

## Comparative evaluation of malaria antigen test and peripheral blood smears in diagnosis of malaria

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### Abstract

**Introduction:** Malaria is a serious, sometimes fatal, parasitic disease posing a major public health problem in India. Microscopic detection of blood though considered the gold standard for malaria diagnosis for decades is quite labor-intensive and requires adequate technical skill and manpower. This has spurred the development of malaria rapid detection tests (RDT) based on the detection of malarial antigen in whole blood.

**Aim:** In this study, malaria antigen test was compared with peripheral blood smears from PUO cases in Tuticorin, Tamilnadu from January 2016 to June 2018. A total of 5603 samples were collected from patients presenting with fever, chills, and rigor throughout 2 1/2 years. Thick and thin blood smears were prepared, stained with Leishman stain and examined. SD BIOLINE Malaria antigen detection kit test used for detection of malaria.

**Results:** Malaria was diagnosed in 422 and 442 patients by malaria antigen test and Leishman staining respectively. The prevalence rate of malaria by card test was 7.5% (422 out of 5603), of these 343 (6.1%) *Plasmodium vivax* positive and 79 (1.4%) *Plasmodium falciparum*. Peripheral smears for malarial parasite 442 (7.9%) and 363 (6.5%) positive for *Plasmodium vivax* and 79 (1.4%) *Plasmodium falciparum*. The blood smear examination could detect 20 positive cases of *Plasmodium vivax* which were not detected by card test.

**Conclusion:** Our evaluation shows that malaria antigen card test is a simple, reliable and rapid test for the diagnosis as well for speciation of the malarial parasite. The test can be a promising alternative to microscopy in remote and rural areas of our country and as a supplement to microscopy in tertiary care centers.

**Keywords:** Malaria, Peripheral blood film, Rapid test.

### Introduction

Malaria a common parasitic disease of humans, spread by female Anopheles mosquitoes. The United States eliminated malaria diseases effectively. India was having the incidence of malaria disease in many states.<sup>1</sup> The challenge in eliminating or controlling malaria in the country like India is the various clinical presentation and inadequate access to quick diagnosis.<sup>2</sup> Confirming malaria needs tests with higher sensitivity and specificity. Smear microscopy is still the gold standard method in confirming malaria than any other newly developed diagnostic methods. Good quality microscope and experience in finding the microorganisms give good results.<sup>3</sup> Rapid diagnostic tests (RDT) are used for identifying the antigens and antibodies. Antibodies may be formed at any time after infection even after a year.<sup>4,5</sup> Lower level <50 parasites/ $\mu$ L is sufficient to detect malaria infection. Malaria antigen tests are extensively used in endemic malaria areas, in which tropical infection and re-infection of parasites are prevalent. There are not enough data about the comparison between malarial diagnostic techniques in Tamilnadu.

### Aims

Comparison of malaria antigen card test with peripheral blood smear examination from Pyrexia of unknown origin (PUO).

### Materials and Methods

This prospective comparison study was conducted in patients attending the outpatient department with fever, chills and rigors. 5603 samples were taken from patients from January 2016 to June 2018. Thick and thin smears were prepared and stained with Leishman's stain and examined for malarial parasites using light microscopy. All the samples were subjected to malaria antigen card test (SD BIOLINE Malaria P.f/P.v kit) according to the manufacturer's instructions. The SD BIOLINE Malaria Ag P.f/P.v test is a rapid, qualitative and differential test for the detection of histidine-rich protein II (HRP-II) antigen of *Plasmodium falciparum* and common *Plasmodium lactate dehydrogenase* (pLDH) of *Plasmodium* species in whole human blood. The test was done using anticoagulated blood.

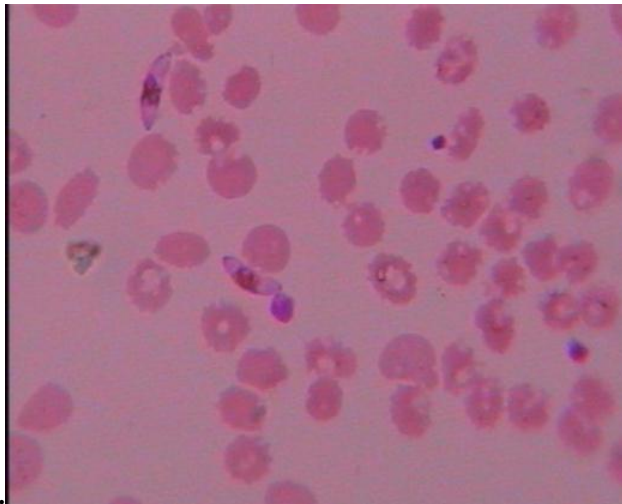
Interpretation of the test result was done as below:

|  |   |
|--|---|
| one control band and two test bands                  | Positive: <i>P.vivax</i> and <i>P. falciparum</i>       |
| one control band and one test band; position of band | Positive: either <i>P.vivax</i> or <i>P. falciparum</i> |
| only control band                                    | Negative  |

