

Evaluation of Falcivax against Quantitative Buffy Coat (QBC) for the Diagnosis of Malaria

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Evaluation of Falcivax against Quantitative Buffy Coat (QBC) for the Diagnosis of Malaria

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Abstract

Introduction: Microscopic detection of appropriately stained blood smear for the diagnosis of Malaria has been the standard diagnostic technique for identifying malaria infections for more than a century. The technique is capable of accurate and reliable identification when performed by skilled microscopists using defined protocols. The problems associated with implementing and sustaining a level of skilled microscopy appropriate for clinical diagnosis have particularly prompted the development of malaria rapid diagnostic devices (MRDDs).

The current MRDDs are based on antigen capture immunoassay methodologies using immunochromatographic strip (ICS) technology. The newer generations of MRDDs are using more antigens like Merozoite protein 2 and circumsporozoite proteins. Further these antigens are obtained using recombinant techniques. This study was done for the evaluation of two commercially available immunossays against QBC for the diagnosis of Malaria.

Aim of the study: The aim of the study is to evaluate Falcivax (Immunochromatographic Strip) test for the diagnosis of Malaria and to compare with Quantitative Buffy Coat (QBC).

Materials & methods: A total of 100 patients attending outpatient department of Kasturba Hospital, Manipal, India, with their own initiative and meeting the inclusion criteria are included in the study. 2ml of blood was collected by venipuncture into tubes (Vacutainer blood collection system) containing EDTA as anticoagulant from all patients. Tests were run in batches of 8 samples at a time for Falcivax, Smear status by QBC, clinical features and relevant laboratory data of each sample was noted down.

Results: Out of 100 patients 70 tested positive for malaria by QBC with *P. falciparum* accounting for 32(45.7%) and *P. vivax* 37(52.9%). In comparison with the study control – QBC in the detection of malaria, Falcivax test showed sensitivity, specificity, positive predictive value and negative predictive value of 90.0%, 100.0%, 100.0% and 81.0% respectively.

Conclusion: Falcivax showed a reduced sensitivity compared to the QBC. Hence QBC still continues to be better option than MRDDs for detection of plasmodium infection in health care facilities with all expertise.

Key Words: Malaria, Immunochromatographic method, Falcivax, Quantitative Buffy Coat

Introduction

Malaria is one of the oldest diseases of mankind caused by a single-cell Apicomplexa of the genus *Plasmodium* and transmitted by biological vectors of the genus *Anopheles*. According to the world malaria report released in 2006 by the World Health Organization, there were 247 million malaria cases, 3.3 billion people at risk, and 881,000 deaths from 109 countries. These deaths were primarily in Africa (91%) and in children under 5 years of age (85%). India had an estimated 1.52 million malaria cases reported in 2008 that account approximately 60% of cases in the WHO South-East Asia Region. The states inflicted are Uttar Pradesh, Bihar, Karnataka, Orissa, Rajasthan, Madhya Pradesh and Pondicherry ¹. Because of immigrant population and resistant to insecticides, this part of Karnataka is witnessing an increasing prevalence of malaria cases over a period of 5 years. In the year 2008 alone, a total of 62,864 cases of malaria and 29 malaria deaths were reported from Karnataka state ². The global impact of malaria has spurred interest in developing diagnostic strategies that will be effective not only in resource-limited areas, where malaria has a substantial burden on society, but also in developed countries, where expertise in malaria diagnosis is often lacking because they do not come across adequate cases of malaria and are not properly trained to report cases ^{3,4}. Endemic malaria, migration, and foreign travel all contribute to the malaria diagnostic problems faced by the laboratory that may not have appropriate microscopy expertise available. Changing patterns of accepted morphologies appearances of malaria species, possibly due to drug pressure, strain variation, approach to blood collection, and have created diagnostic problems that can't be easily resolved merely by references to an atlas of parasitology ⁵. The accurate diagnosis of malaria infection is important in order to reduce severe complications and mortality.

Microscopic detection of appropriately stained blood smear for the diagnosis of malaria has been the standard diagnostic technique for identifying malaria infections for more than a century. The technique is accurate and reliable when performed by skilled microscopists using defined protocols ^{6,7}. The problem associated with implementing and sustaining a level of skilled microscopy appropriate for clinical

diagnosis; particularly has promoted the development of Malaria Rapid Diagnostic Devices (MRDD)^{8,9}. The current MRDD are based on antigen capture immunoassays methodologies using immunochromatographic strip (ICS) technology. Most of the ICS will contain monoclonal antibodies directed against antigens such as histidine rich protein (HRP-2) and Plasmodium lactate dehydrogenase (pLDH) immobilized on a nitrocellulose strip¹⁰. The newer generations of MRDD are using more antigens like merozoite protein 2 and circumsporozoite proteins. Further these antigens are obtained using recombinant techniques. This study was done for evaluation of Antigen detection (Falcivax) against detection of parasites by QBC for the diagnosis of malaria *P. falciparum* & *P. vivax*, in patients attending Kasturba Hospital, Manipal.

