

## CENTRIFUGED BUFFY COAT SMEAR: AN ALTERNATIVE TO CONVENTIONAL PERIPHERAL BLOOD SMEAR FOR DIAGNOSIS OF MALARIA

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**ABSTRACT: INTRODUCTION:** Malaria continues to be a global public health challenge with more than 200 million deaths annually, specially in the tropical and subtropical countries.<sup>(1)</sup> In India malaria is endemic throughout the country, problem accounting for 1-2 million cases and 1100 deaths per year.<sup>(1)</sup> The commonly employed method for diagnosis of malaria involves the microscopic examination of Romanowsky stained blood films.<sup>(2)</sup> For decades light microscopy of blood smears has been the gold standard in the diagnosis of malaria.<sup>(3)</sup> It is labor intensive and requires considerable expertise for its interpretation, particularly at low level of parasitaemia.<sup>(4)</sup> The diagnostic modalities which are available for malaria range from conventional thick and thin smears, Quantitative buffy coat smears(QBC), to rapid and more reliable diagnostic modalities like antigen detection tests for detecting parasitic antigen like Histidine – rich protein-2 (HRP-2), Plasmodium lactate dehydrogenase (pLDH) and pan specific aldolase. These techniques have variable sensitivity and specificity.<sup>(5)</sup> Each of these methods have their own advantages and disadvantages. A previous study from India<sup>(6)</sup> had developed standardized and reported on using Centrifuged buffy coat smear (CBCS) examination for diagnosis of malaria, in which wide bore 4 ml tube instead of a Wintrobe's tube has been used to obtain a buffy coat. This technique has advantage over the existing method. The purpose of the present study was to assess the usefulness of CBCS technique in comparison to peripheral blood smear and antigen detection.

**AIMS:** Detection of malarial parasite by Centrifuged buffy coat smear (CBCS) was compared with conventional thin and thick peripheral smear and antigen detection by commercially available card test. The purpose of this study was to evaluate the usefulness of CBCS in comparison to peripheral blood smear and antigen detection.

**METHODS, MATERIALS AND RESULTS:** A total of 837 patients were tested for malaria by all the three techniques. The maximum number of cases were positive by antigen test (230, 37.9%), followed by CBCS (214, 35.3%) and Peripheral smear (171, 28.2%). However, antigen test could not detect 04 cases, out of which one was picked up by both PS and CBCS and 3 by CBCS only. Antigen test was exclusively positive in 17 cases. A definite relationship was found with degree of parasitaemia. At high parasite level of >1000/microl, all the three tests detected malaria equally, but at lower level of parasitaemia (<200 parasites/microl) PBS could not detect in comparison with CBCS and antigen test in 28 and 25 cases respectively. Similarly at moderate parasitaemia (200-1000 parasites/microl) PS failed to detect 6 and 5 cases in comparison with CBCS and antigen test. The results of CBCS and antigen test correlated well. Thus by adding centrifugation to conventional PS, we could detect 43 more cases which included 26 with low level of parasitaemia.

**CONCLUSION:** Though malaria antigen detection is considered gold standard for diagnosis of malaria, CBCS method has an advantage over peripheral blood smear in a country like India where antigen test and QBC are costly options.

**KEYWORDS:** Malaria, CBCS, Peripheral blood smear.

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**INTRODUCTION: SUBJECTS AND METHODS:** The present study is a prospective, assessor blind, comparative study evaluating the three methods used for the diagnosis of malaria. The study was carried out over a period of 5 months from June 2014 to October 2014 in a 600 bedded tertiary care hospital of Central India.

**PARTICIPANTS:** Patients of either sex and all age groups with a clinical suspicion of malaria were included in the

study. Informed consent was taken from all patients who participated in the study. A single sample was collected in K2 EDTA Vacutainer.

**Sample Processing:** First, thin and thick smears were prepared and stained by standard Field's Staining method and examined at x1000 magnification (7) Levels of parasitaemia calculated, using the thick smears, by counting asexual parasites against a fixed number of oil immersion fields (200) and assuming each patient had WBC count of 8000/microl.<sup>(7)</sup>

Second, CBCSs were prepared as described previously.<sup>(8)</sup> This consisted of collecting blood in a wide bored EDTA vacutainer, centrifuging at 2500 rpm for 15 minutes. The supernatant plasma is discarded, the buffy

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coat and equal thickness of RBCs layer just below the buffy coat was picked, smeared and stained by standard Field's Staining method; 200 oil immersion fields were examined before considering the smear as negative. Level of parasitaemia was calculated if PBS was negative.

Third, Antigen detection was performed using commercially available card, Malascan by Zephyr Biomedicals as per manufacturer's instruction.

**RESULTS:** During study period total of 837 samples were received for malaria testing. Out of this 587 were IPD patients and 250 were OPD patients; 621 were males and 216 were females. Samples were tested by the three diagnostic modalities which gave varied results. (Table No-1) Total number of malaria positive cases was 234 (38.6%). Of these 130 (55.5%) were P. Vivex, 101 (43.1%) were P. Falciparum and 03 (1.2%) were mixed infection caused by both P. Vivex and P. Falciparum species. The maximum number of cases were positive by antigen test (230, 37.9%), followed by CBCS (214, 35.3%) and PS (171, 28.2%). However, antigen test could not detect 04 cases, out of which one was picked up by both PS and CBCS and 3 by CBCS only. Antigen test was exclusively positive in 17 cases.

