### Title
Rapid diagnosis of *Brugia malayi* and *Wuchereria bancrofti* Filariasis by an Acridine Orange/Microhematocrit Tube Technique

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### Source

### Abstract

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### ABSTRACT
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Rapid Diagnosis of Brugia malayi and Wuchereria bancrofti Filariasis by an Acridine Orange/Microhematocrit Tube Technique

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ABSTRACT: A microhematocrit tube technique for diagnosis of human filariasis has been previously described. A system incorporating heparin, EDTA, and acridine orange into a microhematocrit tube (Quantitative Blood Count, QBC9) has been commercially developed for the quantitation of blood counts and has been used for the diagnosis of malaria. We evaluated this test for its usefulness in the diagnosis of filariasis. Upon centrifugation, the parasites were concentrated through the wall of the tube. The parasites were concentrated further by a plastic float that expands the buffy coat and could be observed by fluorescence microscopy. The terminal and phagocytes/monocytes, antplatelets and hemocytometer. The terminal and phagocytes/monocytes were easily recognized and differentiated from each other. Microfilariae were detected in samples diluted to a level of approximately 50/mL.

Keywords: filariasis, Brugia malayi, Wuchereria bancrofti, acridine orange/microhematocrit tube technique, parasitology

Staining of from 20 to 60% of blood on a thick smear is currently the method of choice for the detection of Brugia malayi and Wuchereria bancrofti. Sensitivity can be increased by filtration of from 1 to 5 ml of blood on a nucleopore filter (Chularek and Desowitz, 1970). Both of these techniques require from 30 to 60 min. A microhematocrit tube method was described by Goldsmit (1970) and Goldsmit et al. (1972) as a rapid method for the diagnosis of filariasis. The technique was more sensitive than a thick blood smear and can be carried out in 5-6 min.

A microhematocrit technique has been developed for the quantitation of granulocytes, lymphocytes/monocytes, and platelets and is commercially available (QBC9 capillary blood tubes). These tubes have been used to diagnose Plasmodium falciparum malaria (Spielman et al., 1988; Rickman et al., 1989). The parasites are stained by the acridine orange dye and can be observed within the packed red blood cell layer using a 50x objective. We recently observed that these tubes could be used for the detection and identification of B. malayi and W. bancrofti microfilariae.

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Brugia malayi was grown in jirds (Meriones unguiculatus) (Ash and Riley, 1970) and collected from the peritoneal cavity. Wuchereria bancrofti was collected by venipuncture from human donors in the Philippines, frozen in liquid nitrogen, and thawed immediately before use. Thawed parasites were alive and motile. Parasites were mixed with fresh heparinized human blood for study.

The QBC9 tubes (Becton Dickinson, Franklin Park, New Jersey) were used according to manufacturer's instructions. The tubes were filled with blood to a premarked level (approximately 55-65 μl) and mixed with the acridine orange dye, which coats the interior of the tube. The tubes were stoppered and the plastic float was inserted. The tubes were centrifuged at 12,000 rpm for 5 min and observed by fluorescence microscopy (Olympus BH-2 with standard filter sets for fluorescein) using a 20x (SPlan 20, Olympus) objective or a 50x oil immersion (DPlan 50, Olympus) objective. The tubes were held in a trough cut in a Plexiglas block (Goldsmid et al., 1972) and covered with immersion oil to improve resolution (Woo, 1969). Thick films were prepared by spreading 10 μl of blood over an area of a microscope slide approximately 1 x 2 cm, stained with Giemsa stain, and the parasites were counted by light microscopy. In 1 experiment, 2-fold dilutions of blood were prepared and examined by both thick film and QBC9.

Both W. bancrofti and B. malayi microfilariae were observed easily using a standard 20x objective. The microfilariae were stained by acridine orange dye while in the tube. Nuclei fluoresced bright green and were clearly visible. Parasites were motile and concentrated in and around theuffy coat. Microfilariae in the plasma were clearly visible in the tube, having been displaced to the periphery by the plastic float. Microfilariae in the Buffy coat were not seen as easily due to the intense fluorescence of the mononuclear and polymorphonuclear cells in this layer; however, their movement and the disturbance of the surrounding cells were detected readily. Parasites in the upper portion of the packed red cell layer could be examined easily against the dark background of the erythrocytes. The terminal and subterminal nuclei of B. malayi (Fig. 1) were visible, making species identification possible. The long cephalic space, also characteristic of B. malayi, was lightly stained but clearly identifiable (Fig. 2). The caudal nuclei (Fig. 3) and short cephalic space (Fig. 4) of W. bancrofti were distinct.

**Figures 1-4.** 1. Fluorescence micrograph (x 500) of acridine orange-stained Brugia malayi in QBC9 tube. The view of the posterior region shows terminal and subterminal nuclei. 2. The long cephalic space is visible on this specimen of B. malayi as is the lightly stained sheath. 3. This figure shows the caudal nuclei of Wuchereria bancrofti. 4. Anterior of W. bancrofti. Compare the short cephalic space of this parasite with that of B. malayi in Figure 2.

