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# Comparison of an Immuno-chromatographic Technique-based Kit (Malaria Card) with Microscopy for Rapid Diagnosis of Malaria in and Around Loni, Maharashtra

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**Abstract**

**Although *Plasmodium vivax* is the major species responsible for malaria in most parts of India, *P. falciparum* infections have now increasingly been reported from some parts of India. A study was therefore, undertaken to compare immuno-chromatographic technique (ICT) based Malaria card test with microscopic examination of peripheral blood film in diagnosis of malaria in and around Loni, Maharashtra. A total of 143 (about 24%) samples of 590 febrile patients tested were detected positive for malarial infection- 74 samples for *P. vivax* (approx. 52%) and 69 for *P. falciparum* (approx. 48%). With Malaria card test, the sensitivity and specificity of 93.8% and 92.6% were recorded for *P. vivax* and 95.2% and 95.5% for *P. falciparum*, respectively. Therefore, the test is a valuable adjunct in an emergency for rapid diagnosis of malaria, though microscopy remains the mainstay for routine use in countries, like India.**

**Key Words :** Malaria, peripheral blood film, Malaria card, Comparison

## INTRODUCTION

Malaria is caused by a blood-borne protozoan parasite transmitted by the mosquito that remains as one of the major parasitic diseases causing high morbidity and mortality all over the world, particularly in many developing countries, including India. *Plasmodium vivax* is the major species responsible for malaria in most parts of India, although *P. falciparum* has shown predominance over the former species, especially in North-East region of the country.[1-3] Based on clinical symptoms alone however, malaria including different species of *Plasmodium* and non-malarial febrile cases

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cannot be differentiated.[4] Therefore, microscopic examination of blood smears is a widely-used method in detection of malarial parasites that remains the gold standard for diagnosis of malaria.[5] However, it is laborious and requires considerable expertise for its interpretation, particularly in cases having parasitaemia at low levels. In addition, the infection may sometimes be missed especially, in *P. falciparum* that is not present in peripheral blood due to sequestering of the parasites.[6] Hence, correct diagnosis and proper treatment thereon have an important role to play as one of the salient features of the Global Malaria Control strategy.

The development of rapid diagnostic tests (RDT) based on immunochromatographic technique (ICT) is a hall-mark that has revolutionized the diagnostic facility for malaria.[8] The ICT is a RDT that was initially devised to diagnose *P. falciparum* infection based on the parasite specific HRP II antigen which was subsequently modified to identify simultaneously the malarial infection at the species level also.[9-11] As *P. falciparum* infections have increasingly been reported also from some other parts of India, therefore a study was undertaken to compare ICT-based Malaria card test with microscopic examination of blood film in diagnosis of malaria in and around Loni, Maharashtra.

## MATERIALS AND METHODS

The study was conducted in 590 febrile patients attending Pravara Rural Hospital, Loni, Maharashtra from the month of July, 2009 to September, 2009 after getting ethical clearance from the institutional ethical committee. About 55% of the patients had history suggestive of malaria i.e., fever, chills and rise of high temperature followed by fall in temperature with profuse sweating. Remaining roughly 45% of the patients presented only with history of irregular fever, body and joint pain, and jaundice. The blood samples of the patients were collected by finger-prick using sterile lancet. Thick and thin blood smears were prepared and stained with Giemsa according to the standard procedure. The thick smears were used to detect the parasite infection, while thin smears were utilized for the parasite species identification. The patient was considered negative for malaria if no parasite was detected in 100 fields of an oil immersion (x 1000 magnification) objective lens of a microscope.[12] At the same time, about 5µl of blood from finger-prick of the patients were transferred to the sample pads for testing by Malaria card test kit (Biomed Industries, India, Lot No: 90201, Mfg. Dt: Feb., 2009 and Exp. Dt: Jan., 2011). Alternatively, 5µl of blood sample collected from veni-puncture in anti-coagulant was added to the pad of sample well. Malaria card utilizes monoclonal antibody specific to parasite lactate dehydrogenase (p LDH) of *P. falciparum* (test line/band F for Pf. specific) that is released from the parasitized erythrocytes of infected individuals by the addition of assay buffer. The card test, in addition, detects the presence of pan malaria p LDH (test line/band P for remaining three species) so released from the parasitized erythrocytes with the anti-pan p LDH antibody. The colloidal gold-labeled anti-malarial specific monoclonal antibodies complex migrates through the nitro-cellulose strip by capillary action and forms the pink-purple line/band on meeting with corresponding second antibody i. e., anti-human immunoglobulin immobilized on nitro-cellulose strip. The test was read after 20 min. as per the given specifications. The tests