Table No. 2 shows demographic profile of positive cases as regards to their age, sex and admission status. Out of 234 positive cases 135 (57.6%) were males and 99 (42.3%) were females. Age distribution of cases was as follows: 26 (11.1) were in 0-12 yrs age group, maximum number of cases was in 13-65 yrs age group 151 (64.5%), 57 (23.9) were more than 65 yrs age group. Number of cases requiring admission was 168 (71.7%) as compared to OPD cases 66 (28.2%).

Age in Year	OPD						IPD					
	Male			Female			Male			Femal		
	PV	PF	MIXED	PV	PF	MIXED	PV	PF	MIXED	PV	PF	MIXED
0-12	2	2	0	0	3	0	7	9	0	2	1	0
13-65	11	7	0	7	13	0	41	25	2	24	19	0
>65	5	3	0	4	7	0	13	8	0	12	4	1
<b>TOTAL</b>	<b>18</b>	<b>12</b>	<b>0</b>	<b>13</b>	<b>23</b>		<b>61</b>	<b>42</b>	<b>2</b>	<b>38</b>	<b>24</b>	<b>1</b>

**Table 2: Demographic profile of malaria positive patients (N=234)**

OPD - Out patient department, IPD - In patient department, P.v- Plasmodium vivex, P.f - Plasmodium falciparum.

Table No. 3 depicts the sensitivity, specificity, positive predictive value, negative predictive value and P value of PS and CBCS in comparison to antigen test. It can be observed that both PBS and CBCS had excellent specificity, sensitivity of PBS was low (74.3%) as compared to CBSC (99.7%). The usefulness of CBCS is further confirmed by a statistically significant difference (P<0.001 by chi-square test).

Test	Sensitivity	Specificity	PPV	NPV	P.Value
PBS	74.3	99.7	99.4	88	<0.001
CBCS	93.1	99.7	98.1	96	<0.001

**Table 3: Sensitivity, specificity and validity of PBS and CBCS in comparison to antigen test**

(PBS, CBCS AND ANTIGEN)				
Sl. No.	PBS	CBCS	ANTIGEN	NO. OF SAMPLES
1	N	N	N	606
2	N	P	N	3
3	N	N	P	17
4	N	P	P	40
5	P	N	N	0
6	P	P	N	1
7	P	N	P	0
8	P	P	P	170
<b>Total</b>	<b>171 (20.4%)</b>	<b>214 (25.6%)</b>	<b>230 (27.9)</b>	

**Table 1: Results of samples by three different method for detection of malaria**

- \* Total no. of patients = 837.
- † Total no. of samples positive = 231.
- ‡ PBS: Peripheral blood smear.
- § CBCS: Centrifuged buffy coat smear.
- || P: Positive.
- \*\* N: Negative.

- \* Taking antigen test - 100% Gold standered.
- † PBS: Pheripheral blood smear.
- ‡ CBCS: Centrifuged buffy coat smear.
- § NPV: Negative predictive value.
- || PPV:-Positive Predictive value.
- \*\* P VALUE: <0.001 (Significant for difference between PBS & CBCS (Chi-square tests).

Table No. 4 depicts the relationship between results of PBS, CBCS and antigen test with the degree of parasitaemia in microscopically positive cases. At high parasite level of >1000/microl, all the three tests detected malaria equally. But at lower level of parasitaemia (<200 parasites/microl) PBS could not detect in comparison with CBCS and antigen test in 28 and 25 cases respectively. Similarly at moderate parasitaemia (200-1000 parasites/microl) PBS failed to detect 6 and 5 cases in

comparison with CBCS and antigen test. The results of CBCS and antigen test correlated well. Thus by adding centrifugation to conventional PBS we could detect 43 more cases which included 26 with low level of parasitaemia.

Parasite Density Parasite/ul	No. of Specimens with Indicated Density as Determined by Antigen Test		
	PBS	CBCS	Antigen Test
<200	2	28	25
200-100	39	45	44
>1000	115	115	115
Gametocytes only	15	26	33
<b>Total -</b>	<b>171</b>	<b>214</b>	<b>217 *</b>

**Table 4: Parasite density in microscopy confirmed malaria infections**

\* Includes the 17 smear - negative antigen positive cases.

† PBS: Peripheral blood smear.

‡ CBCS: Centrifuged buffy coat smear.

**DISCUSSION:** The present study was done to demonstrate the performance of adding centrifugation to conventional PBS. This study demonstrates that addition of centrifugation to PBS could detect 43 more cases of malaria, specially at lower level of parasitaemia (additional 26 cases), moreover the results of CBCS correlated very well with antigen test. The PBS failed to detect malaria in 46 samples, while the CBCS failed to detect malaria in only 3 cases. Similar results were obtained in the study by Akhtar et al.,<sup>[9]</sup> in which out of 120 patients the CBCS detected 6 more cases (49%) as malaria positive as compared with the peripheral smear (44%). Similarly, in another study,<sup>[10]</sup> where the authors used centrifugation-enhanced heparinised capillary tubes for smear preparation and examination found that, out of 100 patients the modified centrifuged buffy coat detected 7 more samples as malaria-positive as compared with the conventional smear technique. The addition of centrifugation to the conventional smear technique improved its sensitivity from 86.79% to nearly 100%.<sup>[10]</sup> In yet another study from North India,<sup>[11]</sup> out of 50 patients clinically diagnosed as cases of cerebral malaria, only 28 patients (56%) were positive by Leishman stained blood smear examination for various stages of *P. falciparum*, whereas QBC and ParaSight-F (antigen) test were positive in 47 (94%) and 46 (92%) patients, respectively.

In this study antigen detection method was used as gold standard, conventional PBS was used as the reference standard. It can be concluded that PBS along with CBCS can be used to improve the sensitivity of malaria detection in a country like India, where antigen tests and QBC method are costly options.

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