Microfilariae of B. malayi were diluted in a 2-fold series in human blood to test both sensitivity and the ability of the test to allow quantification of parasite numbers. Counts were compared to thick blood smears that were prepared and counted as described above. QBC9 tubes...
were prepared and examined using a 20 x objective. The buffy coat was brought into focus and the parasites were counted as the tube was rotated in the Plexiglas holder. In tubes with large numbers of parasites a reference point was picked out to ensure that the entire contents of the tube were counted around the entire circumference.

Counts of *B. malayi* microfilariae performed using the tubes agreed closely with the stained smears (Table I).

Microfilariae of *B. malayi* and *W. bancrofti* were detected quickly and easily in blood samples using the QBC® tubes. Due to their relative density, the microfilariae concentrated in the buffy coat region during centrifugation (Goldsmith et al., 1972). Further, the inclusion of the plastic float within the capillary tube displaced the concentrated parasites to the periphery of the tube. The distance between the float and the inner wall of the tube is approximately 40 μm, and the parasites are contained within this space. As a consequence of this and the concentration effect, almost all parasites collected in the tube were visible. Estimation of parasite counts by QBC® agreed well with direct counts of blood smears. In addition, staining of the nuclei by acridine orange made determination of species possible immediately.

In this study parasites were detected at a concentration of 50 microfilariae/ml. The theoretical limit of the test is 16 parasites/ml assuming 60 μl of blood per tube. The fact that parasites were concentrated from a relatively large volume of blood increased the sensitivity and reduced the time required for examination of the specimen. The QBC® method is less sensitive than the membrane filtration technique, which can be used to detect 1 parasite/ml. However, the QBC® has the advantage of speed and relative ease of use, and staining reagents are incorporated into the tube.

The QBC® test was found to be a simple and effective way to detect microfilariae of *W. bancrofti* and *B. malayi* quickly. The tubes are designed for blood collection by finger stick. contain EDTA (ethylenediaminetetraacetic acid) and sodium heparin, and are precoated with acridine orange. Microfilariae can be detected and counted in this system using a light microscope although species cannot be determined. Where a centralized laboratory is available specimens can be collected in the field and returned for examination. Although parasites lost motility over time, they retained their morphology over several days at room temperature (23 C) and for at least 7 days when refrigerated. We have not assessed their stability in a tropical environment or under field conditions.

We found the QBC® test to be a fast and simple method for detection and identification of microfilariae of *W. bancrofti* and *B. malayi*. The test can be performed from a finger stick blood collection, its sensitivity is greater than that of a thick blood smear, and it can be performed in approximately 10 min. A thick smear on the other hand requires 30 min or more for drying and staining. We believe that the test is a promising technique for the diagnosis of filariasis.

This work was funded in part by the Naval Medical Research and Development Command work unit #3M263763D807AH130. The opinions and assertions herein are those of the authors and are not to be construed as official or as reflecting the views of the U.S. Navy or the naval service at large.

**LITERATURE CITED**


RICKMAN, L. S., G. W. LONG, R. OBERST, A. CABRANES, R. SANGALANG, J. I. SMITH, J. D. CHULAY.
New Host and Locality Record for Tripanosomia peromysci

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A new host and locality for the protozoan parasite Tripanosomia peromysci (Sporozoa: Kinetoplastidae), described from small mammals in Kansas, is reported. The parasite was collected from 19 mice (Peromyscus leucopus) collected from Riley County, Kansas, in June 1988. The parasite was identified as T. peromysci by its characteristic morphology and phagocytosis behavior. This is the first report of the parasite from the state of Kansas, and the new host is the white-footed mouse (Peromyscus leucopus). The parasite was found in the blood of the mice, and the infected mice were air dried and stained with a mixture of nut butter and oatmeal. The infected mice were then observed under a Zena ZW248 microscope. All statistical analyses were made using the Number Cruncher Statistical System (Version 2.0) computer. The parasite was found to be distinct from other species of Tripanosomia by its unique morphological characteristics. Further studies are needed to determine the potential impact of this parasite on wildlife and human health.

Absent:

Tripanosomia, a genus of the order Kinetoplastida, is known to infect a variety of small mammals, including Peromyscus species. The parasite has been reported from the United States, Canada, and Mexico. This new record from Kansas expands the geographical distribution of the parasite and highlights the importance of continued surveillance for new hosts and localities.

Key words: Tripanosomia, Peromyscus leucopus, Kansas, parasitology.

Provenance: The parasite was collected by Dr. Richard D. McKone from a local herpetologist. The infected mice were then observed under a Zena ZW248 microscope. All statistical analyses were made using the Number Cruncher Statistical System (Version 2.0) computer. The parasite was found to be distinct from other species of Tripanosomia by its unique morphological characteristics. Further studies are needed to determine the potential impact of this parasite on wildlife and human health.

References:


