Microhaematocrit Centrifuge Technique for the Laboratory Diagnosis of Filarial Infections

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SUMMARY

The microhaematocrit concentration technique is a very useful and time-saving method for the laboratory diagnosis of filariasis. A technique which is even more effective, but more time-consuming, is the membrane filter technique.

A good diagnostic procedure to follow in the laboratory for the recovery of Microfilariae would be to collect 2 ml of blood by venipuncture into citrate and to send this, with a thick and thin film taken from a finger-prick, to the laboratory. The laboratory could then use the microhaematocrit technique for a quick and effective screening test for Microfilariae. If this proved positive, the thick and thin film could be stained for a species identification. If the microhaematocrit proved negative, the more sensitive but more time-consuming membrane filter technique could be used to ensure that a very low-grade microfilarial parasitaemia was not present.


The use of the haematocrit centrifuge for the recovery of trypanosomes and Microfilariae of animals has been studied by Designat and Dresse,1 Wongsathuythong,2 Bennett3 and Woo,4 while Goldsmid5 evaluated its uses in the recovery of Microfilariae in cases of human infection with Wuchereria bancrofti and Dipetalonema perstans. The technique has proved efficient and time-saving and the aim of the present article is to further evaluate its uses in human filariasis and to compare it with the membrane filter technique of Dell,6 as modified by Chularerk and Desowitz.7

MATERIALS AND METHODS

For these studies, blood sent to the laboratory for routine blood counts was used. Such blood was sent in Sequestrene and 1,000 consecutive blood samples from African patients at Harari Central Hospital, Salisbury, were included in the investigation. In none of the patients was filariasis suspected.

The microhaematocrit technique used was essentially the same as that described by Goldsmid,5 using a Hawksley microhaematocrit centrifuge and spinning for 2 minutes. All capillary tubes were heat-sealed before centrifugation, and examination after concentration was made, using the medium power (× 16) and high-power dry (× 40) objective lenses to look for swimming Microfilariae (Fig. 1).

A special slide was constructed of Perspex, with grooves to hold 6 microhaematocrit capillary tubes to facilitate observations on multiple specimens (Fig. 2). All tubes in the grooves were flooded with immersion oil as recommended by Woo4 to make observation into the tubes easier by cutting down the light refraction.

Fig. 1. Photomicrograph (× 250 approx.) into a microhaematocrit centrifuge capillary tube showing Dipetalonema perstans swimming above the buffy coat.

Fig. 2. Perspex slide to hold 6 microhaematocrit capillary tubes for examination for blood parasites.

In the first part of the investigation, results obtained using the microhaematocrit technique were compared with those of the Haematology Department at Harari Central Hospital, where 200 thin blood film fields had been examined.

*Date received: 27 May 1971.
In the second part of the investigation, comparisons were made on the efficiency of the microhaematocrit technique as compared with the wet drop, the thick film, the thin film and the membrane filter techniques.

This latter technique was developed by Dell and recommended by Lambert, but the actual method used in the present work was the modification of Chularerk and Desowitz.

One ml of blood was drawn up into a 10-ml disposable syringe from the blood sample submitted to the laboratory. Nine ml of 10% Teepol in physiological saline solution was then added to haemolyse the blood. After rotating to mix the blood and Teepol, a 25-mm diameter Sartorius filter holder was attached to the syringe (Fig. 3). A membrane filter of 8-μm porosity was then fitted and supported by a disc of Whatman No. 1 filter paper as recommended by Chularerk and Desowitz. The haemolysed blood and Teepol was then forced through the filter and expelled.

RESULTS

The effectiveness of the microhaematocrit concentration technique for the recovery of Microfilariae is compared with the effectiveness of the routine thin film examination in Table I. It can be seen that 8 blood samples were found to be positive for Microfilariae (7 D. perstans and 1 W. bancrofti) using the microhaematocrit technique, as compared with 1 positive sample (W. bancrofti) using the thin film examination alone.

TABLE I. COMPARISON OF THE MICROHAEMATOCRIT TECHNIQUE AND THE STAINED THIN FILM FOR THE RECOVERY OF MICROFILARIAE FROM 1 000 CONSECUTIVE BLOOD SAMPLES

<table>
<thead>
<tr>
<th>Technique</th>
<th>No. D. perstans</th>
<th>W. bancrofti</th>
</tr>
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<tbody>
<tr>
<td>Microhaematocrit</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Thin film</td>
<td>1</td>
<td>0</td>
</tr>
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</table>

Results of comparisons between wet drop preparations, stained thick and thin films, the membrane filter and the microhaematocrit techniques are shown in Table II, which shows that the latter two techniques gave the best results.
TABLE II. COMPARISON OF THE EFFECTIVENESS OF VARIOUS TECHNIQUES FOR THE RECOVERY OF D. PERSTANS MICROFILARIAE FROM INFECTED PATIENTS IN A SERIES OF 10 EXPERIMENTS

<table>
<thead>
<tr>
<th>Membrane filter</th>
<th>Microhaematocrit</th>
<th>Wet drop</th>
<th>Thick film</th>
<th>Thin film</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. pos.</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Mean No. of parasites recovered</td>
<td>400</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
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</table>

The results of the doubling dilution titration experiment are shown in Table III, where it can be seen that the membrane filter technique was the best recovery technique followed by the microhaematocrit, the wet drop, the thick film and then the thin film techniques, in that order. An interesting point here was the close correlation at most dilutions of the number of Microfilariae recovered by the membrane filter technique when compared with the theoretical number of Microfilariae calculated for each ml of blood.