Materials & Methods

The present comparative study was done from February 2008 to July 2009.

Hundred symptomatic patients attending outpatient department of Kasturba Hospital meeting the specific inclusion criteria were enrolled for the study.

The inclusion criteria were:

- 1) Symptoms of fever $> 38^{\circ}\text{C}$, or headache, or history of fever within the past 72 hrs
- 2) Age ≥ 15 years

The exclusion criteria were:

- 1) Patients who had been on anti-malarial therapy
- 2) Treated with anti-malaria therapy within last 2 weeks

Study group 1- 70 Patients suspected of Malaria and are positive for malarial parasites (*P. falciparum* or *P. vivax*) by QBC.

Study group 2- 30 Patients suspected of Malaria but negative for malarial parasites (*P. falciparum* or *P. vivax*) by QBC.

Approximately 2ml of blood was collected by venipuncture into vacutainer containing EDTA as anticoagulant from all patients in study group 1 & 2. Tests were performed following manufacturer's instructions on 8 samples at a time using both Falcivax (Zephyr biomedical) & Anti-Malaria profile (Euroimmun).

Smear status by QBC, clinical features and relevant laboratory data of each sample was noted down.

Statistical Analysis

Validity of tests was statistically analyzed in terms of sensitivity, specificity, positive and negative predictive values. Results were analyzed by Mc Nemar's test by using SPSS computer package.

Results

A total of 100 patients enrolled in the present study were belonging to the age group of the 15 to 65 years. Out of total 100 patients, 76 were males and 24 females. Among the tested 70 were positive and 30 were negative by QBC for malaria. Out of 70, 32 (45.7%) were due to *P. falciparum* and 37 (52.9%) were due to *P. vivax* and one (1.4%) of had mixed infection with *P. falciparum* as well as *P. vivax* (Table 1).

Table 1: Results of QBC for diagnosis of malaria

Malaria	Number	Percentage (%)
Positive for <i>P. vivax</i>	37	37.0
Positive for <i>P. falciparum</i>	32	32.0
Mixed infection	1	1.0
Negative	30	30.0
Total	100	100.0

Grading of malarial parasites was done by *plus method*. Most of the patients with *P. falciparum* infection had a lower parasite load of 1+ (39.4%) where as in contrast; majority of patients with *P. vivax* had a higher parasite load of 4+ (34.21%) (Table 2).

Table 2: Results of QBC for estimating relative quantity of parasites for *P. vivax* and *P. falciparum*

QBC	Number	Percentage (%)	QBC	Number	Percentage (%)
PV1+	7	18.42	PF1+	13	39.4
PV2+	8	21.05	PF2+	5	15.15
PV3+	10	26.32	PF3+	10	30.30
PV4+	13	34.21	PF4+	5	15.15
Total	38	100.0	Total	33	100.0

The Falcivax test showed 63 samples positive out of 100 in which 35 (55.5%) were *P. falciparum*, 26 (41.3%) for *P. vivax*, 2 (3.2%) cases tested positive for both *P. falciparum* and *P. vivax* (Table 3).

Table 3: Results of Falcivax test for diagnosis of malaria

Malaria	Number	Percentage (%)
Positive for <i>P. vivax</i>	26	26.0
Positive for <i>P. falciparum</i>	35	35.0
Mixed infection	2	2.0
Negative	37	37.0
Total	100	100.0

For QBC in the detection of malaria, Falcivax test showed sensitivity, specificity, positive and negative predictive values of 90.0%, 100.0%, 100.0% and 81.0% respectively. The P value (p=0.04) was statistically significant (Table 4).

Table 4: Comparison of QBC and Falcivax test for detection of malaria

Falcivax test	QBC study control		Total No. (%)
	Positive No. (%)	Negative No. (%)	
Positive	63(90.0)	0 (0.0)	63 (63.0)
Negative	7(10.0)	30 (100.0)	37 (37.0)
Total	70(100.0)	30(100.0)	100 (100.0)

P=0.04 s

Sensitivity-90.0%, Specificity-100.0%, Positive predictive value-100.0%, Negative predictive value-81.0%

In comparison with the study control QBC, the sensitivity, specificity, positive and negative predictive values of Falcivax test in detection of *P. vivax* were 73.68%, 100.0%, 100.0% and 86.2% respectively. The P value (0.004) is statistically very significant. In present study, comparing the sensitivity, specificity, positive and negative predictive values of Falcivax test in comparison with QBC in detection of *P. falciparum* were 100.0%, 97.01%, 94.02% and 100.0% respectively.

