

**COMPARATIVE STUDY OF RDTs v/s MICROSCOPY FOR THE DIAGNOSIS OF MALARIA IN CHILDREN**

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**ABSTRACT:** Malaria presents a diagnostic challenge in most tropical countries. Rapid detection of the malaria parasite and early treatment of infection still remain the most important goals of disease management. Therefore, performance characteristics of the indigenous RDTs was determined among children with suspected malaria fever attending pediatrics OPD or admitted in indoor of UP RIMS n R Saifai central India, to assess whether this rapid diagnostic test (RDT) could be used for diagnosis of malaria and results were compared with Gold Standard microscopy test. We also assessed the logical utilization of RDTs to monitor treatment outcome. **MATERIALS AND METHODS:** 03 months to 12 years old children who were presented with acute fever without any focus to the OPD or IPD of our department from May 2011 to April 2013 were selected for the study. A finger prick blood sample was collected from each clinically suspected case of malaria to prepare blood smear and for testing with the RDT after taking informed consent. The blood smears were read by an experienced microscopist blinded to the RDT results and clinical status of the subjects. The figures for specificity, sensitivity, accuracy and predictive values were calculated using microscopy as gold standard. **RESULTS:** Analysis revealed that overall sensitivity, specificity and accuracy of the RDT were approx. 90%, while RDT is useful to confirm the diagnosis of new symptomatic cases of suspected malaria infection, the persistence of parasite antigen leading to false positives even after clearance of asexual Parasitaemia has limited its utility as a prognostic tool. The study showed that RDTs was easy to use, reliable and cheap for diagnosing new malaria cases, and is an appropriate test for the use in the fields and remote areas.

**KEYWORDS:** Diagnosis, Plasmodium, Malaria, Microscopy, RDTs.

**INTRODUCTION:** Malaria is an important tropical disease, affecting 350 – 500 million patients annually and over one million deaths worldwide.<sup>1</sup> 70% of endemicity is contributed by Sub Sahara Africa, and it is an important cause of morbidity and mortality in south Asia. Presently 2 million cases and 1000 deaths due to malaria are reported annually in India.<sup>1</sup> Out of 4 main species of plasmodium, vivax and falciparum are commonest in India.<sup>2</sup>

Malaria presents a diagnostic challenge in most tropical countries<sup>3</sup> and diagnosis of malaria still relies predominantly upon clinical presentation and the century old technique of microscopic examination of blood smears. Diagnosis by clinical symptoms alone is highly unreliable.<sup>4</sup> Microscopy requires significant skills, usually not available in most of the rural centers and remote areas and also takes time, which causes delay in the treatment of malarial cases.

These diagnostic limitations affect the medical care provided, as Malaria is a potentially fatal disease, usually curable if diagnosed quickly.<sup>3,4</sup> The urgency and importance of obtaining results quickly from the examination of blood samples from patients with suspected acute malaria is now

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made possible with the introduction of rapid malaria diagnostic tests [RDTs].<sup>4</sup> Few studies have evaluated rapid diagnostic tests in different epidemiological settings with different observations.<sup>5</sup> A new indigenous test, Para HIT and Para HIT total for the diagnosis of *P falciparum* and non-*falciparum* respectively, has recently been introduced in the national programme for malaria control in remote and inaccessible villages of Central India.<sup>4</sup> We conducted a study to assess if this RDT is reliable, simple and practical and comparable to gold standard microscopic examination for the diagnosis of malaria in our hospital setting.

**MATERIALS AND METHODS:** This study is a diagnosis accuracy test, conducted in the department of Pediatrics UP RIMS and R Saifai Etawah, from May 2011 to April 2013. Children of 3month to 12 years of age who presented with clinical features suggestive of malaria were subjected to microscopic test, both thick and thin films, and also tested with RDTs.

The accepted laboratory practices for the diagnosis of malaria is the preparation and the microscopic examination of blood film stained with Giemsa, Wright's, or Field's stain.<sup>6</sup> Rapid Diagnostic Test (RDT), is a device that detects malaria antigen in a small amount of blood, usually 5–15 µl, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. The result, usually a coloured test line, is obtained in 5–20 min.<sup>7</sup>

The three main groups of antigens detected by commercially available RDTs are:

- Histidine rich protein 2 [HRP2], specific to *P falciparum*.
- Parasite specific plasmodium lactate dehydrogenase (pLDH)
- PMA -Pan malaria Aldolase (pan specific)

Sensitivity and specificity is approx 90% and 95% respectively, of good quality and properly stored RDTs, with parasitemia more than 100 parasites/microliter.<sup>8</sup> The Para hit total is based on Pf HRP2 and PMA which is detect Plasmodium *falciparum* respectively. It can detect >40–100 parasites per microl of *falciparum*, non-*falciparum* species, but cannot differentiate non-*falciparum* species.<sup>1</sup>

The Rapid Diagnostic Test, Para HIT total dipstick test (Code No. 25988, 25988A Span diagnostic Ltd., Surat India) was compared with microscopy for the diagnosis of malaria in children, where microscopy is considered as gold standard.

Children suspected to have malaria who presented with fever or history of fever without any obvious foci, at OPD or Indoor of Pediatrics department of UP RIMS n R Saifai on every Wednesday from May 2010 to April 2011 were the subjects. This hospital is the largest medical facility in Etawah District of central UP and serves both as a general hospital for the local people and as a referral hospital for the adjoining districts.

**Ethical Clearance:** The study protocol was approved by the Ethics Committee of the UP RIMS n R Saifai Etawah UP.

**Consent:** Verbal informed consent was taken from the patients or the guardians.

**Sampling:** Blood obtained by pricking a finger or earlobe is ideal for the sample because the density of developed trophozoites or schizonts is rich in this capillary rich are.<sup>9</sup> A finger prick blood sample was collected from each case, after verbal informed consent was obtained. This sample was used to prepare thick and thin smears and for testing with the ParaHIT total test. The smears were checked

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by a microscopist blinded to the clinical status of the subjects and to the results of the diagnostic tests. Parasitaemia was determined from the thick films by counting the number of parasites against 200 leucocytes and assuming that each subject had 8000 leucocytes/ $\mu$ l.

The Para HIT total test was performed as per manufacturer's instructions. The standard reading time is between 15-20 minutes. All the children with uncomplicated malaria were administered the standard oral dose of chloroquine followed by primaquine and with complicated malarial cases were treated with artemisin based combination therapy. Infants were not given primaquine.

**Data Analysis:** The data were recorded and analyzed using statistical software (SPSS version 10.0 SPSS inc. Chicago 12). Once all the samples had been tested, specificity, sensitivity, predictive values and accuracy to the Para hit total were estimated using microscopy as gold standard. The mixed infection of *P. vivax* and *P. falciparum* was treated as *P. falciparum* and non-falciparum cases were treated as vivax for the purpose of analysis.

**RESULTS:** Out of the 452 patients screened, 242 were male while 210 were female children. Age wise distribution shows that 23% fall in 03 months to 3 yrs. age group, 29% children fall in 3yrs to 6yrs age group and 48% children belonged to 6 to 12 yrs. age group. The male and female ratio and mean age groups for falciparum and non- falciparum groups were comparable. The mean duration of illness ranged from  $6.3 \pm 2.5$  to  $10.2 \pm 4.4$  days and least in complicated falciparum illness.

Clinical, biochemical and hematological profile of children is mentioned in table1. For practical purposes the malaria positive cases were divided in two broad groups like falciparum and non- falciparum malaria, and in this region of our country non falciparum malaria fever is almost always caused by plasmodium vivax species. Mixed infection by both falciparum and non- falciparum parasites were considered as falciparum infection for the ease of calculation.

Variables	Uncomplicated <i>P. falciparum</i> (n-46)	Complicated <i>P. falciparum</i> (n-39)	<i>P. vivax</i> cases (n=177)
Fever (%)	99	83	94
Anemia (%)	35	47	28
Splenomegaly (%)	21	36	27
Seizures (%)	2	52	12
Parasite density(/ $\mu$ l blood)	786.49 $\pm$ 155.47	11458.3 $\pm$ 929.84	2517.42 $\pm$ 188.76

**Table 1: Clinical, Biochemical and hematological profile of malaria cases**

Complicated malaria defined as malaria positive cases presented with Cerebral malaria, Severe anemia, Hemoglobinuria, Acute Respiratory Distress Syndrome (ARDS), shock, Acute kidney failure, Hypoglycemia and Hyperparasitemia with or without fever.<sup>2</sup>

Fever was the commonest clinical presentation and found in almost all the uncomplicated Plasmodium falciparum and Plasmodium vivax cases and 83% of the complicated plasmodium falciparum cases. Splenomegaly was present in almost one fourth of cases and more in complicated

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plasmodium falciparum cases. Seizures were found in half of the complicated falciparum cases and 12% in vivax positive children suggesting possible cerebral malaria by plasmodium vivax also.

