COMPARISON OF THE QUANTITATIVE BUFFY COAT TECHNIQUE WITH THE CONVENTIONAL THICK BLOOD FILM TECHNIQUE FOR MALARIA CASE DETECTION IN THE FIELD

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ABSTRACT

The quantitative buffy coat (QBC) technique was compared with the conventional thick blood film technique in a malaria survey carried out in a mesoendemic area of malaria in Betau, Pahang, Malaysia. The QBC technique was found to be a rapid technique but had a sensitivity of about 56% and a specificity of 95%, using the thick blood film method as the "gold standard". Malaria species identification was unsatisfactory with the QBC technique as it could identify parasites correctly in only about 60% of specimens. It was unable to detect as positive about 58% of specimens which had parasite counts ≤ 500 per ul but could detect about 94% of those with counts > 500 per ul. This difference in positive detection rate was significantly different ($p < 0.05$). It cannot quantify parasitemia easily and the specimens cannot be stored for future reference and for quality control purposes. It is therefore concluded that the QBC technique cannot replace the classical thick blood film technique for use in malaria control programmes. Its use may be appropriate in situations like busy blood banks and outpatient clinics where rapid screening of malaria infection is needed but where experienced malaria microscopists may not be available.

Keywords: Quantitative buffy coat technique, malaria survey, case detection

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INTRODUCTION

There are a number of reasons why case detection is important in a malaria control programme. An obvious one is to identify the infective reservoir so that treatment of cases can be instituted to reduce the chance of the infection being transmitted to others. Species identification of malaria parasites is also important so that appropriate treatment can be given.

Issues that are pertinent in case detection, be it for management of the individual patient or for malaria control, are the rapidity, sensitivity and specificity of the detection methods.

A number of techniques are available for the demonstration of parasitemia. Some, like the classical thick and thin blood film technique, are well established, while newer techniques like the quantitative buffy coat (QBC) analysis system[1] and the use of DNA probes have only been recently introduced[2,3].

The thick blood film stained with Giemsa, is the most commonly used technique for the demonstration of parasites both at the field and hospital laboratory situation. This together with the thin film to aid in morphological identification of the parasite, has provided control programmes with an affordable, sensitive and specific technique to determine parasite rates, parasite densities and species involved in endemic areas. Another advantage of the conventional thick film technique is that specimens can be stored, re-examined, counter-checked or kept for future reference. However, this technique depends heavily on trained microscopists and it takes about 10 minutes to screen through the 200 microscope fields at 100X objective that is the practice. In addition, counting of parasites and species identification in positive slides will lengthen the time to screen the slide.

The QBC analysis system for the demonstration of malaria parasites is based on the principle of centrifugal stratification of malaria parasites and other blood cell components in a capillary tube precoated internally with acridine orange and potassium oxalate. A cylindrical float is inserted into the capillary tube filled with about 60 ul of capillary blood before being centrifuged at 12,000 rpm for 5 minutes. The thrombocyte, leucocyte and top red blood cell layers are enlarged and easily identified as the float occupies about 90% of the interior of the tube in these areas[1]. The adaptation of the technique for the demonstration of malaria parasites is based on the fact that infected red blood cells are less dense than normal ones, and therefore concentrate at the top of the red cells just below the leucocyte layer. As the parasite DNA takes up the acridine orange, and the red cells do not, they are easily visible under UV light. The special UV attachment, the centrifuge needed for the tubes and the coated capillary tubes are available commercially. As the parasites are concentrated in a defined area of the tube, the examination time for parasites is greatly reduced and it has been said that in positive QBC tubes, it takes less than a minute for a diagnosis to be made[4]. In view of the reported attractiveness of this new technique for case detection, we decided to compare its use with that of the conventional blood film technique during a malaria survey in the field.

MATERIALS AND METHODS

Survey location and methodology

A malaria parasite survey was carried out in a known

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mesoendemic area in an aborigine resettlement scheme in Betau, Pahang, in the central part of Peninsular Malaysia. The houses in the resettlement scheme were sprayed with residual DDT at 6 monthly intervals (though irregularly), for the past 2 years and positives were treated with either standard doses of chloroquine or sulfadoxine/pyrimethamine and primaquine. Previous blood surveys for malaria during the past year have shown prevalence rates of 25-30% (unpublished data).

A house to house census was carried out and total coverage of all inhabitants in the blood survey was attempted. Thick and thin blood films as well as QBC(R) (Becton Dickinson) capillary tube specimens were prepared from finger-prick blood. The QBC tube is a capillary tube measuring 75 mm long, internally coated with acridine orange, potassium oxalate, sodium heparin and EDTA. A 20 mm long cylindrical float is placed inside the tube containing 55-65 ul blood[4].

The blood films were dried and stained with Giemsa while the QBC tubes were processed as recommended by the manufacturers within 3 hours of collection (Technicians involved in the survey were all given at least a month's prior training in the use of the QBC system). The blood films were read by a different group of technicians from that reading the QBC tubes. Both groups read their specimens "blind". All technicians involved in the study were experienced, practising malaria microscopists with at least 5 years of experience. Blood films were examined under 1000x magnification and 200 fields were examined for malaria parasites before a film was declared as negative. When malaria parasites were present, quantification of parasitemia was carried out by counting the number of parasites present against 500 leucocytes, and then estimating the parasite count per ul blood, assuming the presence of 8,000 leucocytes per ul of blood.

The QBC tubes were read as recommended by the manufacturers. Essentially, the centrifuged tubes were placed in a special lucite block and examined under ultraviolet light, at just below the buffy coat layer, using a special UV attachment supplied by the manufacturer. The tube was examined at four quadrants by turning the tube and at least 1-4 minutes was spent on each tube before it was declared negative. No attempt was made to quantify the parasitemia in the QBC tubes.

