

DETECTION OF MALARIA PARASITES IN FEVER PATIENTS UNSUSPECTED OF MALARIA : A QBC STUDY

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ABSTRACT : The QBC technique for the detection of malaria uses a micro-hematocrit tube coated with anticoagulant and a fluorescent dye : acridine orange. Blood is filled into the capillary tube. A plastic float which has a density equal to the buffy coat is then inserted into the blood-filled tube. After centrifugation, parasitized RBC are concentrated around the base of the float. These red cells can be examined directly under a modified UV objective lens attached to a regular microscope. The study was performed on 76 adult patients with fever, from the out-patient department of Maharaj Nakorn Chiang Mai Hospital. With the QBC technique, malaria parasites were detected in 29 (38%) of these unsuspected patients while only 4 (5%) were positive when examined with the Wright-Giemsa thick blood film. These data suggested that the QBC technique is much more sensitive than thick film in the detection of malarial parasites

บทคัดย่อ : การตรวจหาเชื้อมาเลเรียในผู้ป่วยที่มีไข้ที่ไม่ได้นึกถึงโรคมาเลเรีย. เดชา ร่มไทรย์*, อัมพรัตน์ ร่มไทรย์*

การตรวจแบบ Quantitative buffy coat (QBC) เป็นเทคนิคใหม่สำหรับตรวจหาเชื้อมาเลเรียในเลือด โดยใช้หลอดแก้วที่เคลือบ Acridine orange และสารกันเลือดแข็งชั้นตอนประกอบด้วยสารบรรจุเลือดตามปริมาณที่กำหนดเข้าไปในหลอดแก้วดังกล่าวก่อนตามด้วยการสอดแท่งพลาสติก ที่มีความหนาแน่นเท่ากับ Buffy coat เข้าไปในหลอดแก้วนั้น แล้วนำไปปั่น จากนั้นจะตรวจหาเชื้อมาเลเรียบริเวณรอบแท่งพลาสติกใต้ชั้นของเม็ดเลือดขาว ด้วยกล้องจุลทรรศน์ธรรมดาที่ติดตั้งเลนส์พิเศษ

ในการศึกษานี้ได้ตรวจเลือดผู้ป่วยที่มีไข้จำนวน 76 ราย ซึ่งแพทย์ได้ส่งตรวจเพียงการนับเม็ดเลือดเท่านั้น แสดงว่าไม่มีเหตุให้แพทย์สงสัยถึงการติดเชื้อไข้วสัน ผลการศึกษาพบว่าเลือดผู้ป่วยดังกล่าวให้ผลบวกกับวิธี QBC ถึง 38% ในขณะที่ให้ผลบวกกับ Thick Film เพียง 5% แสดงว่าประสิทธิภาพของการตรวจหาเชื้อมาเลเรียของ QBC นั้นดีกว่า Thick Film และโรคไข้วมาเลเรีย ก็ยังคงเป็นปัญหาของภาคเหนืออยู่

คำรหัส : มาเลเรีย, QBC เทคนิค

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especially on those fever patients unsuspected of malaria. It should be useful screening system in particular to Northern Thailand where malaria is still a major problem.

Keyword : malaria, QBC technique

INTRODUCTION

Quantitative buffy coat analysis (QBC) is originally designed for the determination of complete blood count (CBC). With modification, the QBC technique was adapted for the detection of blood parasites.(1) After the centrifugation of the blood specimen with an inserted plastic float (density approximately the same as the buffy coat) in a capillary tubes coated with anticoagulant and acridine orange, the tubes were examined for malaria parasites utilizing the specially designed objective lens of the microscope. Blood cells are separated according to their densities (Figure 1). The float occupies 90% of the interior of the tube so that only 40 microns of distance exist between the float exterior and the interior walls of capillary tube.

Parasitized red blood cells which have lower density (because their cell contents are metabolized by the plasmodia) are located in the area

surrounding the float. The tube is then examined at the red cell/white cell interface for the ring form and at the lymphocyte monocyte layer for gametocytes and schizont forms.

The microscope used for the QBC examination is an ordinary light microscope equipped with the specially designed UV microscope adapter fiber optic system. This system consists of a 60x, oil immersion objective lens and a fiber optic illuminator. The objective lens is attached directly to the microscope and connected to a light source via the optical fiber. Filters inside the lens permit epi-illumination of UV light directly on the blood-filled QBC tube. Under the QBC lens, parasites stained with acridine orange appears as brilliantly green fluorescence.(3)

The float is an important part of the test system. Without the float (Figure 2) we can visualize only red cells located peripherally but not those located in the central part of the tube. With the float in place, all red cells are pushed to the side of the tube and all of the low density red cells can be examined. The morphology of parasites seen with QBC technique is similar to those in the thick and thin films but much easier to identify.

The current study was performed to examine malaria parasites in the blood of patients who have fever but not indicated for malaria. Both the QBC technique and conventional thick blood film were used.

MALARIALS AND METHODS

I. Specimens

EDTA blood specimens were obtained from 76 patients who have fever. The patient population is so chosen when the physician requested for CBC only but not for malaria test, indicating that the patient is not suspected to have malaria.

II. QBC Technique(2)

The blood specimen was filled into a QBC tube which is coated with acridine orange. After mixing the blood in the tube by inversion, a plastic cap is put onto one end. A small plastic rod, called "float" which has the same density as buffy coat, was inserted into another end. The tubes were then centrifuged for 5 minutes at 12,000 rpm. The blood sample is considered to be negative for malaria when the entire buffy coat and expanded red cell area has been investigated and no parasites are found. Each blood specimen takes approximately 2-5 minutes to screen for malaria.

III. Thick Blood Film Examination

The thick blood film was prepared from the same blood specimen as those for the QBC technique. After the blood films were dried, they were stained with Wright-Giemsa stain, diluted 1:9 in pH 6.4 phosphate buffer. The stained thick blood films were examined under oil immersion objective lens. Each thick film was examined for 200 oil power fields before reported as negative. Each blood specimen takes approximately 15 minutes to screen for malaria.

RESULTS

Out of the 76 patients which were unsuspected of malaria, 29 cases (or 38%) were positive for ring form of malaria by the QBC technique, while the thick blood film technique was positive in only 4 cases (5%) (Table 1). No gametocytes or Schizonts were found in these specimens by both techniques used.

**DISTRIBUTION OF PLASMODIA
THROUGH QBC TUBE**

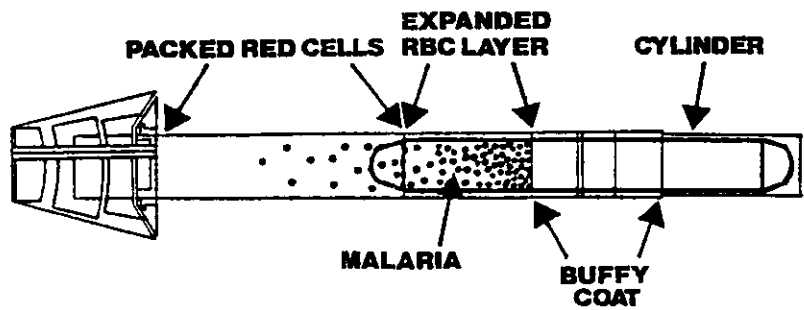


Figure 1 : diagram of layers of cells visible under UV light after centrifugation

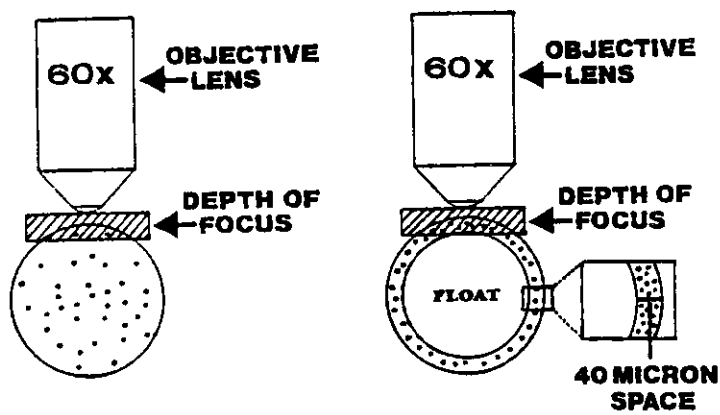


Figure 2 : Cross section of a QBC tube
(With and without float)

Table 1 Malaria positive in patients with fever

		QBC TECHNIQUE		
		POSITIVE	NEGATIVE	TOTAL
Thick Film	Positive	4	0	4
Technique	Negative	25	47	72
	Total	29	47	76

DISCUSSION

The data reported in this study suggest that thick blood film examination alone is grossly inadequate for parasite detection in patients with very low level of parasitemia. It can detect only one out of 7 cases of patients with malaria. It also suggested that malaria is still endemic in Chiang Mai province but it was not detected by using the thick film for blood examination. By using the QBC technique, the microscope examination time is reduced by 5 times for each blood specimen.

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