after 3 – 5 minutes to reveal the control band and one or two others (through the viewing window) for a positive identification for *P. falciparum*. A negative result shows the control band only. The whole test takes less than 10 minutes compared to the more than 30 minutes required for microscopy.

The slides were read blindly by two workers without any knowledge of the results of the test. The parasite density was determined (on the basis of 8,000 leukocytes/ul of blood) by counting the number of parasites/200 leukocytes and extrapolating accordingly. A slide was declared negative only after examining a total of at least 200 oil-immersion fields.

The results showed that by microscopy 29 of the 77 subjects (37.7%) had *P. falciparum* infection with a parasite density ranging from 32 parasites/ul to 200,000 parasites/il. The immunodiagnostic test gave a malaria prevalence of 35.1% (27/77). Thus if Giemsa stain is used as a reference standard, the immunodiagnostic test had a sensitivity of 93.1% (27/29). Two cases tested negative by slides but positive by the immunodiagnostic test; that is 'false positives'. Conversely two cases tested positive by Giemsa stain but negative by the immunodiagnostic test, and had parasite densities of 40 parasites/ul and 512 parasites/ul respectively. One of the false positive subjects had taken chloroquine three days before hospital, and one of the false negative subjects had been taking daraprim weekly.

Other samples were correctly diagnosed by the immunodiagnostic test, including five subjects who had very low parasite densities, between 32 parasites/ul and 400 parasites/il. For parasitaemias above 520 parasites/ul, the test had a sensitivity of 100% and for lower parasitaemias of between 30 parasites/ul and 519 parasites/ul, the sensitivity was 71%. Bechem *et al.* [6], reported a sensitivity of 100% for the test for parasite densities above 50 parasites/il among Cameroonian children.

Of the 48 samples that were negative for *P. falciparum* by microscopy, 46 tested negative by the immunodiagnostic

test giving a specificity of 95.8% which compares favourably with that (96.2%) reported by Garcia *et al.* [2] and higher than that (48%) obtained by Singh et al. [7].

Table: Immunodiagnostic test

	Positive	Negative	Total
Slide Positive	27	2	29
Negative	2	46	48
Total	29	48	77

A possible limitation of the test could be the lack of a method to show the intensity of infection, similar to the plus (+) system in microscopy. The intensity and width of the band generated by the immunochromatographic test may not indicate a corresponding intensity of infection. Craig and Sharp [4] have demonstrated the cost effectiveness of the test over microscopy. At approximately 150 Naira unit cost, it is more expensive than microscopy but it is also simple to perform directly at the field, rapid and immediate treatment can be provided.

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