

AD-A222 965

①

1a.
 2a.
 1b. RESTRICTIVE MARKINGS

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE
 3. DISTRIBUTION/AVAILABILITY OF REPORT
 Approved for public release;
 distribution is unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
 NMRI 90-36
 5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION
 Naval Medical Research
 6b. OFFICE SYMBOL
 (if applicable)
 7a. NAME OF MONITORING ORGANIZATION
 Naval Medical Command

6c. ADDRESS (City, State, and ZIP Code)
 Bethesda, Maryland 20814-5055
 7b. ADDRESS (City, State, and ZIP Code)
 Department of the Navy
 Washington, D.C. 20372-5120

8a. NAME OF FUNDING/SPONSORING ORGANIZATION
 Naval Medical Research and Development Command
 8b. OFFICE SYMBOL
 (if applicable)
 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State, and ZIP Code)
 Bethesda, Maryland 20814-5055
 10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
63763A	3M26373D807	AH-130	DA301600

11. TITLE (Include Security Classification)
 Rapid diagnosis of Brugia Malayi and Wuchereria bancrofti Filariasis by an Acridine Orange/
 Microhematocrit Tube Technique

12. PERSONAL AUTHOR(S)
 Long GW, Rickman LS, Cross JH

13a. TYPE OF REPORT
 journal article
 13b. TIME COVERED
 FROM TO
 14. DATE OF REPORT (Year, Month, Day)
 1990
 15. PAGE COUNT
 4

16. SUPPLEMENTARY NOTATION
 Reprinted from: Journal of Parasitology 76(2):278-291, 1990

17. COSATI CODES

FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
 Filariasis
 Acridine orange
 Brugia malayi
 Diagnosis
 Wuchereria bancrofti

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DTIC
SELECTED
S JUN 19 1990 **D**
D
Co

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT
 UNCLASSIFIED/UNLIMITED SAME AS RPT. DTIC USERS
 21. ABSTRACT SECURITY CLASSIFICATION
 Unclassified
 22a. NAME OF RESPONSIBLE INDIVIDUAL
 Phyllis Blum, Information Services Division
 22b. TELEPHONE (include Area Code)
 202-295-2188
 22c. OFFICE SYMBOL
 ISD/ADMIN/NMRI

—, AND A. J. NAPPI. 1988. Immune response of arthropods. ISI Atlas of Science: Plant and Animal Sciences 1: 15-19.

—, AND W. A. ROWLEY. 1978. Observations on the laboratory biology and maintenance of *Aedes trivittatus*. Mosquito News 38: 9-14.

—, D. R. SUTHERLAND, AND L. N. GLEASON. 1984. Defense reactions of mosquitoes to filarial worms: Comparative studies on the response of three mosquitoes to inoculated *Brugia pahangi* and *Dirofilaria immitis* microfilariae. Journal of Invertebrate Pathology 44: 267-274.

—, AND J. W. TRACY. 1989. Arthropod-transmitted parasites: Mechanisms of immune interference. American Zoologist 29: 387-395.

FORTON, K. F., D. R. SUTHERLAND, AND B. M. CHRISTENSEN. 1985. Ultrastructure of the melanization response of *Aedes trivittatus* against inoculated *Dirofilaria immitis* microfilariae. Journal of Parasitology 71: 331-341.

HAYES, R. O. 1953. Determination of a physiological saline for *Aedes aegypti* (L.). Journal of Economic Entomology 46: 625-627.

LI, J., J. W. TRACY, AND B. M. CHRISTENSEN. 1989. Hemocyte monophenol oxidase activity in mosquitoes exposed to microfilariae of *Dirofilaria immitis*. Journal of Parasitology 75: 1-5.

NAPPI, A. J., AND B. M. CHRISTENSEN. 1986. Hemocyte cell surface changes in *Aedes aegypti* in response to microfilariae of *Dirofilaria immitis*. Journal of Parasitology 72: 875-879.

—, AND J. W. TRACY. 1987. Quantitative analysis of hemolymph monophenol oxidase activity in immune reactive *Aedes aegypti*. Insect Biochemistry 17: 685-688.

—, AND M. SILVERS. 1984. Cell surface changes during parasite encapsulation and tumorigenesis in *Drosophila*. Science 225: 1166-1168.

RIZKE, T. M., AND R. M. RIZKI. 1983. Blood cell surface changes in *Drosophila* mutants with melanotic tumors. Science 220: 73-75.

