

# Studies on the laboratory diagnosis of human filariasis: Preliminary communication

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**SYNOPSIS** Five laboratory methods used for the recovery of microfilariae from the blood were compared for efficiency of recovery and time involved. The methods used were thin blood films, thick blood films, wet preparations, the Polyvidone technique, and the microhaematocrit technique. The last proved superior in both efficiency and saving time.

The present investigation was undertaken to compare the efficiency of the microhaematocrit technique with other methods used routinely in the laboratory for the diagnosis of human infection with *Acanthocheilonema (Dipetalonema) perstans* and *Wuchereria bancrofti*, the two species of human filarial worm commonly encountered in Rhodesia. The technique was originally developed for trypanosomes by Devignat and Dresse (1955) and then further investigated by Bennett (1962) for haematozoa of birds. It does not seem to date to have been used for the diagnosis of human filariasis.

## Materials and Methods

Specimens were obtained from two groups of patients: (1) patients known to be infected with either *A. perstans* or *W. bancrofti*, and (2) 17 randomly chosen patients, this being the entire population of a camp in the Mana Pools area of the Zambesi Valley of Rhodesia.

The techniques used in the study were: (1) the standard thin blood film, (2) the standard thick blood film, (3) the wet drop technique, (4) the Polyvidone technique, and (5) the microhaematocrit technique.

The thick and thin blood films were stained for 20 minutes with Giemsa stain diluted 1 in 20 with buffered distilled water (pH 7.0).

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For the wet preparation, a drop of blood was placed on a clean microslide, covered with a coverslip, and then examined for moving microfilariae.

The Polyvidone technique used was that developed by Mr F. L. Goldring of the Department of Medical Microbiology of the University College of Rhodesia for the recovery of *Trypanosoma rhodesiense*. For this technique, 10 ml of 30% Polyvidone solution (May and Baker Ltd) was poured into a centrifuge tube and then 1-2 ml of citrated blood was gently layered on top. The erythrocytes were allowed to settle below the surface of the Polyvidone solution leaving the buffy coat at the surface. A drop of this layer was placed on a slide using a Pasteur pipette and examined under the low power of the microscope for moving microfilariae. The technique can be extended by removing the buffy coat which is then suspended in 10 ml of saline, the suspension centrifuged at 1,000 rpm for 10 min, the supernatant decanted, and the sediment examined for the parasites. The extended method was not used in this investigation.

For the microhaematocrit technique, citrated blood was drawn up to fill a microhaematocrit capillary tube to three-quarters of its capacity which was then sealed either with sealer or by burning off with a bunsen. The tubes were then spun for two minutes in a Hawksley microhaematocrit centrifuge and the tubes examined under the low power (80-160 × magnification) of a microscope. The microfilariae could be seen

moving on the plasma side of the buffy coat or in the outer layers of the buffy coat (Figs. 1 and 2). Occasionally they could be found among the erythrocytes just beneath the leucocyte layer. For a species identification, the tubes were cut just below the buffy coat on positive tubes, and the junctional layer of erythrocytes, buffy coat, and serum was made into a thick or thin film and serum was made into a thick or thin film and stained with Giemsa stain.

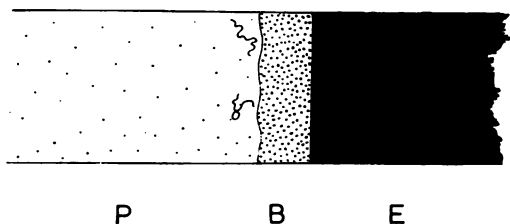


Fig. 1 Diagrammatic representation of a microhaematocrit capillary tube showing the region in which the microfilariae were usually found after centrifugation of the blood (P = plasma layer; B = buffy coat; E = packed red cell layer).

## Results

The results of the investigation are shown in Tables I to V.

Tables I and II and Figs. 3 and 4 show the number of positives obtained for the various techniques using blood from known infected patients.

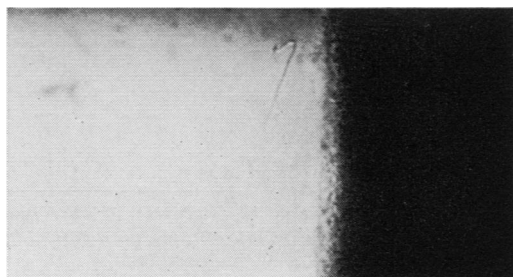


Fig. 2 Photomicrograph into a microhaematocrit tube ( $\times 200$ ) showing a microfilaria of *A. perstans* swimming just above the buffy coat as represented diagrammatically in Figure 1.

