MICROSCOPIC DIAGNOSIS AND USE OF ICT AS A TOOL FOR DIAGNOSIS OF MALARIA- A COMPARATIVE STUDY IN A TERTIARY CARE CENTER IN EASTERN BIHAR

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HOW TO CITE THIS ARTICLE:

ABSTRACT: BACKGROUND: Rapid antigen detection methods have been developed for situations in which reliable microscopy may not be available. The study was undertaken to evaluate the sensitivity and specificity of immunochromatographic test (ICT) malaria P.f/P.v using microscopy as the gold standard for diagnosis. MATERIAL AND METHODS: Three hundred and forty five patients of both the sexes and all age groups with clinical suspicion of malaria were studied. Venous blood was collected for microscopy and ICT. Thick and thin blood films were prepared and stained with Leishman's stain and examined. ICT was performed and interpreted according to manufacturer's instructions. RESULTS: A total of 345 cases were studied, including 190 males and 155 females between 1-60 years. Forty three (12.4%) cases had parasitaemia. On microscopy 42 (12.5%) cases had asexual stage parasitaemia and 1 (0.28%) had P. falciparum gametocytes only. Twenty seven cases (64.2%) were infected with P. falciparum, 11 (26.1%) with P. vivax and 4 (9.5%) had mixed infection. For P. falciparum the ICT was 96.8% sensitive, 98.0% specific, with positive predictive value (PPV) of 83.7% and a negative predictive value (NPV) of 99.7%. For P. vivax the sensitivity was only 90.9%, specificity 99.1%, PPV was 76.9% and NPV 99.7%. CONCLUSION: Rapid tests are commercially available in kit form which can be easily performed without extensive training or equipment to interpret the results. Nevertheless, microscopic examination of blood is economical, accurate if performed by skilled technologists and films remains gold standard for diagnosing malaria.

KEY WORDS: Immunochromatography test (ICT), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)

INTRODUCTION: Malaria presents a diagnostic challenge to laboratories in most countries. The urgency and importance of obtaining results quickly from the examination of blood samples from patients with suspected acute malaria render some of the more sensitive methods for malaria impractical for routine laboratory use. Clinical diagnosis of malaria alone is unreliable and microscopic examination of stained thick and thin blood film is the standard method of malaria diagnosis. Majority of malaria cases are found in areas where cost-effectiveness is an important factor and ease of diagnostic test performance and training of personnel are also major considerations. Secondly, its reliability is questionable particularly with low levels of parasitemia and in the interpretation of mixed infection.

In recent years multiple studies have found that rapid dipsticks have excellent sensitivity and specificity when compared with conventional microscopy. Immunochromatographic test kit capable of detecting antigens of Plasmodium falciparum and P. vivax (P.f/P.v) has been introduced. This test is based on detection of P. falciparum specific antigen and pan-malarial antigen. The test
uses two colloidal gold labeled antibodies - one antibody is specific for the histidine rich protein2 (HRP-2) antigen, that is produced by asexual stages and gametocytes of P. falciparum. The other antigen is an enzyme of the parasite glycolytic pathway expressed by the blood stage of P. falciparum as well as the non-falciparum malarial parasites.

The purpose of the present study was to determine the sensitivity, specificity, PPV and NPV of ICT malaria P.f/P.v using microscopy as a gold standard for the diagnosis of malaria.

MATERIALS AND METHODS:

Study Population: The study population included patients of both sexes and all age groups attending the outpatient and inpatient departments of a medical college hospital in eastern Bihar. Three hundred and forty-five patients with clinical suspicion of malaria having history of fever (temperature > 37.5°C) at the time of presentation associated with shivering in some cases and with other non-specific symptoms like bodyache, headache, fatigue and abdominal discomfort were included in the study. All patients with other known causes of fever were excluded from the study.