J. Parasitol., 76(2), 1990, p. 278-281
© American Society of Parasitologists 1990

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

Gary W. Long, Leland S. Rickman, and John H. Cross*, Infectious Diseases Department, Naval Medical Research Institute, Bethesda, Maryland 20814-5055. *Division of Tropical Public Health, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20892

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, QBC®) 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 in the area of the buffy coat and could be observed through the wall of the tube. The parasites were concentrated further by a plastic float that expands the buffy coat and confines the parasites to the periphery of the tube. Acridine orange stains the DNA of the parasite, and morphologic characteristics can be examined by fluorescence microscopy. The terminal and subterminal nuclei and long cephalic space of *Brugia malayi*, as well as the short cephalic space and caudal nuclei of *Wuchereria bancrofti*, were easily recognized and differentiated from each other. Microfilariae were detected in samples diluted to a level of approximately 50/ml. **Keywords:** *Brugia malayi*, *Wuchereria bancrofti*, filariasis, acridine orange, microhematocrit tube technique, parasites, biology.

Brugia malayi. 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 Goldsmid (1970) and Goldsmid et al. (1972) as a rapid method for the diagnosis of human 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 (QBC® 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.

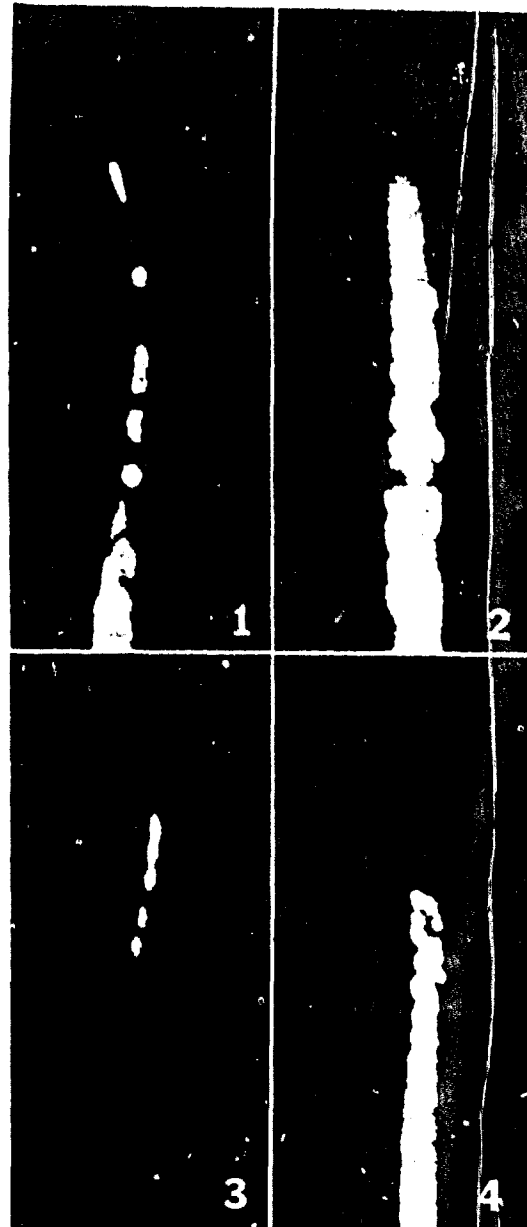
Parasitology, Diagnosis, *Brugia malayi*, *Wuchereria bancrofti*, Filariasis, Acridine Orange/Microhematocrit Tube Technique, Parasites, Biology

90 06 18 274
56

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 QBC[®] 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 μ l) 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 20 \times (SPlan 20, Olympus) objective or a 50 \times 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 μ l of blood over an area of a microscope slide approximately 1 \times 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 QBC[®].

Both *W. bancrofti* and *B. malayi* microfilariae were observed easily using a standard 20 \times 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 the buffy 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 ($\times 500$) of acridine orange-stained *Brugia malayi* in QBC[®] 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. QBC[®] tubes



A-1 | 20 |

were prepared and examined using a 20× 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 (Goldsmid 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 sev-

TABLE I. Comparison of QBC® and thick smear techniques for quantitation of microfilariae of *Brugia malayi*.

Thick smear		QBC® tube	
Total microfilariae counted/10 µl	Parasites/ml*	Total microfilariae counted/tube	Parasites/ml†
15	1,500	‡	—
7	700	42	701
4	400	20	334
1	100	8	133
0	—	3	50

* Determined from thick film count (count times 100).

† Determined by multiplying the QBC® count by 16.7 (assuming 60 µl blood/tube).