Statistical analyses

The field data were analysed using dBASE STATS software (SPSS Inc, Torrance, California, USA). Where appropriate, chi square tests were used to calculate for statistical significance which was taken as $p < 0.05$.

RESULTS

A total of 928 persons aged 10 months to 70 years (mean ± SD = 18.68 ± 16.81 years) out of approximately 1,000 inhabitants in the area were screened for parasitemia using the blood film technique. Of these, 146 (15.73%) were positive for parasitemia. Infections were due to *Plasmodium falciparum* (96), P. *vivax* (39), P. *malariae* (3) and both *P. falciparum* and *P. vivax* (8).

Only 374 subjects were examined by both the thick blood film and QBC methods (Table I). Of the 59 positive by the thick blood film, only 33 were positive by the QBC technique, giving a QBC sensitivity rate of 55.93%. Of the 315 negative by thick blood smear, only 299 were negative by QBC, giving a specificity of 94.92%. Of the 49 positive by the QBC technique, only 33 were positive by the thick blood film, giving a positive predictive value of 67.35% by the former test. Only 20 (58.82%) out of the 34 specimens found to be positive for *Plasmodium falciparum* by blood film were reported as positive by QBC and of these only 15 (75.0%) were correctly identified to the species level. Only 11 (57.89%) out of 19 specimens identified as *Plasmodium vivax* on blood film were correctly reported as positive by QBC and of these 4 (36.36%) were correctly identified. Of the 3 specimens positive for *Plasmodium malariae* by blood smear, only 1 (33.33%) was reported as positive but wrongly identified as P. *falciparum* by QBC. Similarly, of the 3 positive for mix infection by blood film, 1 (33.33%) was reported as positive but wrongly identified as *P. falciparum by QBC.*

Table I - Detection of malaria infection by the Quantitative Buffy Coat (QBC) and thick blood film techniques in Betau, Pahang, June 1991

Results by Blood Film examination	Results by QBC*				Total
	Negative	P.f.	P.v.	Mix	
Negative	299	11	5	-	315
P.f.	14	15	4	1	34
P.v.	8	6	4	1	19
P.m.	2	1	-	-	3
Mix	2	1	-	-	3
Total	325	34	13	2	374

* P.f. = Plasmodium falciparum; P.v. = Plasmodium vivax; P.m. = Plasmodium malariae; Mix = P. falciparum + P. vivax

Table II - Distribution of positives by total parasite count as detected by blood film and Quantitative Buffy Coat (QBC) techniques, in Betau, Pahang, June 1991

Results by QBC	Total parasite count/ul by blood film			Total
	0	1-500	≥501	
Negative	299	25	1	325
Positive	16	18	15	49
Total	315	43	16	374

* Statistical significant difference between positive detection of specimens with counts ≥ 501/ul (93.75%) compared to counts ≤ 500/ul (41.86%) (Chi square = 4.87, df = 1, $p < 0.05$)

Of the 59 specimens positive by blood film, 43 and 16 had total parasite counts ≤ 500 and ≥ 501 respectively (Table II). Of these, 18 (41.86%) and 15 (93.75%) were detected as positive by the QBC technique respectively. This difference in positive detection rate is significantly different ($p < 0.05$).

DISCUSSION

The quantitative buffy coat (QBC) technique has been evaluated in a number of countries including the Philippines[4], Ethiopia[5], and Thailand[6]. In the field study in the Philippines, the QBC technique was compared with the thick smear technique where 200 fields were screened. The QBC technique had a sensitivity of 70% and a specificity of 98.4%, using the blood smear technique as gold standard. Species identification was only correct in 77%. In Ethiopia, where the study was carried out in a laboratory setting, and only 100 fields of the thick film were read, the QBC technique identified 10% more positives. In Thailand, field specimens were collected but read at central or hospital laboratories where 30, 100 or 200 fields of the films were read. The sensitivity was found to be 99.5% and the specificity was 94.6%. Species identification was correctly made in 78% of specimens. In the studies in the Philippines and Thailand, the QBC technique was found to detect parasites at as low as 3-5/ul blood. In the present study, we have found the technique not very sensitive

(55.93%) and could only detect parasites at 40/ul blood. Specificity was found to be 94.92% and species identification was correctly made in only 57.58% of specimens. The QBC technique reported as negative 25 out of 43 (58.14%) specimens which had total parasite counts of ≤ 500 per ul. It detected 15 out of 16 (93.75%) specimens which had parasite counts of ≥ 501 parasites per ul.

The above findings have created doubts as to its real sensitivity when compared to the conventional thick blood film technique under field conditions. We believe that some important reasons why the sensitivity of the QBC technique reported in the present study was lower compared to those of other studies[4-6] are because our technicians are very experienced malaria microscopists and as is our normal practice, we screen 200 microscope fields under 1000x magnification during the examination for parasites. Some other disadvantages are the cost of each individual test, the necessity for fluorescence microscopy, its limitation in estimating parasite density, and the difficulty of storing and sending field specimens for quality control checking at regional or central laboratories. The last point is of importance as the monitoring of the quality of work of field staff must be an important aspect in any malaria control programme.

In view of the above findings, it is concluded that the QBC technique cannot replace the blood film technique in the malaria control programme. However, it may have a place in situations where a rapid screening technique for malaria infection is needed, as in busy blood banks and outpatient clinics. An obvious advantage is that malaria infections with parasite counts greater than 500 per ul blood are very rapidly detected even by fairly inexperienced operators. However, it cannot be relied on to detect infections with parasite counts less than 500 per ul, which an experienced microscopist would have no difficulty in doing so from a thick blood film.

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