

## A Comparison of Smear versus QBC and Rapid Malaria card tests as diagnostic tool for Malarial parasites.

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### Abstract:

**Introduction:** In malaria patients, a prompt and accurate diagnosis is the key to effective disease management. In order to identify a quick and reliable technique for accurate diagnosis of malaria this study has been conducted. **Methods:** A total of 3603 patients who were suspected with malaria were screened for malarial parasites by various methods like Thick and thin peripheral blood film(PBS), Quantitative buffy coat (QBC) and Rapid diagnostic card test.

**Results:** Out of 3603 clinically suspected patients, Two hundred ninety four patients were infected with *P. vivax* and 297 were infected with *P. falciparum* by QBC method. However, QBC was able to identify 294 *P. vivax* and 288 *P. falciparum* followed by 291 *P. vivax*, 288 *P. falciparum* and 245 *P. vivax* and 266 *P. falciparum* by Advatange mal card and peripheral blood smear respectively.

**Conclusion:** QBC remains the high sensitive test for the diagnosis of Malaria due to high volume of blood concentration of parasites by centrifugation and fluorescent staining of parasites.

**Keywords:** Quantitative buffy coat(QBC), peripheral blood film(PBS), *Plasmodium falciparum*, *Plasmodium vivax*

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### I. Introduction

Malaria, sometimes called the “King of Diseases”, is a serious, sometimes fatal disease caused by protozoan parasites of the genus *Plasmodium*, characterized by fever, chills, and anaemia and is caused by a parasite that is transmitted from one human to another by the bite of infected gravid *Anopheles* mosquitoes. The most serious and sometimes fatal type of malaria is caused by *Plasmodium falciparum*. The other human malaria species, *P. vivax*, *P. ovale*, *P. malariae*, and sometimes *P. knowlesi* (commonly found in South Asia) can cause acute, severe illness but mortality rates are low [1].

The disease now occurs in more than 90 countries worldwide, and it is estimated by The national Vector Borne disease Control programme (NVBDCP) of India about 4 lakhs positive malaria positive cases till June 2017 of this year of which about 2,5 lakhs were *Plasmodium falciparum* [2].

The gold standard for malaria detection is microscopic detection which is tedious and dependent on technical expertise. Therefore rapid antigen detection card for malaria and Quantitative buffy coat (QBC) have been introduced. In view of seriousness of malaria infection and paucity in current availability of diagnostic facilities across India [3], the present study was conducted to compare the efficacy of peripheral blood smear, Rapid diagnostic tests(RDTs) and QBC.

### II. Material And Methods

The present study was a prospective, comparative study evaluating various techniques used for diagnosis of Malaria.

The study was carried out in Paras Pathology lab, Jhansi for a period of three and half years (June 2012 to January 2016), Overall, 3,603 patients of either sex of all ages with fever or history of fever within the prior 24 hours were screened.

**Sample collection:** Blood for study purpose was obtained by venopuncture collected in EDTA (anticoagulant coated) tube was used to prevent alteration in the morphology of white blood cells and Malaria parasite.

#### Methodology:

##### 1. Microscopic examination using stained Thin and thick Peripheral Blood smear(PBS):

**Thick smear:** The thick smear was prepared by placing a small drop in the centre of the slide and spreading it out with a corner of another slide to cover an area about four times its original area. The thick smear of correct thickness is the one through which newsprint is barely visible. The smear was dried for 30 minutes at 37°C

**Thin smear:** Air dry the thin smear for 10 minutes. After drying, the thin smear was fixed in methanol by dipping the thin smear into methanol for 5 seconds. While fixing the thin smear, all care should be taken to avoid exposure of the thick smear to methanol.

Thick and thin film was stained by Giemsa method and examined under oil immersion lens by light microscopy.