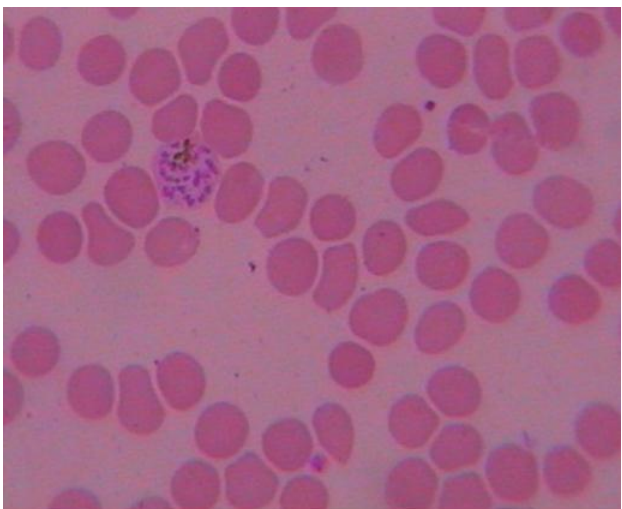
### Results

Malaria was diagnosed in 422, and 442 patients by a Malaria antigen test (Chart 1) and Leishman staining respectively. Out of 5603 samples out of 422 positive Malaria cases by rapid malaria antigen card test 341(80%) were male patients, and 81(20%) were female

(Table 1). Out of 422 positive malaria cases by malaria antigen test maximum number of patients 146(34%) in the age group of 21-30 followed by 31-40 age group 91(22%) (Chart 2). The prevalence rate of malaria by card test was 7.5% (422 out of 5603) of these 343 (6.1%) were *Plasmodium vivax* positive and 79(1.4%) were *Plasmodium falciparum* positive. Peripheral smears positive for malarial parasite 442(7.9%) and 363(6.5%) were positive for *Plasmodium vivax*, and 79(1.4%) were positive for *Plasmodium falciparum*. (Fig. 1&2). The blood smear examination could detect 20 positive cases of *Plasmodium vivax* which were not detected by card test. Two cases were positive by card test and negative by blood smear examination.

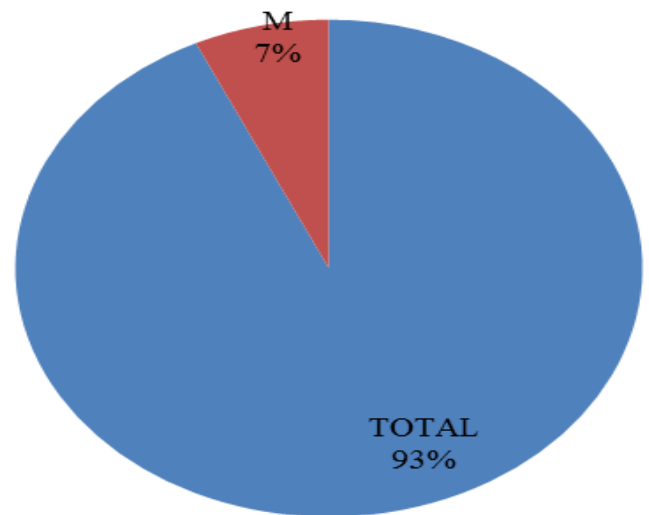


**Fig. 1: Peripheral smear-Gametocytes of *Plasmodium falciparum***



**Fig. 2: Peripheral smear-Trophozoites of *Plasmodium vivax***

**Chart 1: Positive malaria cases**



### Discussion

Malaria Rapid detection test provide an easy-to-use, relatively inexpensive, and low-expertise methodology to quickly diagnose mostly *P. falciparum*, and more recently, *P. vivax* infections within minutes.<sup>6-8</sup> Whereas expert personnel with substantial training are required for both PCR-based and microscopic diagnosis, Rapid detection test can be used with less than a few minutes of training and in low-resource settings, and are thus an invaluable tool in the arsenal to fight malaria.<sup>9</sup> The utility of microscopy and PCR are limited in resource constrained areas by old or non-existent equipment and lack of reliable power, compared to Rapid detection test, which require no electrical supply, special training, or bulky and costly laboratory equipment. The main obstacle in Leishman stained blood smear examination is using very minimal quantity of sample blood (10 $\mu$ l in thick smear and 1 $\mu$ l in case of thin smear) is used in the staining process, 40-60% of loss of parasites is there. Lower Parasitemia cases are undiagnosed, Leishman stained thick blood film detects malarial parasite when there are 5-20 parasites/ $\mu$ l, and thin blood film detects malarial parasite only when there are 50 parasites/ $\mu$ l of blood.<sup>10</sup> In our study, though we have detected positive cases by peripheral smear and rapid tests, peripheral smear had more sensitivity. This is comparable with other studies by Manjunath et al.<sup>11</sup> Variability in usage, storage, and end-user standardization have resulted in a range of reported RDT sensitivities for *P. falciparum* from 88.0–100% (for all antigens). RDT sensitivities for *P. vivax* range between 77.4–97.2%, at Parasitemia greater than 500 parasites/ $\mu$ L (~ 0.01% Parasitemia). WHO recommends lab training to be advantageous to standardization of RDT interpretation in endemic areas. Storage and proper execution of manufacturer instructions is crucial to reduce lot-to-lot variation in performance of RDT kits.<sup>12,13</sup>

## Conclusion

To conclude, our evaluation shows that Malaria antigen card test is a simple, reliable and rapid test for the diagnosis. Sensitivities and specificities are reaching high levels, and meeting WHO guidelines for RDT procurement, which are product-specific. RDTs are rapid, have become increasingly inexpensive, and do not require overhead investment. As a result, they are of increasing utility in both endemic and traveller populations as alternatives or supplementary instruments for Malaria diagnosis.

**Conflicts of Interest:** None.

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