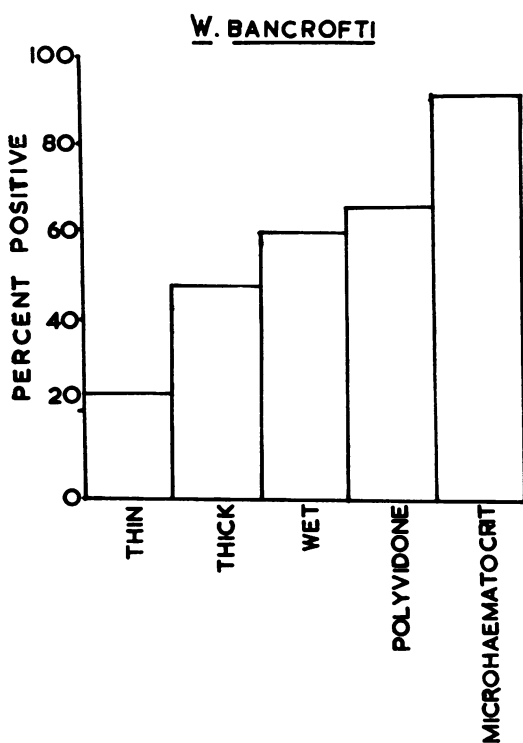


Fig. 3 Histogram showing the percentage of specimens positive for microfilariae for the various laboratory techniques using blood from patients infected with *W. bancrofti*.

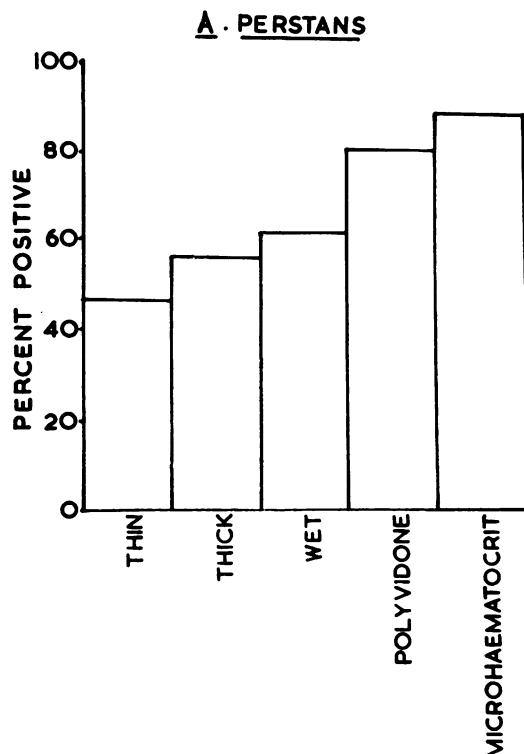


Fig. 4 Histogram showing the percentage of specimens positive for microfilariae for the various laboratory techniques using blood from patients infected with *A. perstans*.

	Thin	Thick	Wet	Polyvidone	Microhaematocrit
Total +	6	12	15	2	23
Total done	25	25	25	3	25
% +	24	48	60	66.6	92

Table I Recovery of microfilariae from blood collected into citrate from patients infected with *Wuchereria bancrofti*

	Thin	Thick	Wet	Polyvidone	Microhaematocrit
Total +	16	20	31	12	40
Total done	35	35	50	15	45
% +	45.7	57.1	62	80	88.8

Table II Recovery of microfilariae from blood collected into citrate from a patient infected with *Acanthocheilonema perstans*

Degree of Concentration	Technique				
	Thin	Thick	Wet	Polyvidone	Microhaematocrit
+	6 (100%)	11 (91.6%)	15 (100%)	2 (100%)	14 (60.8%)
++	0	1 (8.4%)	0	0	5 (21.7%)
+++	0	0	0	0	4 (17.5%)
Total	6	12	15	2	23

Table III Degree of concentration of *Wuchereria bancrofti* using various recovery techniques

Degree of Concentration	Technique				
	Thin	Thick	Wet	Polyvidone	Microhaematocrit
+	16 (100%)	20 (100%)	31 (100%)	12 (80%)	34 (75.5%)
++	0	0	0	2 (13.4%)	8 (17.7%)
+++	0	0	0	1 (6.6%)	3 (6.8%)
Total	16	20	31	15	45

Table IV Degree of concentration of *Acanthocheilonema perstans* using various recovery techniques

	Time (min)				
	Thin	Thick	Wet	Polyvidone	Microhaematocrit
Preparation	20	20	5	20-30	5
Examination	5-10	5-10	5	5-10	1
Total time	30	30	10	30	6

Table V Time required for complete examination of blood specimens for microfilariae of *A. perstans* and/or *W. bancrofti*

The concentration of the microfilariae was estimated using the symbols: + = 1 or few parasites in slide or tube; ++ = 1-2 parasites every two to five fields; +++ = numerous parasites every field.