Evidence of hemolysis or possible hepatic involvement is also shown by presence of clinical jaundice which was present in 12% of complicated falciparum and 4% of vivax malaria cases with deranged LFT in 8% and 3% cases respectively. Parasitemia range from 500 parasites per microliter of blood to approx 100000 parasites and average parasitemia was approx 2500 parasites/ $\mu$ l of blood and maximum parasitemia was found in complicated malaria cases.

Out of 452 children subjected to microscopy and RDTs, 270 children were found positive by RDT, of which 178 were P vivax and 92 were P falciparum. Out of 262 malaria positive cases on microscopy only 241(92%) were positive by RDT, while 29 children which were negative for malaria on microscopy were found positive on RDT. All P. falciparum were also positive by RDT.

However, results of the RDT were not used to guide treatment and antimalarial therapy was prescribed on the basis of clinical severity. The asexual parasitemia ranged from 500-100000 parasites/ $\mu$ l. The sensitivity of the test was 92% (95%CI, 87-96) with 85% specificity (95%CI.78.4-94).

The PPV and NPV were 89% (95%CI. 83.45-94.52) and 88.4% (95%CI. 77.6-94.7) respectively. The accuracy of the test was 89% and I-index 0.77 in comparison with gold standard. Table 2 shows comparative study of the two tests.

TEST	MICROSCOPY POSITIVE	MICROSCOPY NEGATIVE	TOTAL
RDT POSITIVE	241	29	270
RDT NEGATIVE	21	161	182
<b>TOTAL</b>	<b>262</b>	<b>190</b>	<b>452</b>

Table 2: RDT vs. Microscopy results

Based on our study, the ParaHIT -total test is an objective and rapid antigen detection assay for the detection of P. falciparum infection, as well as pan malarial species with very high sensitivity and specificity, comparable to conventional microscopy. This is in concordance with hospital and field studies done in India and various part of the world.<sup>10</sup> It is especially true for severe and complicated cases, as the test was able to detect the antigen in 5 cases of cerebral malaria, which were negative by conventional microscopy.

**DISCUSSION:** The present study was performed in different epidemiological settings i.e. within the routine of tertiary Medical College hospital receiving children with varying clinical severity and allowing the evaluation of the performance of the RDTs in all children suspected to have malaria whatever their history. Therefore, the results obtained reflect the performance of the tests in a real situation. In our study the asexual parasitemia ranged from 500-100000 parasites/ $\mu$ l which is comparable to other studies conducted in India and abroad.

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accuracy of the test was 89% and I-index 0.77 in comparison with gold standard. The above results are comparable to other studies<sup>11,12</sup>

Out of 262 microscopy positive children, 21 were found to be negative on RDT and the possible reason for this false negativity may be occasional failure of rapid test to detect high parasite densities as recorded earlier.<sup>10-12</sup> Pf HRP-2 deleted mutants have been reported but their prevalence is not known.<sup>7</sup> Of the 270 children, who were positive on RDT, 241 were positive on microscopy and 29 children were negative on microscopic examination.

The reason for smear negativity in these cases could have been prior administration of antimalarial drugs or inadequate doses before presenting to the hospital, causing partial clearance of the parasites as also mentioned in other studies.<sup>12</sup> In this study also we detected persistent positivity of RDT in 24.4% of treated patients without asexual Parasitaemia on day 10.

Thus the test has limited utility as a prognostic tool. Because the relatively high rate of persistent false positive tests in recently treated cases, the value of predictability of a test band may be limited to new, untreated cases. However, high NPV allow us to confidently diagnose negative test patients as non-malaria patients in all epidemiological settings.

Despite some limitations, RDTs was easy to use, decreased stress and potential delay in the diagnosis of falciparum malaria in field. If the present observations are validated in larger multicentre, clinical trials, the test may prove to be a useful alternative to microscopy, particularly in places where the facilities for microscopy are poor or nonexistent.

**CONCLUSION:** With this study we can conclude that the Para HIT -total test is an objective and rapid antigen detection assay for the detection of *P. falciparum* infection, as well as pan malarial species with very high sensitivity and specificity, comparable to conventional microscopy. RDT in conjunction with microscopy should improve diagnosis of malaria. However, RDTs are more suited to investigators/health workers in situations where health services are deficient or absent. Therefore, it is reasonable to consider future use of the RDTs as an epidemiological tool for the rapid screening of malaria.

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