

# Comparative Evaluation of Microscopic and Rapid Diagnostic Test (RDT) For Diagnosis of Malaria in a Tertiary Care Hospital in Bathinda

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## ABSTRACT

**Introduction:** The commonly employed method for diagnosis of malaria involves the microscopic examination of Romanowsky stained blood films. In recent years, numerous rapid techniques like acridine orange (AO) stain, quantitative buffy coat (QBC) and Rapid diagnostic tests (RDT's) have been developed for diagnosis of malaria.

**Aim & Objective:** To conduct a comparative study of the commonly employed diagnostic techniques in diagnosis of malaria, i.e., microscopy of thin smear and antigen detection.

**Materials & Methods:** A total of 553 whole blood samples were examined by preparing thin smear blood films and staining with Leishman stain. All the blood samples were also subjected to rapid diagnostic test (RDT) for detection of antigen. Results of both leishman stained smears and RDT were compared.

**Results:** Out of 553 blood samples processed, 62 (11.21%) samples were microscopy positive for malarial parasite and 59 (10.60%) were RDT positive for malaria antigen. 60(96.77%) out of 62 samples; positive by microscopy were positive for *P. vivax* and only 2 (3.22%) were positive for *P. falciparum*. 57(96.61%) out of 59 samples positive by RDT were positive for *P. vivax* and only 2 (3.38%) were positive for *P. falciparum*.

**Conclusion:** RDTs are rapid, do not require expertise and are useful in routine diagnosis. The sensitivity of antigen detection test is lower (97.4%), specificity 100% when compared to microscopy. Microscopy is simple, economical, sensitive and specific, hence still remains the gold standard method for malaria diagnosis.

**Keywords:** Microscopy, Rapid diagnostic test, positivity

## INTRODUCTION

Malaria presents a diagnostic challenge to the medical community worldwide. Its occurrence is noted in more than 90 countries. It is estimated that there are more than 50 million cases and 1.1-2.2 million deaths due to malaria every year. In India, in the year 2005, there were approximately 1.8 million cases of malaria reported of which 44.5% were caused by *Plasmodium falciparum*.<sup>[1]</sup> Due to the

serious nature of *P. falciparum* infections, prompt and accurate diagnosis of the condition is essential for effective management. The diagnostic modalities which are available for malaria range from conventional thick and thin smear to rapid modalities like fluorescent staining and antigen detection tests detecting parasitic antigens like histidine-rich protein-2 (HRP 2), plasmodium lactate dehydrogenase (pLDH) and pan-specific aldolase. All these

techniques vary in their sensitivity, specificity, positive and negative predictive values. [2]

### Aim & Objective

Keeping in mind the seriousness of the condition and the current availability of diagnostic facilities across India, we decided to conduct a comparative study of the commonly employed diagnostic techniques in diagnosis of malaria, i.e., microscopy of thin smear and antigen detection.

### MATERIALS AND METHODS

The present study was conducted in the Parasitology Laboratory of Microbiology department of AIMS (Bathinda) on IPD & OPD blood samples suspected of malaria of all age group coming to AIMS. This work was started after the approval from AIMS Research Committee & Institutional Ethical committee. A total of 553 whole blood samples were examined by preparing thin smear blood films and staining with Leishman stain. They were examined for malarial parasite by light microscopy. A thin blood smear was examined for 15 minutes. All the blood samples were also subjected to rapid diagnostic test (RDT) for detection of antigen. Results of both leishman stained smears and RDT were compared.

#### Microscopic diagnosis of malaria:

Thin blood smears were prepared as per the standard method. The smears were stained with Leishman's stain. Approximately 80-100 fields were examined over 8-10 minutes

**Antigen detection by RDT:** Antigen was detected by immunochromatographic (ICT) method with PAN+Pf CARD malaria antigen test kit, manufactured by J. Mitra & Co. Pvt. Ltd. [3] Test procedure was done as per the instructions given in the kit manual.

### RESULTS

A total of 553 blood samples suspected to be of malaria cases were received from various departments

(Medicine, Pediatric, Diabetic centre, OBG, ICU, Emergency) in the Parasitology laboratory of Microbiology department, AIMS over a period of one year.

Out of 553 blood samples received, 62 (11.21%) samples were microscopy positive for malarial parasite and these samples were subjected to rapid diagnosis, 59 (10.60%) were RDT positive for malaria antigen. In only 3 cases, discordance was observed which were positive by microscopy but negative by RDT. In none of the cases, microscopy was negative and RDT was positive. (Table 1)

Table 1: Percentage positivity rate by Microscopy and RDT

Method	Positive samples	Percentage Positivity
Microscopy	62/553	11.21%
Rapid Diagnostic test	59/553	10.60%

60(96.77%) out of 62 samples; positive by microscopy were positive for *P. vivax* and only 2 (3.22%) were positive for *P. falciparum*.

57(96.61%) out of 59 samples positive by RDT were positive for *P. vivax* and only 2 (3.38%) were positive for *P. falciparum*. (Table 2)

Table 2: Microscopy and RDT results for the two species of malaria

Method	Positive for <i>P.vivax</i>	Positive for <i>P.falciparum</i>
Microscopy(n=62)	60(96.77%)	2(3.22%)
Rapid diagnostic test(n=59)	57(96.61%)	2(3.38%)

### DISCUSSION

In our study, the microscopy results of blood smears indicated a positivity of 11.21%. Our study compares well with that of Parija et al, [2] Bhatt et al, [4] Sathpathi et al, [5] who reported positivity of direct microscopy varying from 9.32 to 12%. However, slightly higher positivity (17.40%) has been reported by Salmani et al. [6]

In present study the Rapid Antigen Detection Assay results indicated a positivity of 10.6%.

Our figure of 10.6 % positivity defines well with that of Prabhu et al, [7] Bhat et al, [4] Dougnon et al, [8] who

reported positivity as 7.4%, 11.9% and 9.6% respectively. Higher positivity (12.9%) has been reported by Salmani et al.

In the present study, prevalence of *P. vivax* detected by microscopy was 96.77% and that of *P. falciparum* was 3.22%.

The positivity of *P. vivax* in our study ; 96.77 % stands well with that of Naveen et al [9] and Chayani et al [10] and Bansal et al [11] who reported positivity of this species as 78.6 %, 92.3%, 99.3% respectively.

Positivity of *P. falciparum* in the present study was 3.23% which is comparable to that of Bansal et al, [11] Naveen et al [9] and Acheapmong et al, [12] who have reported positivity as 0.6%,4.5% and 8.2% respectively. Higher positivity (21.4%) of *P.falciparum* has been reported by Chayani et al [10]

The Rapid Antigen Detection Assay results indicated a positivity of 10.6% while microscopy indicated the total positivity of 11.21%. The result clearly indicated that HRP- 2 antigen detection test had slightly lower sensitivity as compared to microscopic analysis method. Thus, RDT's may give false negative results. This showed that microscopic examination of blood smears remains the "gold standard" for diagnosis of malaria. Microscopy is considered accurate and reliable for diagnosis of malarial parasite.

## CONCLUSION

RDTs are rapid, do not require expertise and are useful in routine diagnosis. The sensitivity of antigen detection test is lower (97.4%), specificity 100% when compared to microscopy. Microscopy is simple, economical, sensitive and specific, hence still remains the gold standard method for malaria diagnosis. This method has the advantage of high sensitivity, quantifiable results and accurate speciation, though it is fairly time-consuming. In evaluating the methods for malaria diagnosis, sensitivity, rapidity availability and cost are to be taken into consideration. Microscopy meets these

requirements and still remains the gold standard method for malaria diagnosis.

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