

The Quantitative Buffy Coat Technique (QBC) in Early Diagnosis of Malaria: The Santo Tomas University Hospital Experience

Ronaldo H. Estacio, M.D.,* Emmanuel Edwin R. Dy, M.D.,** Shirley Cresswell, M.D.,** Remedios Fabra Coronel, M.D.**** and Angeles Tan Alora, M.D.*****

(*Resident and PSMID-Unilab Junior Awardee, 1993; **Senior Resident; Department of Medicine, ***Visiting Professor and Laboratory Chief, Michigan State University (Saginaw Campus), Michigan, U.S.A., ****Associate Professor of Medicine and *****Professor of Medicine, Faculty of Medicine and Surgery, University of Santo Tomas, Espana, Manila)

The quantitative buffy coat (QBC) technique is a method of diagnosing hematoparasites based on microcentrifugation, fluorescence and density gradient of infected red blood cells. This study compared the QBC technique and the conventional thick-and-thin smear in the diagnosis of malaria. There were 25 patients clinically diagnosed to have malaria. All patients included in the study presented with fever: 23 (92%) had history of travel to an endemic area while 2 (8%) had history of blood transfusion. Fifteen patients were male (60%) and 10 were female (40%). The age of the patients ranged from 5 to 70 years with a mean of 20 years. The smears did not reveal any malarial parasite while the QBC showed malarial parasites present in all the patients. Treatment was not started until the diagnosis was confirmed. Twenty-three patients has *Plasmodium falciparum* while 2 had *Plasmodium vivax*. The QBC offered several advantages: (1) concentration of parasites is ensured in a narrow zone for ease of examination, (2) enhanced sensitivity and (3) ease of performance. This study showed that the QBC was more sensitive than the conventional method in the early detection of malarial parasite. [*Phil J Microbiol Infect Dis* 1993; 22(2):56-59]

Key Words: malaria, quantitative buffy coat, thick and thin smears, *Plasmodium falciparum*, *Plasmodium vivax*

Malaria causes changes in most organs ranging from very mild to very severe and sometimes can be fatal. It is caused by one (or combination) of 4 species of *Plasmodium*: *falciparum*, *vivax*, *ovale* and *malariae*. The first two account for more than 95% of malaria cases in the world.¹ The clinical presentation varies from mild to complicated according to the specie s involved, the patient's state of immunity, intensity of infection and presence of concomitant conditions such as malnutrition. The disease tends to be more severe in children and among pregnant individuals. In the Philippines, Tagle et al² reported that 63% of malaria cases were due to *P. falciparum* while 20% were due to *P. vivax*. In 1987, malaria ranked sixth in the list of ten leading causes of morbidity. From 1982 to 1986, 121,097 cases (21.1% per 100,000 population) affected mostly residents from the hinterlands and newly opened settlement areas. The number of deaths from malaria recorded in 1987 was 1,226 or a mortality rate of 2.1% per 100,000 population.

In 1992, Tagle and Cabanban conducted a retrospective study regarding severe and complicated malaria at the San Lazaro Hospital.² The study revealed a fatality rate of 6.3% (29 out of 457 cases). Among the various modalities of clinical presentation, cerebral malaria was noted in 89.6% of fatal cases (26 out of 29 cases).

The demonstration of malarial parasite is a requisite for definitive diagnosis. The conventional method used for the diagnosis is the thick-and-thin smears. This method costs less, is easy to handle and does not pose any transport difficulty. On the other hand, it has several disadvantages, most notably: (1) the competence of the reader, (2) the inevitability of artifacts which may hinder proper differentiation of the parasites, and (3) the unnecessary tediousness of the procedure.

The inconsistent reliability of the smears has led to the practice advocated by some clinicians to simply employ a therapeutic trial of anti-malarial agents, out of utter desperation from the oftentimes reported negative malarial smear. This has been a reality the clinician has

learned to accept (and abuse) and work around, often fearing (for the patient's welfare) that the drug might not work.

In a country where malaria is endemic, a procedure which is sensitive enough to detect malarial parasite in a short span of time is a very much welcome diagnostic tool. This facilitates early diagnosis and thus timely therapy, averting the florid manifestations of malaria thus reducing morbidity and mortality.

The quantitative buffy coat technique (QBC) [Becton Dickinson, Baltimore], conceptualized as far back as 1974, employs microhematocrit centrifugation which is an effective means of concentrating hematoparasites (e.g. malaria) prior to direct examination. It employs a precisely constructed capillary tube which is internally coated with EDTA and acridine orange.^{3,4} The use of the dye is based on the premise that infected red cells appear to be less dense than uninfected ones, and concentrate primarily within the zone at the interface - a small 1-2 mm region near the top of the RBC column. These parasites fluoresce green and orange objects because of the uptake of the dye.

