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# Evaluation of Blood Smears, Quantitative Buffy Coat and Rapid Diagnostic Tests in the Diagnosis of Malaria

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## Abstract

Rapid diagnosis of malaria is important for the administration of effective treatment, to reduce the morbidity and mortality. The present study was carried out to compare the efficacy of quantitative buffy coat (QBC) and rapid diagnostic test (RDT) with conventional peripheral blood smears. Blood samples from 100 patients were obtained with symptoms suggestive of malaria. A total of 74(74%) cases were positive by blood smears, while 80(80%) and 71(71%), were positive by QBC and RDT(Falcivax). Blood smears indicated that 74% (55 0f 74) of the patients were positive for *P.vivax* and 25% (19 of 74) were infected with *P.falciparum*. QBC showed that 75 % (60 0f 80) were positive for *P.vivax* and 25% (20 of 80) were infected with *P.falciparum*. Falcivax identified 74 % (53 of 71) were positive for *P.vivax* and 25 % (18 of 71) of *P.falciparum*. QBC had a sensitivity and specificity of 74.3% and 80.7% for *P.vivax* and 100% and 98.7% for *P.falciparum*. Falcivax had a specificity of100% and sensitivity of 96.3% and 94.7%.

**Keywords:** Malaria diagnosis; QBC; RDT

## Introduction

Malaria, a widely prevalent parasitic disease affects 500 million people each year and is associated with 2-5 million deaths [1]. One of the most pronounced problems in controlling the morbidity and mortality is limited access to effective diagnosis and treatment in areas where malaria is endemic [2]. Microscopic examination of blood smears is the widely used method for detection of malaria parasites and remains the gold standard for malaria diagnosis [3]. But microscopic examination is laborious and time consuming and requires considerable expertise for its interpretation particularly at low levels of parasitemia [4]. Rapid and early detection of malarial parasite and early treatment of infection still remains the most important goals of disease management [5]. A key feature of the World Health Organization global malaria control strategy is the rapid diagnosis of malaria at the village and district level so that effective treatment can be administered quickly to reduce morbidity and mortality. There is therefore an urgent need for a field test which is simple, rapid and accurate. These RDT's have a number of important limitations, including suboptimal sensitivity at low parasite densities, to quantify infection rate and a higher unit cost relative to microscopy [6].

## Materials and Methods

This study was conducted in the department of microbiology, Kasturba Medical College Hospital, Ambedkar circle, Mangalore, during the period from July 2005-2007. The study was cleared by the Institutional ethics committee. Patients attending the hospital, with symptoms and signs suggestive of malaria formed the study group. A total of 100 patients were included in the study. Blood sample collected from the patients were subjected to thick and thin smear (Traditional microscopy), Quantitative buffy coat (QBC) and Immuochromatographic test (ICT) Falcivax. Thick and thin smear were stained with Giemsa stain and observed under 100 X microscopy. Thick smear was used for the identification and thin smear for the speciation of the parasite. According to standard practice, thin smear was examined for 15 minutes and thick smear 200 fields were visualized.

### Quantitative buffy coat

The QBC capillary tubes were filled with blood by capillary action and were centrifuged at the rate of 1200g for 5 min after proper balancing. The tubes were examined under fluorescence microscope. The ring forms appeared as apple green with or without an orange dot at one side, schizonts as dark brown in colour, and gametocytes as yellowish green sickle shaped bodies.

### Immunochromatographic test

Falcivax [Tulip diagnostics pvt ltd, Goa, India], is a rapid self performing, qualitative, immunoassay used for the detection of *P.falciparum* specific histidine rich protein-2 (HRP-2) antigen and *P.vivax* specific lactate dehydrogenase (PLDH).The test was performed according to the manufacturer's instructions, all the kit components were brought to room temperature, the whole blood was centrifuged, and 2-3 drops of serum was dispensed into the sample port, followed by 5 drops of buffer solution provided along with the kit. The results were read at the end of 15 minutes. A pink purple band appeared at the region 'Pv' in the test window 'T' in addition to the control band it was considered as *P.vivax* positive. A pink purple band appeared at the region 'Pf' in the test window 'T' in addition to the control band, it was considered as *P.falciparum* positive.

To measure the agreement between Blood smears, QBC and Falcivax, Kappa statistics was used and statistical significance was assessed.