Before reporting a negative result, 200 oil immersion visual fields at a magnification of 1000× were examined on both thick and thin smears. The level of parasitemia was expressed by the percentage of parasitized erythrocytes. In non-falciparum malaria, parasitemia rarely exceeds 2%, whereas it can be considerably higher (>50%) in falciparum malaria. In nonimmune individuals, hyperparasitemia (>5% parasitemia or >250 000 parasites/μl) is generally associated with severe disease.

2. *Modified Quantitative Buffy coat (QBC):*

QBC Test, developed by Becton and Dickenson Inc., employs microhemocrit centrifugation which is effective method for identifying the malarial parasite in the peripheral blood.

**Principle of QBC:** Acridine orange binds deoxyribonucleic acids and ribonucleic acids. The malaria parasite binds acridine orange in the nucleus and the cytoplasm and emits green and red fluorescence when excited by blue light (at 460 nm) allowing the detection and examination of parasite morphology by fluorescent microscopy. The nuclei of the parasites emit yellowish green fluorescence whereas the cytoplasm exhibits bright red fluorescence. RBCs are not stained by the dye, while the brightly fluorescent parasites are easily seen. The outlines of stained parasites are well preserved and the general morphology is similar to that in specimens stained by the Giemsa stain.

**Methods:** In QBC, QBC tubes were filled with 55 μl blood. Stopper and float was placed at either end of the tube and then centrifugation was done in RM-12 REMI centrifuge. The centrifuged tube was placed in the paraviewer tube holder and examined under 60 X objective of UV microscope manufactured by Olympus pvt. Ltd.

**Interpretation:** Parasite nucleus fluoresces bright green, while cytoplasm appears orange. The total examination time was seven to ten minutes. Results were reported in terms of the following: presence or absence of parasites and morphology.

3. *Evaluation of Malaria Rapid Diagnostic tests (RDTs):*

Advantage P.f Malaria Card by J Mitra & Co Pvt. Ltd, India. was used in the study.

**Principle:** Advantage Malaria card is an immunoassay based on the “sandwich” principle. Colloidal gold is conjugated to P.f specific monoclonal anti-HRP-2 antibody and monoclonal anti-pan specific pLDH antibody. The test uses monoclonal anti-P.f. HRP-2 antibody (test line F) & monoclonal anti-P.v. specific pLDH antibody (test line V) immobilized on a nitrocellulose strip.

**Methods:** It requires five μL of whole blood to be collected with a pipette provided by the test kit. Test results need to be read after 20 minutes.

**Interpretation:** (i). Appearance of three pink colored line one each in P.v. region (V), P.f. region (F) & Control region (C) indicates that the sample is reactive for P. falciparum and P. vivax. (ii). Appearance of two pink coloured line one each at V & C region only indicates that the sample is reactive for P. vivax only. (iii). Appearance of two pink coloured line one each at F & C region only indicates that the sample is reactive for P. falciparum only. (iv). The test is invalid, if no line appears after the completion of test. The test should be repeated using a new card.

### III. Results And Discussion

**Participants:** A total of 3603 patients who were suspected with malaria were screened for malarial parasites by thick and thin PBS (peripheral blood smear) from May 2013 to April 2017. 568 patients were positive for malarial parasites; Out of 568 positive patients, 268 patients were infected with *Plasmodium falciparum*, and 282 were infected with *Plasmodium vivax* as shown in **Table 1**.

The age of the patients diagnosed with falciparum malaria ranged from 18 to 70 years, (31.94 ±11.37) and that with vivax was 32.01 ± 12.21.

**Discussion:**

The accurate diagnosis of malaria is important for the timely treatment of febrile patients with anti-malarial drugs to reduce their mortality and morbidity and also to effectively manage non-febrile illness. PBS microscopy is tedious and time consuming. Various sensitive methods have been employed for the simple,

reliable, and rapid diagnosis of malaria. The most promising of these were the rapid diagnostic tests and QBC. [4]

QBC failed to detect two PBS falciparum positives which may be due to low parasitaemia. First time users may also fail to identify parasites especially when the parasite concentration is low [6].