Routine tests for identification of parasites: Venous blood was collected by the standard venipuncture procedure into EDTA tubes for microscopy and immunochromatographic testing. The blood samples were immediately processed to prevent alteration in the morphology in the white blood cells and malarial parasites. Both thick and thin blood film were prepared. The thin film was air-dried and the thick film was dehaemoglobinised before being stained with Leishman’s stain and examined at 1000X magnification. Immunochromatographic test was performed according to the manufacturer’s instructions using ICT ParaHIT TOTAL malaria P.f/P.v rapid diagnostic device (Span Diagnostics, Surat, India) and the results were read individually by two persons, who were blinded to the microscopy results. The sensitivity of the rapid diagnostic test is more than 100 parasites/µl of blood. This test is based on the principle of immunochromatography in which nitrocellulose membrane is coated with anti-HRP-2 antibody (capture antibody) which is specific for P. faciparum and anti-aldolase antibody which detects the presence of any of Plasmodium species (P. faciparum, P. vivax, P. ovale& P.malariae). When the test sample along with reaction buffer flows through the nitrocellulose membrane, colloidal gold coupled with anti-HRP2/anti-aldolase antibodies (detection antibody) binds to plasmodium antigens released from the lysed test sample. This antigen-antibody complex moves further through the nitrocellulose membrane and it binds to the corresponding immobilized antibodies to HRP-2/aldolase (capture antibody) leading to the formation of magenta colored band or bands with indicative reactive results. The control band appears irrespective of reactive or non-reactive samples and serves to validate flow of the reaction mixture. Appearance of magenta colored band at the test region in addition to magenta colored band at the control region indicates positive test results.

Data analysis: Performance indices were calculated for malaria as a whole (diagnosis of either species), P. falciparum malaria and P. vivax malaria. For sensitivity and specificity, the ICT malaria test results were compared with microscopy as a gold standard. Variables measured were the number of true positives (TP), number of true negatives (TN), number of false positives (FP), and number of false negatives (FN). Sensitivity was calculated as TP/(TP+ FN), specificity was calculated as TN/(TN+FP), the PPV was calculated as TP/(TP+FP) and NPV was calculated as...
When analyzing the test performance of ICT for detection of P. vivax, those samples showing mixed infection by microscopy were considered true negative for P. vivax by ICT and true positive for P. falciparum only. This was done taking into consideration the clinical implications of P. falciparum infection. When analyzing the test performance of ICT for detection of P. vivax, those samples showing mixed infection by microscopy were considered true negative for P. vivax by ICT and true positive for P. falciparum only. This was done taking into consideration the clinical implications of P. falciparum infection.

RESULTS: Of the 345 patients who met the case definition for malaria, 190 were males and 155 were females. The age range was between 1 to 60 years. Forty three cases (12.4%) were found to have parasitemia amongst which 42 (12.1%) cases had asexual-stage parasitemia and 1 (0.28%) had P. falciparum gamatocytes only. Of the 42 patients with asexual-stage parasitemia, 27 (64.2%) were infected with P. falciparum as detected by microscopy, 11 (26.1%) were infected by P. vivax and 4 (9.5%) were infected by asexual forms of both P. falciparum & P. vivax (mixed infection).

The results of parasite detection by microscopy and ICT are compared in Table 1. The sensitivity and specificity of ICT for P. falciparum was 96.8% and 98.0% respectively. The PPV was 83.7% and NPV was 99.7% respectively. The corresponding sensitivity, specificity, PPV and NPV for the diagnosis of P. vivax malaria were 90.9% and 99.1%, 76.9% and 99.7% respectively. Table 2 Six cases of P. falciparum and 3 cases of P. vivax were false positive by rapid test. One subject each with P. falciparum & P. vivax infection were not detected by either of the HRP-2 or pan-malarial antigens.

DISCUSSION: Conventional microscopy has undergone very little improvement since its development in the early 1900s. The recommended method and the current gold standard used for routine laboratory diagnosis of malaria is the microscopic examination of stained blood films. This method requires a trained microscopist, and sensitivity and specificity may vary greatly on the competence of the microscopist. It is however, inexpensive and reliable especially in resource limited countries like India. Even though microscopy is adequate for diagnosis of malaria in patients presenting with fever many technicians tend to miss P. falciparum parasites with monocular microscopes especially with low level of parasitemia. Identification of P. vivax is never a problem for most of the laboratory technicians and pathologists. Fluorescent microscopy and PCR has proven to be sensitive but difficult to perform in routine laboratory practice. Therefore, the development of easy, rapid and accurate tests for the detection of plasmodium infection is highly desirable.

