

EVALUATION OF QUANTITATIVE BUFFY COAT TECHNIQUE IN THE DIAGNOSIS OF MALARIA: A STUDY FROM NORTH EAST INDIAPranita Medhi¹, Swagata Dowerah², Aparna Dutta³**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: Malaria is one of the major public health problems in north eastern region of India. The conventional method used for the diagnosis is the thick and thin smears. This study was carried out to evaluate the Quantitative Buffy Coat technique against the conventional Giemsa stained smears in diagnosis of malaria parasite. **MATERIALS AND METHODS:** This study was carried out in a hundred febrile cases with presumptive diagnosis of malaria. Twenty healthy controls were included in the study. The blood samples of the cases and controls were evaluated for malaria parasite by light microscopy with Giemsa stained thick and thin smears and Quantitative Buffy Coat fluorescent microscopy technique. **RESULTS:** Out of the total 100 febrile cases examined by both QBC and Giemsa stained smears, only 70 cases were diagnosed to be malaria microscopically. The prevalence of *P falciparum* (75.7%) was more than *P vivax* (21.4%) and that of mixed infection was 2.9%. Taking Giemsa stained films as gold standard, the sensitivity and specificity of QBC technique was found to be 98.5% and 85.7% respectively with positive predictive value of 92.7% and negative predictive value of 96.8%. Concordance of the two tests was found to be 94%. In 5 blood films where no parasite could be detected initially, QBC technique was able to detect parasite. The QBC technique was seen to be less sensitive in the detection of vivax than falciparum malaria. The average time required to examine a positive QBC tube was 1.12 minutes whereas GTF method required an average of 10 minutes to examine.

KEYWORDS: Quantitative Buffy Coat, Malaria, Giemsa-Stained Thick Film.

INTRODUCTION: Malaria is one of the major public health problems in north eastern region of India. However, it presents a diagnostic challenge to laboratories in most countries. The conventional method used for the diagnosis is the thick and thin smears. This method costs less and easy to handle but on the other hand, it has several disadvantages.^[1] Rapid diagnosis of malaria is pre-requisite for effective treatment and reducing mortality and morbidity of malaria. The Quantitative Buffy Coat (QBC) technique involves the use of special fluorochrome dye to highlight malaria parasite at predictable location of a specially prepared capillary tube. This method, though faster and easier is however much more costly and species identification is difficult by this technique.^[2] This study was carried out in a teaching hospital in North-east India to evaluate the QBC technique against the conventional Giemsa stained smears in diagnosis of malaria parasite.

MATERIALS AND METHODS: This study was carried out in a hundred febrile cases with presumptive diagnosis of malaria. Twenty healthy controls were included in the study. The blood samples of the cases and controls were evaluated for malaria parasite by light microscopy with Giemsa stained thick and thin smears and Quantitative Buffy Coat fluorescent microscopy technique. Haematological and other biochemical investigations were carried out in the positive cases Giemsa stained thick blood films were taken as gold standard against which the QBC technique was evaluated.

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Quantitative Buffy Coat Technique (QBC): Quantitative buffy coat technique (BD Diagnostics) was employed for the detection of malarial parasites in blood. Specially designed microhematocrit tubes coated with acridine orange were used. Approximately, 55-60 μ l of blood was loaded into the tubes and stopper and float were applied at either ends; the tubes were centrifuged at 12000 RPM in a pre-programmed centrifuge as per the manufacturer's instructions. The interpretation was done using a standard microscope fitted with Para Lens ultraviolet microscope adaptor and a \times 60 objective connected to fiber optic ultraviolet light module. The parasites were seen in buffy coat layer and the interface between RBC and WBC regions.^[3]

RESULTS AND OBSERVATIONS: Out of the total 100 febrile cases examined by both QBC and Giemsa stained smears, only 70 cases were diagnosed to be malaria microscopically. The prevalence of *P falciparum* (75.7%) was more than *P vivax* (21.4%) and that of mixed infection was 2.9%. Of these 70 diagnosed cases of malaria, 64 cases were positive by both techniques. (Table 1) Of the remaining 6 cases, 5 were positive only by QBC technique and 1 case was positive only by Giemsa stained thick film (GTF). Taking GTF as gold standard, the sensitivity and specificity of QBC technique was found to be 98.5% and 85.7% respectively with positive predictive value of 92.7% and negative predictive value of 96.8%. Concordance of the two tests was found to be 94%. None of the controls showed positivity by either technique. On re reading and examining 400 fields of GTF in those films which were QBC positive but GTF negative, it was seen that 7.1% of discordant results fell to only 2.8%.