## Results

A total of 100 samples were examined for malaria parasites by quantitative buffy coat and Falcivax and the results were compared with peripheral blood smear examination. Blood smear results indicated that 74 cases were found to be positive for malaria parasites and the rest 24 were negative. Among the positive patients *P.vivax* was detected in 55 cases (75%) and *P.falciparum* in 19 cases (25%).

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Correspondingly QBC method detected, 80(80%) of total malaria cases, of which 60 (75%) cases were positive for *P.vivax* and 20 (25%) cases were positive for *P.falciparum* (Table1). QBC detected five cases of *P.vivax* and one case of *P.falciparum* that were negative by blood smear.

Falcivax indentified 71(71%) of total malaria cases, of which 53(74%) and18 (25%) cases were positive for *P.vivax* and *P.falciparum* infections (Table1). Two cases of *P.vivax* and one case of *P.falciparum* positive by blood smears were not detected by Falcivax.

Sensitivity, specificity, positive and negative predicative value of QBC for *P.vivax* were 91.6,100, 100 and 88.8% respectively and for *P.falciparum* were 95,100, 100 and 8.7% where that of Falcivax were 100, 95.7, 96.3 and 100% for *P.vivax* and 100, 98.7, 94.7 and 100% for *P.falciparum* (Table 2).

On comparing, QBC test with blood smear examination for *P.vivax* (K=0.898, P<0.0001) and for *P.falciparum* (K=0.968, P <0.0001) which is statistically significant. Comparison of Falcivax with peripheral blood smear examination for *P.vivax* (K=0.960, P< 0.0001) and for *P.falciparum* (K=--, P <0.0001) which is also statistically significant.

## Discussion

Malaria is a well-known disease and it continues to be a major public health problem at the start of new millennium. Reliable diagnosis of malaria requires laboratory confirmation of the presence of malaria parasites in the blood of a febrile patient [7]. Although microscopic examination of blood smear continues to be the gold standard, it has a drawback that it is time consuming and requires an expert microscopist and less sensitive in cases of low parasitemia [8]. Various sensitive methods have been employed for the simple, reliable, and rapid diagnosis of malaria, the most promising of these is the rapid diagnostic test and quantitative buffy coat [9]. We employed these tests and compared with Giemsa stained peripheral blood smear for the diagnosis of *P.vivax* and *P.falciparum* infections.

The QBC and RDT identified 80% and 71% as malaria positive while blood smears detected 74% of the positive cases. Five cases of *P.vivax* and one case of *P.falciparum* negative by blood smear were detected by QBC indicating a higher sensitivity and specificity of QBC. High sensitivity of QBC might be due to concentration of parasites below the buffy coat. Parzy et al found QBC to be more sensitive than blood smear examination and advocated its use for urgent diagnosis [10]. In our study the sensitivity and specificity of QBC for *P.vivax* was74.3% and 80.7% and for *P.falciparum* was100% and 98.67% respectively our results are in agreement with the results reported by various studies. Study by (Ye Htut et al. 2002) had a sensitivity of 82.8% and 100% for *P.falciparum* and *P.vivax* and specificity of 97.1% and 98.6% [11].

One of the major advantages of the QBC technique is rapidity and reliability in diagnosis of malaria even under field conditions. In addition, it requires less training and experience than blood smears. Its chief drawback is its high cost and in the identification of *Plasmodium* species. Ring stages of *P. falciparum* and *P. vivax* are difficult to distinguish by the QBC. This problem is particularly important in endemic areas where *P.falciparum* coexists with *P. vivax* [12].

| Blood smear | QBC | | Falcivax | |
|---|---|---|---|---|
| ***P.vivax*** | + | - | + | - |
| Positive-55 | 55 | 0 | 53 | 2 |
| Negative-45 | 5 | 40 | 0 | 45 |
| Total-100 | 60 | 40 | 53 | 47 |
| ***P.falciparum*** | | | | |
| Positive-19 | 19 | 0 | 18 | 1 |
| Negative-81 | 1 | 80 | 0 | 81 |
| Total-100 | 20 | 80 | 18 | 82 |

**Table 1:** Comparison of peripheral blood smears with other methods for the detection of malaria parasites

| | QBC | | Falcivax | |
|---|---|---|---|---|
| | ***P.vivax*** | ***P.falciparum*** | ***P.vivax*** | ***P.falciparum*** |
| Sensitivity (%) | 91.6 | 95 | 100 | 100 |
| Specificity (%) | 100 | 100 | 95.7 | 98.7 |
| Positive predictive value (%) | 100 | 100 | 96.3 | 94.7 |
| Negative predictive value (%) | 88.8 | 98.7 | 100 | 100 |