After filling up with venous or capillary blood to a predetermined level and after centrifugation the QBC tube shows different regions representing different blood components depending on their specific gravity (Figure 2). Because the float occupies 90% of the interior of the lumen of the tube, the leukocyte and thrombocyte band width and the top most area of the RBCs are enlarged to 10% normal. The hematoparasites are concentrated into a small 1-2 mm region near the top of the erythrocyte column, i.e. the buffy layer. The parasites present in the buffy layer are held close to the wall by the plastic float which has the same specific gravity as that of the buffy layer. Essentially, this method automatically prepares a concentrated smear about 40 μ thick which is the distance between the float and the walls of the tubes and into which all the parasites become concentrated. In practice, since the area where the parasite is present in highest concentration is predictable hematoparasites can be detected within 15 seconds of focusing the tube, while 7 to 9 minutes are required to ensure a negative sample. The QBC has been used primarily as a screening method for malaria in highly endemic Africa. The centrifuge machine has been designed to accommodate 30-40 capillaries at any time and the facility of examination takes a shorter time than the conventional means.

Recognizing the advantages and disadvantages of the accepted standard method of diagnosing malaria in the local setting as well as the promise of a better screening tool for recognizing the same disease, this study compares the two techniques now available at the Santo Tomas University Hospital.

PATIENTS AND METHODS

All patients clinically diagnosed to have malaria and admitted at the Santo Tomas University Hospital from January to December 1992 were included in the study. They were screened for malarial parasite using the conventional thick and thin smear and the quantitative buffy coat technique by any of the 3 authors (RIDE, EERD, SC). The conventional procedure in performing malaria thick and thin smears was followed. The quantitative buffy coat (QBC) technique was as follows:

1. Prick patient. Fill QBC tube with blood from the end with EDTA (blue-lined end).
2. Mix blood with acridine orange dye at the other end by ensuring the dye is washed out. When this is done, cap the blue-lined end.
3. Insert the plastic float on the other end.
4. Spin the tube in parafuge machine.
5. Place the centrifuged QBC tube into the groove or the paraviewer tube holder.
6. Place the paraview tube holder on the stage of the microscope. Using 19x wide field, high-point eye piece and 50x (60x) objective lens, add 2-3 drops of fluorescence optical immersion oil. Bring buffy coat area of the tube to a focus.

7. Move the microscope stage until the top of the expanded RBC layer is in the field of view. Examine sample.

Results for both techniques were reported in terms of the following: (1) presence or absence of parasite (2) morphology, and (3) quantitation (if possible).

RESULTS

The study population was composed of 25 patients. Fifteen patients (60%) were male while 10 (40%) were female. Most patients were between 19 to 35 years old with a mean of 20 years (range 5-74 years).

All patients had a history of fever. Twenty-three (92%) of the patients had a travel history to an endemic area for malaria. Table 1 would show that most cases came from Bulacan (5.20%) followed by Cabanatuan (4.16%) and San Pablo, Laguna (3.12%). The remaining 2 patients (8%) did not come from any endemic area but had previous blood transfusion immediately before the onset of fever.

Thick-and-thin blood films of all patients did not reveal any parasite. On the other hand, the QBC revealed the presence of malarial parasite. *Plasmodium falciparum* was identified in 23 of the subjects (92%) while *Plasmodium vivax* was present in 2 (8%) of the subjects who had blood transfusion. Table 2 shows the extensive differentiation between features of the QBC and the thick smear.

The treatment of malaria was not started until diagnosis was documented. Of the 25 patients, 19 (76%) showed clinical improvement after being given appropriate treatment. Six patients (24%) expired while on treatment. Five of these had concomitant septicemia while one had myocarditis.

Of the 19 improved cases, 16 (84%) had lysis of fever after conventional chloroquine treatment while 3 patients (16%) still needed other anti-malaria (pyrimethamine-sulfadoxine and quinine) before improvement was noted.

Table 1. Area distribution according to Plasmodium infection among patients with positive QBC at the STUH

Areas	P. falciparum	P. vivax	Total	(%)
Bulacan	4	1	5	(20)
Cabanatuan, Nueva Ecija	4	0	4	(16)
Isabela	4	0	4	(16)
Malabon	3	0	3	(12)
Manila	0	1	1	(4)
Negros Oriental	1	0	1	(4)
Palawan	1	0	1	(4)
Pangasinan	2	0	2	(8)
Tarlac	2	0	2	(8)
Zambales	2	0	2	(8)
Total	23 (92%)	2 (8%)	25	(100%)

Table 2. Comparison of features between the thick smear and the quantitative buffy coat technique (QBC)