QBC method also failed to detect one PBS vivax positives. This may be due to the fact that the specific gravity of late trophozoites of *P. vivax* is similar to the leukocytic layer of the buffy coat. They also become difficult to identify as they get compacted and lose their amoeboid shape during centrifugation [5]. [Table 2]

The sensitivity of QBC has been reported to be as high as 90%, by Gurung et al. [7], 96.22%, by Bhandari et al. [8], and 99.7%, by Benito et al. [9]. Similar findings were obtained for modified QBC in our study. The sensitivity and specificity in vivax was 98.5% and in falciparum arm was found to be 99.02% and 96.1%, respectively, in the present study, which makes it a good diagnostic test.

QBC in our study detected 27 samples as false positive for *P. vivax* and thirteen as false positives for *P. falciparum* which were negative by microscopy and Adv. Mal Card.

Results were available in just twenty to 25 minutes which is drastically reduced time as compared to PBS. The resources for training were also reduced as trainees with 3–5 days of training could produce results comparable to an experienced microscopist. QBC holds promise as a good alternative to PBS due to its speed, sensitivity, and specificity. Routinely QBC method should be employed, especially where the patient load is extremely high.

In the present study, Advantage mal card showed sensitivity of 97% for *P. vivax*, thus making it a good diagnostic tool in areas where the predominant species is *P. vivax*. However this study shows that Advantage mal card has a very high sensitivity and specificity for both *P. vivax* and *P. falciparum* which may be attributed to improved diagnostic technology, where there is inappropriate facilities and lack of advanced set of labs.

**Table 1: Identification of Plasmodium species: Comparison between different diagnostic tools.**

	QBC	Advantage mal card	Peripheral blood film
<b>Plasmodium Vivax</b>	294	291	245
<b>Plasmodium falciparum</b>	297	288	226

**Table 2: 2 X 2 Contingency table showing Accuracy of Plasmodium diagnostic tools**

	QBC		Adv. Mal Card		PBS	
	Positive	Negative	Positive	Negative	Positive	Negative
<b><i>Plasmodium vivax</i></b>						
Positive (286)	266 TP	FN 1	264 TP	22 FN	245 TP	41 FN
Negative (3317)	27 FP	3309 TN	5 FP	3312 TN	0 FP	3317 TN
<b>Total</b>	293	3310	269	3334	245	3358
<b><i>Plasmodium falciparum</i></b>						
Positive (282)	282 TP	FN 2	264 TP	18 FN	226 TP	56 FN
Negative (3321)	13 FP	3306 TN	7 FP	3314 TN	0 FP	3321 TN
<b>Total</b>	295	3308	271	3332	226	3887

TP: True positive, TN: True negative, FP: False Positive and FN: False positive

### Conclusion

Globally, around 3.2 billion people are estimated to be at risk of being infected and developing malaria, and 1.2 billion people are at a high risk. According to the latest estimation, over 200 million cases of malaria occur around the world annually, and the disease leads to over 500,000 deaths each year. Early detection could save lives and prevent disease outbreaks, as well as allow parasite enrichment for biology studies, which are

important topics in malaria research.

The conventional microscopic examination of blood smears, antigen-based rapid test and molecular biology-based diagnosis all have some limitations for effective employment in low-resource setting areas.

There is need to employ more sensitive tests which apart from being rapid will also be able to detect low levels of parasitaemia.

QBC was found to be more rapid so, could be the method of choice for laboratory setting in India as an alternative to conventional microscopy as centrifugation causes the malarial parasites to concentrate to a small compact zone below the buffy coat layer.

Considering the advantages and disadvantages of the diagnostic methods, it can be concluded that QBC can replace microscopy in setups where appropriate facilities are available. However, where there is inadequate laboratory backup, simpler and user friendly techniques like Advantage mal card can be employed which has high sensitivity and specificity.

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