

Malaria Diagnosis by Quantitative Buffy Coat Technique

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Summary

The QBC (Quantitative Buffy Coat) method is a rapid diagnostic test for malaria based on acridine orange staining of centrifuged parasites in a microhaematocrit tube. We compared the QBC tube method with the conventional blood film examination in the diagnosis of malaria in a double-blind study in three population groups. When compared with the thick blood smear in 318 hospital patients clinically suspected of having malaria, QBC method resulted in 96.77% sensitivity and 98.73% specificity. The QBC tube method interpreted correctly as negative in all of 800 blood donor samples (free of malaria) demonstrating a specificity of 100%. When used for mass screening in the field study of 339 patients, the test had a sensitivity of 70.97%. Overall, the QBC tube method was found to be highly specific (99.07%). Despite its high cost, inability to quantitate parasitaemia and instability with long storage, the QBC technique definitely has potential use as a rapid screening tool in malaria diagnosis.

Key Words: Malaria diagnosis, Quantitative buffer coat

Introduction

The diagnosis of malaria is conventionally established by microscopic examination of stained thick and thin blood smears. Considerable time and experience are required for adequate preparation and interpretation of the blood smears. A less labour-intensive alternative to this conventional technique is therefore long overdue. In 1983, a method using a capillary tube precoated with acridine orange and containing a float was developed for the rapid quantification of leucocytes in peripheral blood. This method was termed "QBC" for "Quantitative Buffy Coat Analysis"¹. The acridine orange stains all nucleic acid containing cells and the associated fluorescence is observable under blue-violet light through a microscope. This technique was then adopted for detection of malaria parasites as the acridine orange

staining permits differential colouration of green (nuclei) and red (cytoplasm) in stained parasites. Spielman *et al*² found that the QBC method is at least eight times more sensitive than Giemsa stained thick blood smears. Rickman *et al*³ found that the QBC method is easier and faster to perform than the thick smear method yielding a specificity of 98.4%. In a field study, Mak *et al*⁴ found 55.93% sensitivity and 94.92% specificity when the QBC tube method was compared with the blood smears.

The aim of our study is to determine the sensitivity and the specificity of the QBC tube method in the detection of malaria parasite in a hospital and field setting using the blood film method as the standard. This study also explores the feasibility of employing the QBC method routinely for malaria diagnosis in our laboratory.

Materials and Methods

Study groups

Three population groups were studied. The first group, the hospital group, included 318 patients clinically suspected of having malaria who presented to the Hospital Universiti Sains Malaysia (HUSM), Kelantan between May and December 1991. The second group, the field group, consisted of 339 villagers from Gua Musang, Kelantan. The Gua Musang district is a known malaria endemic area and contributed about 40% of the total malaria cases seen in the state of Kelantan in 1990⁵. For practical reasons the subjects were selected from those screened during a mass blood survey made by the Kelantan Vector-Borne Disease Control Unit. They were screened during two visits, three days each, in January and October, 1992. The third group, the blood donor group, comprised all 800 blood donors screened at HUSM blood bank between May and July 1991.

Sample collection and examination

Samples were collected by either capillary finger-prick or phlebotomy. The blood samples were anticoagulated in glass bottles containing ethylenediamine tetraacetate (EDTA). Each sample was processed by i) conventional microscopic examination and ii) QBC tube direct inspection test.

QBC methodology

Acridine orange (AO) 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 at 480 nm allowing the detection and examination of parasite morphology by fluorescent microscopy. The outlines of stained parasites are well preserved and the general morphology is similar to that in specimens stained by the Giemsa stain⁶. In the present study, fluorescent microscopy was through the use of a standard light microscope provided with a battery-powered light and a special blue-violet light. Red blood cells are not stained by the dye, hence remain inconspicuous under fluorescent light while the brightly fluorescent parasites are easily seen. The nuclei of the parasites emit yellowish green fluorescence whereas the cytoplasm exhibits bright red fluorescence.