QBC	Thick Smear
Easily run by technician	Requires high degree after brief training of technical skills, training and experience
Time for screen < 1 minute	17 minutes for 100 fields by experienced technician
Over 100 tubes screened with ease in 4 hours	Diminished attention from fatigue
Parasites maybe detected 12 - 48 hours before visible on thick smears	
Parasites concentrated in narrow zone where they can be viewed with ease	Sensitivity reduced by obscuring debris/poor contrast
No variables - coated tubes	Quality of staining affected by pH and freshness of stain

DISCUSSION

The QBC technique is an attractive alternative in the diagnosis of malaria. A precisely constructed capillary tube is internally coated with EDTA and acridine orange, filled with capillary or venous blood to a predetermined level (60 uL) and capped.² Staining of the nuclear DNA of the parasites by acridine orange makes determination of specimen immediately possible. Morphologic characteristics can be examined by fluorescence microscopy, since nuclei fluoresce bright green. When examination cannot be carried out right away, the parasites retain their morphology over several days at room temperature or for at least 7 days when stored in the refrigerator.

The insertion of a plastic float prior to centrifugation (12,000 g for 5 minutes) concentrates the parasites. The float serves to expand the buffy coat by creating a 40 u wide area within the buffy layer defined by the exterior of the float and the interior of the capillet. Malarial parasites thus confined to the periphery of the tube can be counted in this system using a light microscope, although fluorescence is essential for speciation. As a consequence of displacement and concentration, almost all parasites collected in the tube are visible. Parzy et al⁵ observed that 3 minutes is sufficient to determine a negative QBC. The study also stated that for the malaria smear to be sensitive, it entails a prolonged reading time.

Results of QBC have been shown to agree well with direct counts from positive blood smears. Since the area of the tube where concentration of parasites is predictable after centrifugation, these can be detected within 15 seconds of focusing on the tube in individuals with high parasitemia while 7-10 minutes is required for a thorough examination to ensure a negative sample.³ The fact that parasites are concentrated from a relatively large volume of blood increases the sensitivity and reduces the examination time of the specimen.

The QBC technique, although initially more expensive than the standard blood films, is more sensitive than the smears. Paton et al⁶ showed that the QBC was able to detect levels of parasitemia as low as one parasitized RBC out of 5×10^5 RBC. Spielman et al⁸ had 10% more malaria infection recognized by direct centrifugation than by an experienced microscopist examining conventional Giemsa stain. Rickman et al⁷ observed that positive QBC tubes were never observed in uninfected individuals, yet positive QBC tubes were obtained in several individuals with initially negative smears later becoming positive. It has been said that parasites may be detected 12 to 48 hours using the QBC before becoming visible on the thick smear.

CONCLUSION

The study showed that QBC technique can be a more sensitive means of detection of malaria parasite. With the advantages and disadvantages cited between the fluorescent technique and the conventional method, the QBC can be considered a rapid means of recognizing malaria, a vital requisite prior to early and definitive treatment.

REFERENCES

1. Rashid KM, Kondrashin AV, et al. The clinical management of acute malaria. WHO Regional Publication Southeast Asia. New Delhi, 1986.
2. Tagle R, Cabanban A. Severe and complicated malaria at San Lazaro Hospital. *Phil J Microbiol Infect Dis* 1992; 7:4-10.
3. Levine RA, Wardlaw SC, et al. Detection of hematoparasites using quantitative buffy coat analysis tubes. *Parasitology Today* 1989; 5(4):133.
4. Levine RA, Wardlaw SC. A new technique for examining blood. *American Scientist* 1988; 6:597.
5. Parzy D, Raphenon B, Martet G, et al. Quantitative buffy coat test (QBC test) monoflow kit falciparum: comparative value in the rapid diagnosis of malaria. *Med Trop* 1990; 50:97-101.
6. Paton EL, Philippine Y, et al. A sensitive technique for malaria diagnosis: DNA fluorescence in the expanded buffy coat (a laboratory and field evaluation. 1986. (unpublished).

7. Rickman LS, Oberst R, Sangalang R, et al. Rapid diagnosis of malaria by acridine orange staining of centrifuged parasites. *Lancet* 1989; 862(9):68-71.
8. Spielman A, Perrone JB, et al. Malaria diagnosis by direct observation of centrifuged samples of blood. *Am J Trop Med Hyg* 1988; 15(27):337-342.
9. Kaplow LS. Buffy coat preparatory tube. *Am J Clin Pathol* 1969; 39(5):806-807.
10. Van TH, et al. Severe malaria in a provincial hospital in Vietnam. *Lancet* 1990; 336:1316.
11. WHO Malaria Action Programme. Severe and complicated malaria. *Trans R Soc Trop Med Hyg* 1986; (suppl):3-50.