were considered valid only if the control line/band were observed along with F or P band, and the sensitivity and specificity were calculated as per the criteria described by Mason and Co-workers.[13] Also the kit lot used was invariably checked for the performance in quality assurance so as to validate the test findings.

## RESULTS AND DISCUSSION

Out of 590 blood samples tested by conventional examination of peripheral smears, 143 (about 24%) samples were detected positive for malarial infection-74 samples for *P. vivax* (approx. 52%) and 69 for *P. falciparum* (approx. 48%). Of these 143 positives, 121 (60 samples positive for *P. vivax* and 61 for *P. falciparum)* blood samples were tested by Malaria card; all the 121 samples were found positive correspon-dingly for the species as revealed by P and F bands (Table 1). Also 64 of the 590 samples that were negative for the malarial parasites in peripheral smears additionally yielded positive results for 7 samples - 3 samples for *P. vivax* and 4 for *P. falciparum* in Malaria card test.

As early detection and differentiation of malaria is of paramount importance due to occurrence of cerebral malaria and drug-resistance associated with *P. falciparum* infections, various workers therefore, have reported different degree of sensitivity and specificity in detection of the two species of malarial parasites reported from different localities employing ICT kits manufactured in different countries.[3, 6,14-18] In present study employing Malaria card test based on the detection of parasite specific p LDH and pan antigen, a sensitivity and specificity of 95.2% and 95.5% for *P. falciparum* and 93.8% and 92.6% for *P. vivax,* respectively have been recorded. The three smear- negative cases detected positive for *P. vivax* and 4 cases positive for *P. falciparum* in Malaria card test might either be related to the human error while testing or even persistence of the p LDH (pf specific and the genus specific) following the clearance of parasites on institution of anti- malarial drugs in inadequate doses.[19,20] This is further explained by the fact that increased awareness amongst general public of malarial other similar febrile conditions which might have led to the misuse of anti-malarial drugs that are easily available in the Indian open market. As the present study was conducted based only upon qualitative assessment rather than on quantitative, therefore the counting of parasites was not

**Table 1 : Comparison of Malaria card test with peripheral blood smear examination for detection of malarial parasites**

| Parasite species | Malaria card test | Peripheral smears | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| *P. vivax* | Positive | 60 | 03 | 63 |
| | Negative | 0 | 61 | 61 |
| | Total | 60 | 64 | 124 |
| *P. falciparum* | Positive | 61 | 04 | 65 |
| | Negative | 0 | 60 | 60 |
| | Total | 61 | 64 | 125 |

carried out. However, increasing sensitivity of the ICT kits along with increase in parasite densities is one of the main reasons in detecting parasites even at low densities rather than the human error in testing.[13] Still the information regarding history of self-medication by the patients and the manufacturer's instructions and kit storage conditions along with its expiry date needs consideration to rule out any of such factors essentially required for validity of the results.

Further, it is interesting to note that a general trend in increase of malarial cases, particularly due to *P. falciparum* has been seen for the last few years especially during and after rainy season in and around Loni, Maharashtra (Gupta *et al.*, Unpublished data). Epidemiological studies are therefore, to be undertaken to establish the locus of falciparum malaria in the area including the surveillance under the Global Malaria Control strategy.[7] The present study has thus shown that Malaria card is a simple, sensitive and effective RDT for both *P. vivax* and *P. falciparum* in countries where these species are causing infections and a RDT is essentially required as a screening test for the same purpose. The sensitivity of the test is very close to microscopic examination of peripheral smears and does not require highly skilled personnel to perform and interpret these results. However, the cost of test may not be affordable by many that are a hindrance for its routine use in many of the laboratories in developing countries. Still, it is a valuable adjunct in an emergency for rapid diagnosis, although the microscopy remains the mainstay for routine use in malaria in countries like India.

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