‡ Too numerous to count.

eral 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

- ASH, L. R., AND J. M. RILEY. 1970. Development of subperiodic *Brugia malayi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. *Journal of Parasitology* 56: 969-973.
- CHULAREK, P., AND R. S. DESOWITZ. 1970. A simplified membrane filtration technique for the diagnosis of microfilaremia. *Journal of Parasitology* 56: 623-624.
- GOLDSMID, J. M. 1970. Studies on the laboratory diagnosis of human filariasis: Preliminary communication. *Journal of Clinical Pathology* 23: 632-635.
- , K. MAHOMED, H. MAKANJI, AND M. MUIR. 1972. Microhematocrit centrifuge technique for the laboratory diagnosis of filarial infection. *South African Medical Journal* 46: 171-174.
- RICKMAN, L. S., G. W. LONG, R. OBERST, A. CABBANBAN, R. SANGALANG, J. I. SMITH, J. D. CHULAY,

- AND S. L. HOFFMAN. 1989. Rapid diagnosis of malaria by acridine orange staining of centrifuged parasites. *Lancet* i: 68-71.
- SATELMAN, A., J. B. PERRONE, A. TEKLEHAIMANOT, F. BALCHA, S. C. WARDLAW, AND R. A. LEVINE. 1988. Malaria diagnosis by direct observation of centrifuged blood samples. *American Journal of Tropical Medicine and Hygiene* 39: 337-342.
- WOO, P. T. K. 1969. The hematocrit centrifuge for the detection of trypanosomes in blood. *Canadian Journal of Zoology* 47: 921-923.

J. Parasitol., 76(2), 1990, p. 281-283
 © American Society of Parasitologists 1990

New Host and Locality Record for *Trypanosoma peromysci*

Richard D. McKonn, Steve J. Upton*, Robert D. Klemm†, and Robert K. Ridley, Department of Laboratory Medicine, College of Veterinary Medicine; *Division of Biology; and †Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506

ABSTRACT: *Trypanosoma peromysci* Watson, 1912 (Sarcocystidophora: Kinetoplastida), is described from a new host and locality. One of 20 (5.0%) *Peromyscus leucopus* collected from Pottawatomie and Riley counties in Kansas was found to harbor the parasite. Morphometric and statistical analysis confirmed the trypanosome to be indistinguishable from *T. peromysci*, the only difference being a greater mean flagellar length than reported previously. This is the first reported occurrence of *T. peromysci* in the white-footed mouse (*Peromyscus leucopus noveboracensis* Fischer, 1829) and also the first record of its occurrence in Kansas.

Two species of trypanosome, *Trypanosoma cruzi* and *Trypanosoma peromysci*, have been reported to occur in *Peromyscus* spp. (Wood, 1934, 1942, 1952a, 1952b, 1975a, 1975b; Davis, 1952; Esquivel et al., 1967; Burkholder et al., 1980). *Trypanosoma peromysci* was first described by Watson in Watson and Hadwen (1912) in *Peromyscus maniculatus*, *Peromyscus nebracensis* (now *P. maniculatus nebracensis*), and other species collected from Lethbridge, Alberta, Canada. Since that time, additional hosts and localities for the parasite have been reported, including *P. maniculatus* from New Mexico, Arizona, and California (Wood, 1942, 1952a, 1975a; Davis, 1952); *Peromyscus truei* from Arizona and California (Davis, 1952; Wood, 1952a, 1975a, 1975b); *Peromyscus californicus* from California (Wood, 1942, 1952a); *Peromyscus boylii* from California (Wood, 1942, 1952a; Davis, 1952); and *Peromyscus nudipes* from Costa Rica (Esquivel et al., 1967). The parasite has not been recorded previously from *Peromyscus leucopus*.

During an ongoing survey of protozoa of small mammals in Kansas, 20 *P. leucopus* were collected from 2 locations; 17 mice were collected between 1 May and 30 June 1988 from Pottawatomie County (39°13'N, 96°30'W), and 3 mice

were collected between 1 March and 30 April 1989 from Riley County (39°08'N, 96°29'W). All mice were caught using Sherman Live Traps (H. B. Sherman Traps, Tallahassee, Florida) baited with a mixture of peanut butter and oatmeal. The rodents were transported to Kansas State University where blood samples were obtained by tail bleeding into heparinized microhematocrit capillary tubes (American Scientific Products, McGaw Park, Illinois). Thick and thin blood smears were air dried and stained with either Giemsa stain or a modified Wright's stain (Leuko Stat Stain Kit, Fisher Scientific, St. Louis, Missouri). Of 20 mice, 1 (5.0%), collected from Riley County, was found to be infected. All specimens were elongate trypomastigotes with the nucleus located slightly anterior to the midpoint of the body and both the anterior and posterior ends tapering to a point (Figs. 1, 2). After morphological and statistical analysis it was concluded that the trypanosome species found was *T. peromysci*.

Davis (1952) presented a table listing measurements of 5 parameters from the specimens she examined, and Esquivel et al. (1967) listed measurements for 3 additional parameters. Because a complete description of *T. peromysci* is lacking, a description of the parasite from a new host, *P. leucopus*, is provided.

Twenty-five trypomastigotes were viewed with a Zeiss Standard RA microscope using a 100× oil immersion objective. Actual measurements and morphometric analyses were performed using the Microcom PM Interactive Image Analysis for Planar Morphometry (Southern Micro Instruments, Atlanta, Georgia) in conjunction with a Zenith ZW248-12 computer. All statistical analyses were made using the Number