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A Comparative Study Between Peripheral Smear and QBC Test for Suspected Cases of Malaria

Medical Science

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ABSTRACT

A technique for diagnosis of malaria based on acridine orange staining of centrifuged blood samples in a microhematocrit tube (QBC) was compared with thick and thin peripheral blood smears in 300 samples. Malaria was diagnosed in 42(14%) patients by field's staining technique and QBC method. An additional 6 (2%) cases were diagnosed by the QBC technique. Thus the prevalence rate of malaria during the study was 16%. Although QBC method was superior to the smear for malarial parasite detection, species identification was not possible in 3 (6.25 %) cases. Thus, QBC method has advantages in the terms of speed, sensitivity and ease. However, the age old Romanowsky's stains still appear "Gold" standard for species identification.

Introduction:
The commonly used method for diagnosis of malaria is the microscopic examination of Romanowsky's stained blood films. In the recent years, a variety of rapid and new techniques for malaria diagnosis have been developed one such being the QBC (Quantitative buffy coat) technique. Malarial parasite, unlike their red blood cell host, contains nucleic acid that stains with fluorescent dyes, such as acridine orange. As the parasite within erythrocyte mature, they reduce the buoyant density of infected erythrocyte. These two properties are exploited in QBC technique for malaria diagnosis (1,16)

This study was carried out to compare effectiveness of malaria diagnosis by QBC method with that of peripheral smear examination in terms of sensitivity, reliability, speed and cost effectiveness.

Materials and method:
This study was conducted on 300 blood samples from patients presenting with pyrexia and / or atypical presentations, attending the outpatient department of Civil hospital, B.J. Medical College, Ahmedabad. Blood films were prepared for thick and thin smear examination after staining with Field's stain. Approximately 80 to 100 fields were examined over 8 to 10 minutes by the same microscopist who visualised the QBC system.

In the QBC method, a 75 mm long QBC capillary tube pre-coated internally with acridine orange stain, potassium oxalate, sodium heparin and K2EDTA was filled with 55-65 microliters of whole blood and fitted with a clear plastic closure. A precisely made 20 mm long cylindrical float was inserted inside the tube and then spun in the QBC microhematocrit centrifuge at 12000 rpm for 5 minutes. The tube was then immersed in microscope oil-immersion in a notch recite block and examined for 2 minutes through an ordinary light microscope with Paralens attachment.

The principle of QBC is based on the fact that on centrifugation at a high speed, the whole blood separates into plasma, buffy coat and packed red cell layer.

The float gets buoyed by the packed blood cells and is automatically positioned within the buffy coat layer. Blood cells in the buffy coat layer get separated according to their densities, forming visibly discrete bands; platelets remaining at the top, lymphocytes and monocytes within the middle layer and granulocytes at the bottom.

Since the parasites contain DNA which takes up the acridine orange stain, they appear as bright green (DNA: nucleus) and orange (RNA:cytoplasm) among the non-fluorescing red blood cells. The tube was examined in the region between the red blood cells and granulocytes and within the granulocytes and mononuclear cell layers where parasites are most abundant. As the buffy coat in the QBC tube appears pigmented grey or black in colour in positive samples, an attempt was made to analyse the correlation of buffy coat colour and positivity.

Results:
Three hundred samples were compared by both thick and thin Field's stained smears and QBC tube.

Malarial parasites were detected in 42(14%) cases by smear examination. These cases were also positive by the QBC method. An additional 6 (2%) cases were also diagnosed as malaria by QBC method.

Thus the prevalence rate of malaria was (16%). All the patients (n=252) who were negative by QBC method were also smear negative.

Table 1: Diagnosis of malaria by Peripheral smear examination and QBC method.

Diagnosis	Peripheral smear examination	QBC
Positive	42	48
Negative	258	252

The species of malarial parasites encountered by smear examination were P.vivax (36; 75%) and P.falciparum (9;18.75%). Mixed infection accounted for 6.25% (n=3) cases. By the QBC method, species identification was not possible in 3 (6.25%) cases. It was observed that smear examination required on an average 10 to 12 minutes in contrast to 1 to 2 minutes to report by QBC method.

Table 2: Species detection by Peripheral smear examination and QBC test

Species	Peripheral smear examination	QBC
P.vivax	36	35
P.falciparam	09	08
Mixed	03	02
Total	48	45

Grey to black colour of the buffy coat in the QBC tube was noted before it was examined under the microscope. In 44(91.6%) malarial parasite positive tubes, the buffy coat appeared pigmented and thus served to predict positivity.

Discussion

In this study, a total 300 samples were studied with 42 smear positive and 48 QBC positive cases.

The major advantages of QBC method over peripheral smear examination are its higher sensitivity and greater rapidity. Microscopy is time consuming and needs expertism. Also in the smear examination, the average time to process and report the smear is 40-45 minutes and 10-12 minutes respectively in contrast to 10-12 minutes and 1-2 minutes in case of QBC method.(1,8,9,10,14,15,16)

Another advantage of QBC method is its ease of interpretation and it being technically easy to perform. A technician can be taught to carry out this test accurately in less than 10 days, whereas it may take weeks or months to learn to interpret blood smear properly.(5,7,12,16)

The main drawback of the QBC technique is failure to identify the species. This difficulty is encountered as the morphology of infected erythrocytes which aids in identification of species remain occult.(13,16)

As it is difficult to differentiate the trophozoite forms of different plasmodium species, the specificity of QBC method is questionable.(4,5,6,13,15,16)

Another disadvantage of QBC method is costly instrument and its maintenance. The instrument is easy to use but requires a trained observer. It is not constructed to protect against blood contamination of the centrifuge from events of blood filled QBC tubes breaking or leaking in the machine. Microcoagulation, the adverse effects of heat and humidity, strong background fluorescence, scattered lysed white blood cells and the incorrect positioning of the float have been reported as making it difficult to examine some tubes. The tubes must be centrifuged promptly. Considerable skill and experience are required to process and examine the tubes correctly and confidently.(2,11,16)

The QBC test is also costly and the special disposable capillary tubes are also expensive. So the expense is an important factor especially for developing countries.(2,3,16)

As compared to QBC, the peripheral smear examination is also sensitive, highly specific, easy and cost effective. But it has disadvantages such as the manner in which the film is prepared, the level of training of the observer, the quality of stain material used and the adequacy of maintenance of materials and equipment.

In QBC method, quantitation of Parasitemia relies on a subjective grading system because it can not quantify Parasitemia easily.(5,7,8,14,16)

The smear slides can be stored for long period of time for record purpose, whereas it is difficult to preserve the capillary tubes for more than 2 days.

So the QBC method can not replace the peripheral smear examination since species identification is imperfect with this method and no doubt, the smear examination is still the method of choice in species identification of malaria.(4,5,7,13,16)

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