



## COMPARISON BETWEEN THE SPECIFY AND SENSITIVITY OF BUFFY COAT SMEAR AND DIRECT MICROSCOPY IN MALARIA DIAGNOSIS

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Article Info	ABSTRACT
<p>Received 15/09/2014 Revised 27/09/2014 Accepted 02/10/2014</p> <p><b>Key words:</b> Malaria, Plasmodium, Giemsa stain.</p>	<p>Malaria is a serious disease caused by protozoan parasite Plasmodium of human blood, if left untreated, can be fatal. A number of different diagnostic methods are now in use for diagnosis of malaria, but they are expensive and have less specificity and sensitivity. To that using buffy coat technique to increase the specificity and sensitivity of diagnosis than using The direct microscopy. Blood samples were collected from Khartoum teaching hospital. A total number of 40 samples were examined by The Giemsa stain and the buffy coat technique. 32 samples were found to be negative by Giemsa stain and Buffy coat and eight (8) samples were found to be positive.4 out of 8 samples were found to be positive by microscopy for presence of parasites by Giemsa stain and the same 4 samples were found to be positive by Buffy coat, while 4 of them were positive by Buffy coat only. The sensitivity and specificity of the Buffy coat technique were found to be 100% and88.8% respectively compared to microscopic examination. Conclusion the Buffy coat technique was more sensitive and specific than microscopy, and was simple to apply.</p>

### INTRODUCTION

Malaria is a serious disease caused by a parasite, and if left untreated, can be fatal. It is caused by the protozoan parasite Plasmodium.

Malaria parasite is protozoan parasite belonging to the subclass coccidia; it is a blood parasite that transmits through the bite of infected female anopheles mosquito when it takes a blood meal. There are four species that identified to infect human {*Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. Malaria*}. Malaria affects 300 to 500 million people annually worldwide and accounts for over 1 million deaths [1].

The risk of infection may vary according to the season, being highest at the end of the rainy season or soon after. There is no risk of malaria in many tourist destinations in South-East Asia, Latin America and the Caribbean. According to the annual reports of National

Malaria Administration/Sudan (2009), there is an estimated 600 thousand clinical cases/year and about 20% of the total outpatient attendances and 25% of the total admissions are attributed to malaria [2-4].

Early detection and accurate diagnosis are the best tools for saving lives in regions of endemicity. Correct species identification and accurate diagnosis of mixed infections are of particular importance for proper treatment in regions where multiple parasite species are endemic .Of the four species within the genus *Plasmodium* known to infect humans, *Plasmodium falciparum* is the most deadly, followed by *Plasmodium vivax*, which also causes significant morbidity and some mortality.

Light microscopy remains the gold standard method of malaria diagnosis in regions of endemicity; while microscopy is cost effective and requires little



equipment, a well-trained microscopist essential. A highly trained and experienced microscopist can typically detect parasitemias of as low as 90 to 200 parasites/ $\mu$ l. Misdiagnosis may still occur due to low parasitemia or mixed infection.

When thick and thin blood films are negative in a suspect malaria patient, the Buffy coat method is recommended as an adjunct method. Blood stages can be concentrated using centrifugation of blood collected in EDTA anticoagulant, placed in a Wintrobe tube.

This study was carried out to determine the usefulness of examination of blood Sample using thin blood film and Buffy coat smear for diagnosis of plasmodium Species as well as to determine the sensitivity and specificity of those two techniques.

### MATERIAL AND METHODS

This is comparative study, utilize qualitative approach, the Study design is Cross Sectional study. In Khartoum teaching hospital, Khartoum State-Sudan during the Period from April – May. Using Non probability sample, with sample size 2.5ml of Venous blood is taken in EDTA container from 40 patients. The data are collect using questionnaire. Due to limitation of time, the study make by forty (40) samples.

About 2.5 ml venous blood is collect using disposable syringes from anticubital vein after disinfection the skin with 70% alcohol. Specimen is slowly pour into EDTA containers 'as an anticoagulant' gentle and adequate mixing of these specimen is achieve to avoid haemolysis or clotting.

Test applied:- Diagnosis of malaria is appli by two methods blood film[thick and thin] and Buffy coat technique.