The degree of parasite concentration achieved by the various techniques is indicated in Tables III and IV.

The results of the second half of the investigation were as follows. Of 17 people randomly chosen from the Mana Pools area of the Zambesi Valley, *A. perstans* microfilariae were found in the blood of two (11.8%) by stained thin films; four (23.5%) by both stained thick films and wet preparations; and six (35.3%) by the microhaematocrit technique.

In terms of time needed for the various procedures, approximate times for routine investigations are shown in Table V.

## Discussion

The investigation was aimed primarily at comparing standard laboratory techniques in terms of accuracy and time involved, and thus degree of concentration was of secondary importance except in that the greater the degree of concentration, the better the chance of making a positive diagnosis. With this aim in mind, it must be remembered that the amounts of blood used in the various techniques differed and also that the thick films were prepared in the routine manner which involved the danger of 'peeling' and loss of parasites.

In terms of practical laboratory considerations, the best method for the laboratory diagnosis of human filarial infections seemed to be the microhaematocrit technique, a technique which gave the most accurate results in terms of positive cases diagnosed and time saved. Referring to the latter point, however, in positive cases time needed for examining material from the wet preparations, polyvidone technique, and microhaematocrit technique was extended due to the fact that thick or thin films had then to be made and stained for species identification using such features as the presence or absence of the sheath, details of the nuclear column, fixed measuring points, and the relation of posterior nuclei to the tip of the tail. Such staining was advisable, although the sheath of *W. bancrofti* could often be seen trailing behind living microfilariae (especially with phase-contrast microscopy) because it was not uncommon to find that the microfilariae of *W. bancrofti* had shed their sheaths making staining imperative for identification.

Stained thick or thin preparations could be made from microhaematocrit material by cutting the tubes as mentioned previously and making the smear as for blood films.

The overall results are not, of course, really

statistically comparable, varying amounts of blood being used in the different methods. But the results are of great interest in terms of practical diagnostic laboratory value in ensuring the detection of the maximum number of positive cases in the shortest possible time. It is here that the microhaematocrit technique really scores, the parasites being concentrated in a very small area easily covered in four or five low or medium power fields of the microscope. The technique may even be adapted to field use; a portable microhaematocrit centrifuge adapted to being run off a 12-volt motor car battery has already been tried out in the Zambesi Valley.

While the Polyvidone technique was found to give good recovery figures, it is not at all time-saving and its efficiency is probably somewhat dependent upon room temperature which affects the viscosity of the Polyvidone. It also has the distinct disadvantage that unless the microscopist is experienced, the microfilariae can be missed, their movement being slowed down by the viscous fluid and by the fact that they become coated with sticky erythrocytes, a fact which, together with their reduced activity, can easily lead to their being overlooked.

The above conclusions as to the efficiency of the microhaematocrit technique are in general agreement with those of Bennett (1962) for hematozoa (including filarial worms) of birds and those of Woo (1969) for amphibian trypanosomes. Both these authors, however, centrifuged the blood for longer periods (eight and six minutes respectively), and the latter has developed a modification to make the observations in the capillary tubes easier, cutting down the refraction of light at the sides of the tubes. During the present investigation centrifuging for two minutes was found to be quite adequate for the recovery of microfilariae.

The Knott concentration technique for microfilariae as described by Burrows (1965) was not used in the present investigation, but the microhaematocrit technique would have some advan-

tage over the former technique in being quicker and not requiring such chemicals as formalin or acetic acid—an advantage in field trips where the smaller centrifuge and tubes of the latter technique are also space saving.

### Conclusions

Of five techniques tried out in the laboratory for the diagnosis of human filariasis, the microhaematocrit technique, a technique which does not seem to be widely used in diagnostic medical parasitology laboratories, was found to give the most satisfactory results in terms of efficiency and saving of time. Preliminary results using this technique in two cases of human infection with *Trypanosoma rhodesiense* have been encouraging, trypanosomes being detected in the blood using the microhaematocrit technique, while wet preparation, thick films, and thin films were apparently negative.

I should like to thank Dr T. Ashworth of the Department of Pathology, University College of Rhodesia, for his cooperation in supplying blood from the Zambesi Valley survey, Mr F. L. Goldring for allowing the use of his Polyvidone technique in the investigation, and Professor J. G. Cruickshank for his comments on the script.

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