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Field Evaluation of an Antigen Detection Immunochromatographic Test for Diagnosis of *Plasmodium falciparum* Malaria in India

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Abstract: An immunochromatographic test (ICT Malaria P.f.) based on the detection of *Plasmodium falciparum* HRP-II antigen was evaluated in Gujarat state, India. Whole blood of 148 clinically suspected malaria patients was tested blind by microscopy and ICT simultaneously. Compared with the examination of Giemsa stained thick blood films, ICT test was 99% specific and 92% efficient. It was 100% sensitive for detection of >5000 parasites/μL blood, 90% sensitive (95% CI 72–109) for 1001–5000 parasites/μL, 89% sensitive (CI 69–109) for 501–1000 parasites/μL, 83% sensitive (CI 66–100) for 101–500 parasites/μL and 57% sensitive (CI 31–83) for 22–100 parasites/μL blood. Blood of a patient who had taken some drugs in recent past was negative by film but tested positive on ICT. The test does not require equipment, was easy and rapid (took <8 minutes) to conduct in the field and may be an useful diagnostic and epidemiologic tool in *P. falciparum* dominant areas.

Key words: Malaria, PfHRP-II, Immunochromatographic test, Sensitivity, Specificity

INTRODUCTION

In India over a third of nearly 2.9 million malaria cases are caused by *Plasmodium falciparum* infections. At present malaria diagnosis is based on microscopic examination of blood films. During 1995, about 70 million blood slides were examined. In the countryside a large number of blood slides collected by peripheral health workers are diagnosed at the health centres under the primary health care system. There is often a long time lag between collection and microscopic examination of the blood films, particularly during the main transmission period when diagnosis is required rapidly to prevent morbidity and mortality. A large number of private health clinics do not have malaria diagnosis facility. Since microscopic examination of blood films is labour intensive and requires a skilled microscopist, a simple and rapid diagnostic method is required since long. A recently developed QBC tube test for malaria based on acridine orange staining of centrifuged parasites require expensive equipments and was found to be comparable with examination of thick films but during field screening it was not considered to substitute the latter (Rickman et al., 1989) or was even less sensitive and more inconvenient than thick films (Baird et al., 1992).

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Following the identification of a water soluble *P. falciparum* histidine-rich protein-II (PfHRP-II) secreted by *P. falciparum* infected erythrocytes (Howard et al., 1986; Rock et al., 1987) and the determination of this protein in plasma using a monoclonal antibody against it (Parra et al., 1991), an antigen-capture enzyme linked immunosorbent assay (ELISA) has been developed (Taylor and Voller, 1993). Field evaluations have shown good sensitivity and specificity of ELISA test (Namsiripongun et al., 1993), however it is less practicable in field owing to the need for considerable skill, an expensive equipment and longer period of diagnosis. For rapid diagnosis of *P. falciparum* a dipstick test (Para Sight®-F) based on detection of PfHRP-II antigen has been developed and tested (Shiff et al., 1993; Premji et al., 1994). Although this test is significantly faster than ELISA, but multiple manipulations which include a lysing step prior to running the test can be a disadvantage when working in the field. Recently a simple immunochromatographic test (ICT Malaria P.f.) developed by ICT Diagnostics, Australia and based on detection of circulating PfHRP-II antigen in whole blood was demonstrated to show 100 % sensitivity and 96.2 % specificity to *P. falciparum* in a field trial in Solomon Islands (Garcia et al., 1996). Since further field trials were indicated to determine the value of this test under various field conditions, we evaluated it in 1996 in the Gujarat state, India where 6.5 million blood films were examined during 1995, of which 44,932 were positive for *P. falciparum* and 146,096 for *P. vivax*.

**MATERIALS AND METHODS**

The test principle of ICT Malaria Pf test is described elsewhere (Garcia et al., 1996). In brief, the test uses two antibodies specific for PfHRP-II antigen. One of these antibodies is attached to visible colloidal gold and impregnated into a sample pad. The other one is immobilised in a line on a membrane test strip. Using an EDTA coated capillary tube, 10μL of whole blood drawn through aseptic finger prick is added to the sample pad where lysis occurs and any PfHRP-II antigen present binds to the colloidal gold labelled antibody. On adding the running buffer to the sample pad, the blood and labelled antibody migrate up the test strip crossing the second antibody line. In a positive sample PfHRP-II complexed with the gold labelled antibody is captured by the antibody on the membrane and a pink line forms. No pink line develops in a negative sample. The sample pad provides and antigen positive line as control above the test line. The test card (7.6 cm × 6.3 cm) is packaged in a foil pack and stored at 2°C-8°C until the test is conducted at the room temperature.

**Evaluation**

The evaluation was conducted in malaria endemic villages of Valsad and Panchmahals districts and at the malaria clinic of the Malaria Research Centre (MRC) in Nadiad, Gujarat state. The test kits were carried in the field under cold condition in the containers that are commonly used by the PHC staff for carrying vaccines. After selecting the test subjects based on fever and clinical condition, the ICT test was performed 'blind'. Simultaneously thick and thin blood films were taken in duplicate on microslides. Presumptive antimalarial
treatment (chloroquine 600 mg base, adult dose) was given to all the patients except to those attending the MRC clinic. Based on microscopic examination further treatment to malaria confirmed cases was given following the standard drug policy (total chloroquine 1500 mg, and primaquine 45 mg [P. falciparum] or 15 mg for 5 days [P. vivax]; all adult dosage). Personal details and clinical record of each test subject was entered on a questionnaire.