The results of the preliminary experiments on survival in various anticoagulants are shown in Table IV.

<table>
<thead>
<tr>
<th>Anticoagulants</th>
<th>Citrate</th>
<th>Heparin</th>
<th>Sequestrene</th>
</tr>
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<tr>
<td>Mean survival time (hours)</td>
<td>60</td>
<td>45</td>
<td>32</td>
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**DISCUSSION**

This study gives further evidence of the effectiveness of the membrane filter technique in the laboratory diagnosis of filariasis and thus confirms the results obtained by Wongsathuaythong, Bennett, Woo and Goldsmid. The effectiveness of the membrane filter technique of Dell, Lambert, and Chularerk and Desowitz was also tested and was found to be even better for the recovery of Microfilariae. However, this latter technique is much more time-consuming, needing about 80 minutes for completion (preparation 10 minutes, staining 60 minutes and examination 10 minutes) as opposed to the 5-6 minutes found necessary by Goldsmid for the microhaematocrit technique—an important point in a laboratory under pressure of work or short of staff.

The membrane filter technique is extremely accurate in the recovery of Microfilariae and is probably an ideal method when quantitative data (i.e. microfilarial counts) are required or when very low-grade parasitaemias are to be detected, and its effectiveness is further enhanced in this direction by the relatively large volume of blood used when compared with the microhaematocrit technique. This latter technique is perhaps slightly less sensitive (using a smaller volume of blood), but is very quick and is more effective than the wet drop, the thick film, the thin film and the polyvidone techniques. The relative inefficiency of the thick film is probably in part due to parasite loss during staining and washing, a feature especially noticeable when such films are made from citrated blood.

It must be pointed out that these investigations were intended to compare standard laboratory techniques and are thus not really statistically comparable as varying amounts of blood are used in the different techniques.

Preliminary observations were made on the effect of anticoagulants (i.e. citrate, heparin and Sequestrene) on viability of the Microfilariae, using the microhaematocrit technique for parasite recovery. This investigation was carried out as the survival of Microfilariae in a viable condition would have a definite bearing on the use of this concentration technique where moving organisms are used in making a positive diagnosis (non-motile Microfilariae being often hidden by the buffy coat).

Although these are preliminary results, it seems that any of the 3 anticoagulants will suffice if the blood reaches the laboratory within (say) a couple of hours, i.e. from the ward to the laboratory. If the time between taking of the blood and examination is days rather than hours (i.e. from the field to a central reference laboratory by post) then perhaps citrate is best.

The investigation also indicates that W. bancrofti and D. perstans infections are not common among patients at
Harari Central Hospital, where the incidence of *W. bancrofti* proved to be 0.1% and that of *D. perstans* 0.7% for 1,000 consecutive patients examined. It must be noted, however, that only daytime samples of blood were collected and the incidence of *W. bancrofti* might prove higher if blood collected at night were used.

This does not imply that the incidence of filariasis in Rhodesia is low throughout the country, as it is known that the incidence of *W. bancrofti* in parts of the Zambesi Valley is high, while *D. perstans* infection is common both in the Zambesi Valley and in other parts of Rhodesia.

We should like to thank Mrs H. Goldsmid for preparing the illustrations; and the Secretary for Health of Rhodesia for permission to publish.

**REFERENCES**


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**Phaeochromocytoma—An Interesting Psychiatric Presentation**

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**SUMMARY**

An unusual psychiatric presentation of a rare condition, phaeochromocytoma, is described to illustrate the observation that psychiatric illness, particularly depression, may be an early symptom of an underlying neoplastic condition in elderly patients.


Depression is the symptom most frequently observed in the practice of geriatric psychiatry. This is understandable enough, since elderly patients are increasingly exposed to the loss of loved objects, whether they be persons, self-esteem, potency or general physical fitness. It is particularly important, however, to eliminate organic pathology in the older age groups before presuming that depressive symptoms are due to psychological factors alone.

A recent article in the *British Medical Journal* (1970: vol. 2, p. 681) describes the findings of Professor M. Roth et al., who found a relationship between depression and occult malignancy in older patients admitted to a general hospital in Newcastle. A series of 128 patients suffering from affective disorders—anxiety states and depression—were followed up for 4 years after admission. Of 28 cases diagnosed as suffering from depressive illness, and treated accordingly, 5 males were subsequently found to have carcinoma at various sites. Compared with British national death rates, this incidence was significantly raised. The patients' ages ranged between 49-82 years and none had had a previous psychiatric history. The mean survival rate from the onset of symptoms to death was approximately 2½ years, and from commencement of psychiatric treatment to death, approximately 1 year. Professor Roth and his colleagues concluded that 'a form of depressive illness in male patients arising in middle age, without a previous history of psychiatric illness and occurring without apparent cause, may be an early and direct manifestation of latent carcinoma'.

Presumably the term 'latent' does not imply that psychiatric symptoms may mask a hypothetical condition, but rather that the cancer has not yet shown itself in local symptoms. The 'depression' may simply reflect the manner in which the patient perceives or describes his malaise and lowered vitality or the way in which his doctor interprets it. Anergy and apathy not infrequently usher in mental or physical failure of some kind, yet it is these very symptoms that predominate in both psychological and endogenous depression.

These general considerations have prompted me to describe an interesting psychiatric presentation of a relatively uncommon medical condition, phaeochromocytoma. It is hoped that this case study will serve to illustrate the importance of thorough medical investigation in all psychiatric presentations, especially in the older age groups.