In this study, the performance of ICT (ParaHIT TOTAL) for diagnosis of malaria was compared with traditional microscopy. The kit showed 96.8% sensitivity and 98.0% specificity for P. falciparum, with PPV and NPV of 83.7% and 99.7% respectively while the sensitivity and specificity for detection of P. vivax was 90.9% and 99.1% with a PPV and NPV of 76.9% and 99.7% respectively. Similar results were reported by Harani S Mahadev et al with sensitivity, specificity, PPV and NPV of 97.0%, 98.3%, 78.0% and 99.8% respectively for P.falciparum and 89.7%, 97.9% 70.3% and 99.4% respectively for P. vivax. On the other hand, Zeb J et al reported sensitivity and specificity of Now ICT test kit to be 100% for P. falciparum and sensitivity and specificity for detection of P. vivax was 87.5% and 100%. In contrast, Mishra et al reported sensitivity of 100% for detection of P. vivax and 96.1% for P. falciparum by a different kit (Optimal). The high cost of the ICT (Rs. 200/per test) precludes their routine use but they are extremely useful for smear negative cases, patients with altered sensorium (for exclusion of cerebral malaria) or shock and in pregnant females with malaria.
In the present study, the overall sensitivity of ParaHIT TOTAL P.f/P.v for the detection of P. vivax was lesser compared to P. falciparum. Similar results were reported by Harani S. Mahadev et al where they explained that this is most likely due to low parasitemia levels. The pyrogenic threshold, which is the density of plasmodium required to elicit a febrile reaction in a given individual is on an average lower for vivax malaria than for falciparum malaria.\textsuperscript{11}

In the present study, one subject each with P. falciparum and P. vivax infection were not detected by the ICT kit. Similar findings have also been reported by others where two and three cases of P. vivax and P. falciparum infection respectively were not detected by ICT.\textsuperscript{13} This may be due to low level of parasitemia and gene deletion for the production of HRP-2 antigen.\textsuperscript{11}

Some authors have reported that a single sample that was positive for P. vivax by microscopic method showed as P. falciparum by ICT P.f/P.v. This might be due to mixed infection with P. falciparum and that low-level of P. falciparum may have been obscured or overlooked due to predominance of P. vivax or also due to sequestration of the parasites in internal organs.\textsuperscript{11,13} Such a situation was not encountered in the present study.

**CONCLUSION:** We recommend the use of non-microscopical rapid test for the detection of plasmodial antigen in critically ill-patients who are suspected to have malaria and where parasites may be sequestered in internal organs. ICT for malaria can be used as an adjunct to clinical diagnosis and/or blood film microscopy. They may also be used in selective circumstances when microscopic examination is not available in order to prevent unnecessary use of antimalarials in febrile patients wrongly diagnosed on clinical grounds alone. These tests are rapid, simple to perform and to interpret but not cost effective when compared to microscopy. ICT are also known to give false negative results in low levels of parasitemia (< 100 parasites/µl) and false positive results which may be due to various factors. These tests also have a poor prognostic value due to persistence of HRP-2 in the blood even after 1-3 months of effective treatment. Adequate caution has to be exercised in cases showing negative results especially if there is strong clinical evidence of disease. In such cases a repeat test is advocated.

ICT for malaria therefore cannot replace microscopy which, though time consuming is economical and accurate if performed by skilled technologists and is still considered the gold standard for diagnosing malaria.

**REFERENCES:**


<table>
<thead>
<tr>
<th>Microscopic results (Plasmodium species)</th>
<th>Breakup of ICT P. f/ P.v results</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>P. falciparum</td>
</tr>
<tr>
<td>P. falciparum (asexual stage) N = 27</td>
<td>26</td>
</tr>
<tr>
<td>P. vivax (asexual stage) N = 11</td>
<td>0</td>
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<tr>
<td>P. falciparum + P. vivax (asexual stage) N = 4</td>
<td>4</td>
</tr>
<tr>
<td>P. falciparum (sexual stage) N = 1</td>
<td>1</td>
</tr>
<tr>
<td>Negative N = 302</td>
<td>6</td>
</tr>
<tr>
<td>Total = 345</td>
<td>37</td>
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Table 1: Comparison of ICT P. f/P. v and microscopic examination results (n=345)
Table 2: Performance characteristics of ICT P.f/P.v relative to those of microscopy in patients with a presumptive clinical diagnosis of malaria (n= 345)

<table>
<thead>
<tr>
<th>Test done as a whole/ P.f/P.v</th>
<th>Sensitivity%</th>
<th>Specificity%</th>
<th>PPV%</th>
<th>NPV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>95.3</td>
<td>97.0</td>
<td>82.0</td>
<td>99.3</td>
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<tr>
<td>P. falciparum</td>
<td>96.8</td>
<td>98.0</td>
<td>83.7</td>
<td>99.7</td>
</tr>
<tr>
<td>P. vivax</td>
<td>90.9</td>
<td>99.1</td>
<td>76.9</td>
<td>99.7</td>
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