It is therefore possible that the assumptive "false positives" could actually be true positives if GTF were subjected to prolonged re reading of smears or repeat smearing, thus further enhancing the specificity of the QBC test. On re- examination of the QBC tubes, none of the negative tubes were considered positive. It is evident from this that in 5 blood films where no parasite could be detected initially, QBC technique was able to detect parasite. Although at this point it is difficult to comment on its sensitivity due to unavailability of further higher modes of investigations like PCR, it is clear that QBC detected more positive specimen ($P < 0.001$ by chi square test). Moreover it showed 100% sensitivity with parasite density of ≥ 440 parasites/ μ l of blood. The sensitivity of QBC technique varied in different levels of parasitemia as depicted in Table 2. The sensitivity of the QBC technique with regard to ability to identify species and different stages correctly was 97.1% but in cases of *P. vivax*, the sensitivity was found to be 92.8% as QBC could not make species identification in 1 case of *P. vivax* ring form and it failed to detect the one and only sample that showed *P. vivax* schizonts in the blood film. (Table 3) The results indicate that QBC technique is less sensitive in the detection of vivax than falciparum malaria.

Most of the QBC tubes were read at 30 seconds whereas the shortest time required to read GTF by light microscopy was 1 minute. The average time required to examine a positive QBC tube was 1.12 minutes whereas GTF method required an average of 10 minutes to examine.

DISCUSSION: The recommended method and current gold standard used for the routine laboratory diagnosis of malaria is the microscopic examination of stained thin and thick blood films, particularly with the additional sensitivity offered by examination of thick blood films. The centrifugal quantitative buffy coat combines an acridine orange-coated capillary tube and an internal float to separate layers of WBC and platelets using centrifugation. Parasites concentrate below this layer of cells, appearing in the upper layer of RBC but also sometimes appearing within the layers of platelets

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and WBC. Parasites can be viewed through the capillary tube using a special long-focal-length objective with a fluorescence microscope.^{[3],[4],[5],[6]}

In our study QBC technique was found to have a sensitivity of 98.5%, specificity of 85.7%, positive predictive value of 92.7% and negative predictive value of 96.8%. However on repeated examinations and re reading of 400 microscopic fields of Giemsa stained thick films, specificity was increased from 85.7% to 93.7%. In a study by Parija et al,^[7] it was found to have sensitivity of 78.94%, specificity, PPV and NPV were found to be 98%, 90%, and 95%, respectively. Benito et al^[8] however reported a sensitivity of 99.7%, even higher than in our study.

In relation to parasite density, it was observed that sensitivity of QBC in cases with parasite density of ≥ 400 parasites/ μl of blood was 100% and sensitivity in parasite density of 40-400 parasites/ μl of blood was 91.6%. QBC detected parasite in 5 samples where no parasite was detected by Giemsa stained smears.

The sensitivity of QBC for species detection was found to be 97.1%. Sensitivity was 100% for falciparum malaria but only 92.8% for *P. vivax*. The only false negative result by this technique was found to have *P. vivax* by Giemsa stain while the 5 cases detected positive by QBC which were negative on Giemsa were all *P. falciparum* cases. Parija et al,^[7] also noted that the detection of parasites other than *P. falciparum* was not very sensitive by this technique. In another study by Pinto et al,^[9] species identification was not possible in 7.9% of cases by QBC technique. Concern over the ability of this method in species detection has been expressed with success rate varying from 75% to 93%.^[10]

QBC technique was found to be easier to process and more rapid than GTF. Most of the QBC tubes were read at 30 seconds and the average time required to examine a positive QBC tube was 1.12 minutes whereas GTF method required an average of 10 minutes to examine. The speed of this method in detecting malaria parasites, especially in cases with low parasite levels is an advantage in laboratories processing a large daily load of samples.

An advantage of QBC is its ease of interpretation and it being technically easy to perform. (2) Diagnosis of malaria by the acridine orange staining of centrifuged parasites in microhematocrite tubes is easy to learn. ^[11] A technician can be trained to perform the QBC test and detect malarial parasite accurately, in less than a day, in contrast to smear examination and interpretation where proper training may take weeks.

CONCLUSION: Since malaria is endemic in certain regions of India, we need to employ more sensitive tests, which are also rapid to detect low levels of parasitemia in population. Quantitative buffy coat can be of use in centres where appropriate facilities are available. On the other hand, the need for a sophisticated ultraviolet light microscope, cost, difficulties in species determination and parasite quantification are few disadvantages of QBC technique which need to be kept in mind.

		Giemsa Stained Thick Film		Total
		Positive	Negative	
QBC technique	Positive	64	5	69
	Negative	1	30	31
Total		65	35	100

Table 1: Showing comparison of QBC and GTF methods of malaria detection

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No. of parasites /200 WBCs	No. of blood films	No. of QBC Positive Tubes	QBC Sensitivity
0(parasites not detected)	5(negative films)	5(positive tubes)	?*
1-10	12	11	91.6%
11-100	14	14	100%
≥101	39	39	100%

Table 2: Comparing QBC with GTF for different parasite densities

*sensitivity could not be commented as GTF was taken as the gold standard

Species and Stage Identification by GTF	No. of Blood films	No. of QBC +ve Correct Species Identification	Sensitivity of QBC
Not detected	5	5(all P.F)	?*
PF (ring form)	41	41	100%
PF(gametocyte)	7	7	100%
PV(ring form)	14	13	92.8%
PV(schizonts)	1	Not detected	-----
PF + PV(mixed)	2	2	100%

Table 3: Showing sensitivity of QBC in species detection

*sensitivity could not be commented as GTF was taken as the gold standard

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