**Microscopy and determination of parasitaemia**

The blood films were stained with 10 % Giemsa and examined under oil immersion lens (1000x). Blood films were also taken from 66 (34 adults and 32 children) from the villages to determine average white blood cell (WBC) count. Based on this count (6900 WBCs/µl) parasite density was calculated for each P. falciparum positive film from a count of 200 WBCs reading the thick films. Microscopists counted 300 WBCs before classifying a side negative. A negative slide that tested positive on ICT was re-examined by counting 2000 WBCs. A positive slide that tested negative on ICT was re-examined and confirmed by another person by staining the duplicate film. Sensitivity, specificity, positive and negative predictive values and efficiency of the test were determined. The colour intensity of the test line was further graded from 1 to 4 (i.e., 1 = barely discernible, 2 = readable, 3 = easily readable & 4 = intense). Association of intensity grades with parasite density was determined by the value of the correlation coefficient.

**RESULTS**

The test subjects (n=148) comprised of 73 males and 75 females which included 79 children and 69 adults. From microscopic examination of blood films, 60 patients had P. falciparum infection, 20 had P. vivax and 11 had mixed infections. Among the 60 P. falciparum cases, 50 tested positive on ICT. All the 20 P. vivax tested negative on ICT. Among 11 mixed infections, 10 tested positive on ICT. Among the remaining 57 patients whose thick films were negative, one tested positive on ICT.

Since all the P. vivax tested negative on ICT, all mixed infections were pooled with P. falciparum for analysis. From Table 1 it is seen that the test was 100 % sensitive for detection of >5000 parasites/µL blood, 90 % sensitive (95 % CI 72-109 %) for 1001-5000 parasites/µL blood, 89 % sensitive (CI 69-109 %) for 501-1000 parasites/µL blood, 83 % sensitive (CI 66-100 %) for 101-500 parasites/µL blood and 57 % sensitive (CI 31-83 %) for 22-100 parasites/µL blood. The test gave an overall sensitivity of 85 % (CI 77-93 %), specificity of 99 %, positive predictive value of 98 %, negative predictive value of 87 % and the test efficiency of 92 %.

There was one patient whose blood film was negative but tested ICT positive. It was observed that 11 of 71 patients positive for P. falciparum by thick films tested negative on ICT. Six of these had parasite densities <90/µL blood, another 4 had <600/µL and the remaining one had 1980/µL. For the 60 P. falciparum cases that tested positive both by microscopy and ICT the association of parasite density with ICT intensity scale was positive ($r = +0.57$; $P<0.05$).
Table 1. Sensitivity of immunochromatographic test by parasite density.

<table>
<thead>
<tr>
<th>Parasites/µL</th>
<th>Samples positive by ICT</th>
<th>Samples positive by blood film</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 – 100</td>
<td>8</td>
<td>14</td>
<td>57 (31–83)</td>
</tr>
<tr>
<td>101 – 500</td>
<td>15</td>
<td>18</td>
<td>83 (66–100)</td>
</tr>
<tr>
<td>501 – 1000</td>
<td>8</td>
<td>9</td>
<td>89 (69–109)</td>
</tr>
<tr>
<td>1001 – 5000</td>
<td>9</td>
<td>10</td>
<td>90 (72–109)</td>
</tr>
<tr>
<td>&gt;5000</td>
<td>20</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>&gt;22 (all)</td>
<td>60</td>
<td>71</td>
<td>85 (77–93)</td>
</tr>
</tbody>
</table>

Figures in parentheses are 95% confidence intervals

DISCUSSION

The ICT test showed good sensitivity and specificity to P. falciparum. It could not however detect infections in 11 microscopically positive samples albeit mostly with low parasitaemia except for one with high parasitaemia which is of concern as observed in an earlier study (Beadle et al., 1994). The test showed positive a blood film negative sample taken from a patient who had taken some drugs, including probably antimalarials, which he could not ascertain. That might have caused parasite clearance but ICT was positive due probably to residual antigenaemia in blood following radical cure as reported earlier by Beadle et al. (1994).

To conclude the test showed good efficacy in field against P. falciparum, was rapid, simple, easy to conduct, do not require an equipment. Besides its use for rapid diagnosis of P. falciparum, it may be useful in epidemiological studies and monitoring of new interventions such as the impregnated bednets, vaccines, new drugs etc in P. falciparum dominant areas. Since a large number of malaria cases in India are due to P. vivax and a clinician would not like any P. vivax infection to go undetected, there is a need for a similar test for P. vivax for better acceptance of this new technology at the users' end. Storage of the kit in cool condition until used in field may not be a disadvantage, at least in India, as the primary health centres are equipped with refrigerators. Since even after 9 months of use pink line was visible in all the test cards which gave a positive ICT test, the cards could be stored for future reference. If not needed, the test card can be safely disposed of after use thereby limiting the risk of contamination.

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