The QBC glass capillary tube (Becton Dickinson) is 75 mm in length and 1.677 mm in diameter¹. The tubes are internally coated with EDTA and heparin at the fill end and with acridine orange stain and potassium oxalate at the other end. Samples of blood, 55-65 μ l, were transferred to the QBC tube by capillary action. The tubes were rotated for 10 seconds in order to dissolve the contained residues in the blood. Expansion of the centrifugally separated cell layers is achieved with a 20 mm plastic float. When the plastic float is inserted into the tube, there will be a 40 μ wide space between the float exterior and the float interior. The plastic float having a specific gravity (1.055) that is midway between that of plasma (1.028) and red blood cells (1.090) is positioned. The tubes are then centrifuged at 12,000 g for 5 minutes. The area surrounding the float just beneath the buffy coat was examined under oil immersion. Individual cells within this layer were easily seen by microscopy; the malaria parasites staining green (DNA) and orange (RNA) under blue-violet light. The entire circumference of the tube was examined systematically while moving away from the buffy coat through the erythrocyte layer. Each tube was examined until parasites were detected or for a maximum of 5 minutes.

In the hospital and the blood donor groups all the QBC tubes were centrifuged and examined within an hour of collection and centrifugation respectively. Due to transport delays attributable to poor road access, the QBC tubes in the field group were centrifuged and examined only 8 to 10 hours after collection and reexamined 72 hours later in the central laboratory. The examination was made under x 10 ocular lens and x 60 objective lens.

For the conventional method (referred here as BFMP - blood film for malaria parasite), blood was prepared in paired thick and thin films on microscopic slides. The smears were dried, the thin films fixed in absolute methanol, and both preparations stained by Giemsa stain. Each blood smear was examined microscopically without the knowledge of the QBC test result. The parasite species was determined by examination of the thin blood film smears. In the event that no parasites were seen, the corresponding thick film was reexamined for a further 200 microscopic fields under x 1000 magnification.

In the hospital group, samples from malaria positive patients were obtained and examined daily until each of the patient was documented to be negative for malaria parasite.

For calculations of sensitivity and specificity, the thick blood smear was used as the standard.

The sensitivity and specificity were calculated as follows:

$$\text{Sensitivity} = \frac{\text{Number of specimens positive by both QBC \& thick smears}}{\text{Total number of positive thick smears}} \times 100$$

$$\text{Specificity} = \frac{\text{Number of specimens negative by both QBC \& thick smears}}{\text{Total number of negative thick smears}} \times 100$$

Results

The hospital group

Samples from 16 of the 318 hospital patients were found to be positive for malaria by both blood smears and the QBC technique. Blood samples from the 16 malaria positive patients were obtained daily until each of the patients was documented to be negative for malaria parasites. The results of this daily examinations is shown in Figure 1. It can be seen that the QBC tube is comparable to the thick smears in detecting malaria parasites. In patients 1, 2 and 3 the QBC tube method could detect the malaria parasites 24-48 hours earlier while the blood smears were negative. In patient 2, the QBC could detect the malaria parasites at days 5 and 6 while it was negative with the blood smears. In patients 4 and 5 the thick smears were positive after being found negative by the QBC. A total of 430 specimens were examined daily from these 16 patients. The results are shown in Table I. Sixty out of 62 samples were positive for malaria parasites by both the QBC technique and the blood smear yielding a sensitivity of 96.77% and 99.73% specificity.

The QBC method correctly identified the parasite species in all the positive samples, four being

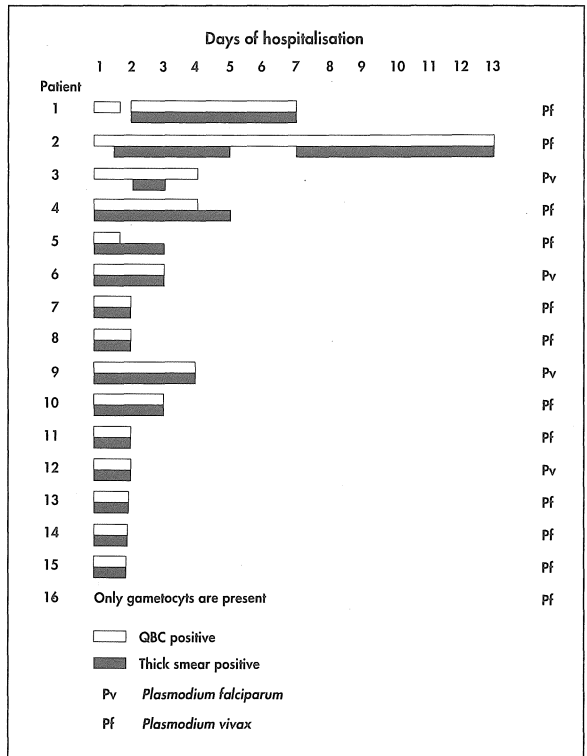


Fig. 1: Comparison of QBC tube test with thick blood smears in 16 hospital patients, HUSM Kelantan, 1991

Plasmodium vivax and twelve *Plasmodium falciparum*.