**Thick and thin blood films:-** using a cotton piece moist with 70%alcohol disinfection is make of anticubital area, then about 2.5 ml of venous blood is collect using disposable syringe, on clean glass side small drop from the venous blood is put, Using the edge of spreader and by angle45 the thin blood film is make, then left to dry, and fix using absolute methanol for 1-2minutes.on the top of the same slide three large drops of the blood is put, using the edge of another slide the blood is rotate in a circular motion and a thick film is make, of 1cm long, left to dry" the thick film is not fix" both thick and thin films are stain with Giemsa stain with concentration of 10% and left to stain in staining rack for10minutes.after staining time they wash with tap water, and left to air dry then examine under the microscope using 100x oil immersion lens.

**Buffy coat smear for malaria parasite:** The EDTA anticoagulated venous blood is transfer into a glass test tube using a small plastic pulp pipette, then the blood is centrifuge at 2000 for 20 min, small part of red blood cell that found only below the Buffy coat part of Buffy coat layer is transfer to the 1/3 of clean glass slide using Pasteur pipette, then the blood is mix by the end of smooth edged spreader and a thin blood film is make. the preparation is let to dry and then fix with absolute methanol for 2 minutes. the fixed thin blood film is stain with Giemsa stain diluted 1:10 with distilled water and let to stain in staining rack for 10 minutes, the slides is wash with clean tap water after the staining time is finish, then place in clean place for air dry. The preparation is examine microscopically using oil immersion lens (100 x objectives) immersion lens.

### RESULTS

**General description:** This study was carried out to determine the usefulness of examination the RBCs under the Buffy coat for the diagnosis of malaria as well as to compare the sensitivity and specificity of this test with conventional method of microscopic examination of Giemsa-stained blood films.

Forty (40) blood samples were examined through the routine microscope examination (Giemsa stain) and Buffy coat test for malaria, nineteen (19) 19% were male and twenty one (21) 21% were female of different age group. The result showed that thirty two (32) samples were found to be negative by Giemsa stain and Buffy coat test, and eight (8) samples examined were found to be positive. Four (4) out of (8) samples examined were found to be positive by microscopy for the prescence of parasite by Giemsa stain, and the same (4) samples were found to be positive by Buffy coat, while four (4) of them were positive by Buffy coat only. (Table 1)

#### Sensitivity and specificity of the Buffy coat:

#### Calculation of Specificity and Sensitivity of the Results:

Sensitivity was estimated by the following formula:

$$\frac{\text{Number of true positive result by new method}}{\text{Number of true positive + no of false (-)ve result}}$$

Specificity was estimated by:

$$\frac{\text{No of true negative result}}{\text{No of true negative result + No of false (-)ve result}}$$

The sensitivity and specificity of the Buffy coat was determined – that the sensitivity and specificity of the Buffy coat were 100% and 88.8% respectively. (Table 2).

**Table 1. Comparison between Diagnosis by blood film by Giemsa stain and Buffy coat**

	Results	Buffy coat		
		Positive	Negative	Total
Giemsa stain	Positive	4	0	4
	Negative	4	32	36
	Total	8	32	40



**Table 2. Sensitivity and specificity of the Buffy coat compared to thick blood film by Giemsa stain**

Buffy coat test		
Sensitivity	$\frac{8}{8 + 0}$	100%
Specificity	$\frac{32}{32 + 4}$	88.8%

**DISCUSSION**

The majority of cases of malaria worldwide are treated on the basis of clinical Diagnosis and microscopy [5,6]. 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 results are poor in cases of low parasitaemia. The sensitivity and specificity of Buffy coat technique test was found to be higher in the present study than reported by Serougi AO et al (1996) due to our selection criteria. We have not included patients of malaria in general and have carried out the tests only where there was strong suspicion

due to various associated clinical presentations of *P. falciparum* infection. This is a baseline study comprising small group of patients.

On comparing Buffy coat technique with conventional blood smear examination Z-values were found to be statistically significant. This indicates that this test may be better option in case of negative blood smear.

**CONCLUSION**

The present study demonstrated that the Buffy coat test is highly sensitive and specific for the diagnosis of malaria infection.

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