**Table 2:** Comparison of sensitivity and specificity of various methods in the identification of malarial parasites

Falcivax failed to detect two cases of *P.vivax* and one case of *P.falciparum* which were positive by blood smears. The sensitivity and specificity of falcivax was 96.3% and 100% for *P.vivax* and 94.7% and100% for *P.falciparum*. The low sensitivity of the Falcivax can be explained by the fact that it detects enzyme pLDH produced by live parasites and the parasites might have been killed and not cleared from the host[4] and also due to low parasitemic levels as observed by Iqbal et al. who observed 75% sensitivity at parasitemia < 100/µl. However, the rapid diagnostic test was found to be user friendly and interpretation was more objective as compared to smear and QBC [13]. Although no single test can replace the conventional method of peripheral blood smear examination, these newer diagnostic tests can be used as supplement to microscopic examination of peripheral blood smear where the diagnosis cannot be made on microscopy and an experienced microscopists are not available. The high cost of the test may prevent routine use in many laboratories. However it is a valuable adjuvant at the time of emergency for rapid diagnosis, although microscopy remains the main stay for the diagnosis of malaria.

## References

1. Gogtay NJ, Dalvi SS, Rajgor D, Chogle AR, Karnad DR, et al. (2003) Diagnostic and Prognostic Utilization of Rapid Strip (OptiMAL and Paracheck), Versus Conventional Smear Microscopy in Adult Patients of Acute Uncomplicated *P.falciparum* Malaria, in Mumbai, India. J Assoc Physicians India 51:762-765.
2. Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, et al. (1998) Evaluation of the optimal Test for Rapid Diagnosis of *Plasmodium Vivax* and *Plasmodium falciparum* malaria. J Clin Microbiol 36: 203-206.
3. Pinto MJ, Pereira NF, Rodrigues S, Kharangate NV, Verenkar MP (1999) Rapid diagnosis of *falciparum* malaria by detection of *Plasmodium falciparum* HRP-2Ag. J Assoc Physicians India 47: 1076-1078.
4. Chayani N, Das B, Sur M, Bajaria S (2004) Comparison of parasite lactate dehydrogenase based immunochromatographic antigen detection assay (OptiMAL) with microscopy for Detection of Malaria Parasite. Indian J Med Microbiol 22: 104-106.
5. Vakharia S, Gopinath N, Kshirsagar NA (1997) The Para Sight F test for detecting treatment failure. Trans R Soc Trop Med Hyg 91: 490-491.
6. Playford EG, Walker J (2002) Evaluation of the ICT Malaria Pf / P.V. and the optiMAL rapid diagnostic tests for malaria in febrile returned travelers. J Clin Microbiol 40: 4166–4171.
7. Nevill CG (1990) Malaria in sub Saharan Africa. Social Science & Medicine 31: 667–669.
8. Dowling MA, Shute GT (1966) A comparative study of thick and thin blood films in the diagnosis of scanty malaria parasitaemia. Bull World Health Organ 34: 249–267.
9. Sing N, Valecha N, Sharma VP (1997) Malaria diagnosis by field workers using a immunochromatographic test. Trans R Soc Trop Med Hyg 91: 396-397.

10. Shujatullaha F, Malik A, Khan HM, Malik A (2006) Comparison of different diagnostic techniques in *Plasmodium falciparum* cerebral malaria. J Vector Borne Dis 43: 186–190.

11. Htut Y, Aye KH, Han KT, Kyaw MP, Shimono K, et al. (2002) Feasibility and limitations of acridine orange fluorescence technique using a Malaria Diagnosis Microscope in Myanmar. Acta Med Okayama 56: 219-222.

12. Bosch I, Bracho C, Perez HA (1996) Diagnosis of Malaria by Acridine Orange Fluorescent Microscopy in an Endemic Area of Venezuela. Mem Inst Oswaldo Cruz 91: 83-86.

13. Parija SC, Dhodapkar R, Elangovan S, Chaya DR (2009) Comparative study of blood smear, QBC and antigen detection for diagnosis of malaria. Indian J Pathol Microbiol 52: 200-202.