Discussion

Malaria is still a major global health problem, killing more than one million people every year. A key to effective management of malaria to reduce mortality and morbidity is accurate and prompt diagnosis. Since the introduction of the MRDD in early 1990s new rapid diagnostic techniques have been developed and evaluated widely in recent years, but the rapid introduction, withdrawal, and modification of commercially available products, variable quality control in manufacturing, and potential decrements in test performance related to the stability of stored test kits have rendered these reviews largely obsolete^{5,14,15}. The World Health Organization (WHO) has recommended a minimal standard of 95% sensitivity for *P. falciparum* densities of 100/μl and a specificity of 95%^{16,17}. The development of easy, rapid, and accurate tests for the detection of plasmodial infection is highly desirable.

In our study out of 100 patients, 70 were positive and 30 were negative for malaria by QBC and 63 patients were tested for malaria by Falcivax test, of whom 35 (55.5%) were for *P. falciparum* followed by 26 (41.3%) for *P. vivax*. Two (3.2%) cases were tested for both *P. falciparum* and *P. vivax*. MRDD are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies; they do not require laboratory equipment. In contrary QBC is although simple, reliable fluorescent staining of malaria parasites; it requires specialized instrumentation¹⁹. Studies of MRDD have demonstrated widely varying sensitivity, ranging from poor to 100%. The sensitivity of QBC for detection of malaria parasites in infections with parasite levels of >100 parasites/μl (0.002% parasitemia) has been reported to range from 41 to 93% and the specificity for infections with *P. falciparum* is excellent (>93%)^{20,21}.

Commercially available antigen detection Falcivax test used to detect (Pf. HRP-2) for *P. falciparum* and specific pLDH for *P. vivax* were used. pLDH is a soluble glycolytic enzyme expressed at high levels in asexual stages of malaria parasites²². It has been found in all four human malaria species^{23,24}. Iqbal et al in their study concluded that pLDH has 97% sensitivity when parasite levels is > 100/μl parasites but failed to detect when parasite load was >50/μl parasites, but microscopy was able to detect²⁵. In our study, sensitivity was 73.68% with the parasite load of >3+ (11-100 parasites per QBC field) in 25 cases out of 37 for detection of *P. vivax*. Several workers have noted that during therapy the clearance of parasites from blood films and decreased pLDH levels parallel each other²⁶⁻²⁸. This advocates the possible use of tests measuring pLDH as valuable tools in monitoring anti-malarial therapy particularly in areas where other facilities not available. Parija et al have found the sensitivity of 70.0 % where as we observed 73.68% of Falcivax test²⁹.

In the study comparing the QBC with the Falcivax test for the detection of *P. falciparum*, the sensitivity, specificity was 100% and 94.02. Most products target a *P. falciparum*-specific protein like HRP 2¹⁷ and HRP-2 from sexual stages of *P. falciparum* is more readily detected than pLDH. HRP-2 antigen detection for detection of *P. falciparum* in blood samples have shown an overall average sensitivity

of 77 to 98% when >100 parasites/μl (0.002% parasitemia), and specificity of 83 to 98% for *P. falciparum* compared with thick blood film microscopy. We observed the sensitivity of 100.0% which is in agreement with the result of Moody et al⁵.

Two cases were negative by QBC but positive by Falcivax test. This could be explained by persistence of antigenemia beyond the clearance of parasitemia in certain cases which reduce the usefulness of the test response⁵.

Among the eight cases which were negative by Falcivax positive by QBC, seven were *P. vivax* and one was *P. falciparum*. This can be explained by certain artifact seen in blood like Howell Jolley bodies that resemble the ring form of *P. falciparum*²⁹ and polymorphism of targeted antigens³⁰.

Conclusion

The study results suggest that MRDD for the detection of plasmodial antigens may develop as an important diagnostic tool and can prove to be a valuable adjunct to clinical assessment of the patient and QBC. These tests are rapid, simpler to perform and to interpret.

The 100.0% sensitivity for identification of *P. falciparum* conveys that this test using HRP-2 (Falcivax test) can substitute for diagnosis of malaria under certain cases but *P.vivax* targeting pLDH antigen (Falcivax test) has shown a lower sensitivity of 73.68% and a higher specificity of 100.0%, thus may rule out false positive.

Thus QBC still continues to be a better option than MRDDs for the detection of *Plasmodium* infections in health care facilities with all expertise. But the limitation of the test is its being poor in species identification⁵. If facilities are available combination of QBC with MRDDs help in rapid diagnosis of malaria and help in monitoring the treatment.

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