The field group

Thirty-one of the 339 subjects evaluated in this group were positive for malaria parasites when examined using 200 fields of a thick blood smear (Table II). Parasites were identified by the QBC tube method in 22 of the 31 subjects yielding a sensitivity of 70.97% and specificity of 97.40%.

Blood donors

All of the 800 blood donor samples examined were interpreted correctly as negative by the QBC tube method yielding a specificity of 100%.

Discussion

Since the introduction of this new technique, the QBC

Table I
Comparative study between QBC and BFMP in 430 samples from 16 patients in HUSM, Kelantan, 1991

		QBC		
		Positive	Negative	Total
BFMP	Positive	60	2	62
	Negative	1	367	368
Total		61	369	430

Sensitivity = 96.77%; Specificity = 99.73%
 BFMP = Blood Film For Malarial Parasite

Table II
Comparative study between QBC and BFMP in the diagnosis of malaria in 339 subjects from Gua Musang, Kelantan, 1992

		QBC		
		Positive	Negative	Total
BFMP	Positive	22	9	31
	Negative	8	300	308
Total		30	309	339

Sensitivity = 70.97%; Specificity = 97.40%
 BFMP = Blood Film For Malarial Parasite

has been evaluated by a number of workers. The sensitivity observed is high if done in a laboratory (hospital) setting but is low if done in a field setting. Spielman *et al*² carried out his study in a laboratory setting and found that the QBC could identify 10% more positives as compared to the thick blood smears. Rickmann *et al*³ found that the QBC could detect as few as 3 parasites/ μ l giving a sensitivity of 96% (when all stages of malaria were considered) and specificity of 93% in his hospital group patients. In a laboratory setting in Thailand, Namsiripongpum *et al*⁷ found the QBC had a sensitivity of 99.13% and a specificity of 96.76%. Species differentiation was found to be 92.98%.

In a field study by Rickmann *et al*³, the QBC technique had a sensitivity of 70% and a specificity of 98.4%. Mak JW *et al*⁴, on the other hand found the QBC to have a sensitivity of 55.93% and a specificity of 94.92% in a field study.

The findings of our study showed that the QBC technique showed a higher sensitivity and specificity in a laboratory setting (97.77% sensitivity and 99.73% specificity) than in the field (sensitivity 70.97% and specificity 97.40%) when compared with blood smears.

The lower sensitivities observed in the field group may be attributable to delayed processing of the specimen after collection. The specimens were exposed to high ambient temperatures and humidity, and were transferred to QBC tubes and centrifuged up to 8-10 hours after collection. On the other hand, in the hospital group, specimens were processed within 2 hours of collection. Similar potentially compromising delays in specimen processing were encountered by Rickmann *et al*³ in his field study.

The major advantages of the QBC tubes over the blood smears are its speed and ease of performance. Once the specimen is obtained, it is a matter of putting the specimens in the specified capillary tube, centrifuge it for 5 minutes and the specimen is then ready for interpretation. Furthermore learning of the QBC technique can be acquired in less than a week whereas it may take weeks or months to learn to interpret thick blood smears accurately.

However there are limitations observed with the QBC system i.e. its inability to estimate parasite density, instability with long storage and the need for expensive equipments.

In conclusion, the QBC tube is not a substitute for the blood smear in the overall detection and control of malaria. However the speed and ease of use of this technique make it an important new tool for the diagnosis of malaria. Once malaria diagnosis has been established by the QBC, blood smears need to be examined for quantification of parasitaemia; an important parameter in the management of malaria afflicted patients. This study underscores the urgency of processing blood specimens without unnecessary delay following collection to optimize the results.

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