A COMPARISON OF THE EFFICIENCY OF THE NUCLEPORE AND MILLIPORE FILTRATION SYSTEMS FOR DETECTING MICROFILARIAE

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INTRODUCTION

In the recent past two membrane filtration systems have been used to detect low level microfilaraemias. The original method was developed by Bell (1967) but was too cumbersome for field application using, as it did, a vacuum filtration system. More recently Chularerk and Desowitz (1970) and Dennis and Kean (1971) have developed filtration systems using positive pressure applied by means of a syringe. Chularerk and Desowitz (1970) used Millipore filter membranes and Dennis and Kean (1971) Nuclepore membranes. We decided to make a controlled laboratory examination of the filtration systems using the two filter membranes.

MATERIALS AND METHODS

Samples of cat blood containing microfilariae of *Brugia pahangi* and dog blood containing microfilariae of *Dirofilaria immitis* were carefully mixed with sheep blood to provide approximately 30 ml of blood containing 15-30 microfilariae of either species per ml.

The Millipore filtration technique : 1 ml of specimen blood was drawn into the 10 ml syringe. 9 ml of 10% Teepol in normal saline was drawn into the same syringe and the syringe rotated or shaken until the blood was completely haemolysed.

A Swinnex filter holder containing a 25 mm Millipore membrane of 5 μm porosity with a supporting washer was connected to the syringe.

The haemolysed blood was passed through the filter by steady pressure on the syringe plunger. The holder was removed and the syringe filled with clean water which was expressed through the membrane. This washing was repeated three times. The syringe was then filled with air which was expressed through the membrane to remove excess fluid. The membrane was removed from the Swinnex holder, placed in hot, not boiling Mayer's haematoxylin stain for 5 minutes, blued briefly in running tap water and dried. It was placed on a clean glass slide, cleared with immersion oil and examined at X 100 magnification and the microfilariae counted.

The Nuclepore filtration technique : 1 ml of specimen blood from the sequestrine container was drawn into a 10 ml syringe. The blood was passed directly from the syringe through a Swinnex filter holder containing a 5 μm pore Nuclepore membrane.

The syringe was filled with physiological saline, reattached to the filter, and saline expressed through the filter. This was repeated once or twice until no more blood was seen through the washings. The microfilariae were retained on the filter. A syringeful of air was blown through the filter followed by 1 ml of methanol. Another syringeful of

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air was blown through to clear any residual fluid.

The Swinnex holder was dismantled, the filter membrane removed, placed onto a clean glass slide with a drop of water and examined immediately under a binocular microscope at a X 100 magnification. The microfilaria which were seen were counted. The membrane filter was then allowed to dry whilst remaining stuck to the slide and stained with 1 in 25 Giemsa stain for one hour. It was rapidly rinsed with tap water, dried, cleared in xylene and re-examined at X 100 magnification and the microfilariae counted.

EXPERIMENTS AND RESULTS

Comparison of the Millipore and Nuclepore membranes

40 one ml aliquots of cat-sheep blood were removed from the well-shaken blood pool. 20 of these were filtered through a Nuclepore filter and 20 through a Millipore filter. The mean count (after staining) for the Nuclepore filters was 14.6 (SE 0.57) and for the Millipore filter was 15.2 (SE 0.73) microfilariae. The difference between these two series of counts was not statistically significant ($t = 0.638$ with 39 degrees of freedom). It, therefore, appears that both techniques are equally effective in detecting microfilariae in blood.

The efficiency of the two filter techniques

We attempted various methods to detect whether the filtration techniques were 100% efficient in detecting microfilariae or not. On no occasion were we able to detect fewer microfilariae on the filters than had been predicted from an exhaustive examination of the samples by the chamber counting technique (Denham *et al.*, 1971). As another test we decided to re-filter all the filtrates from experiment 1.

Filtration of the filtrate from the Millipore membranes revealed two microfilariae while that of the Nuclepore membranes revealed three microfilariae. It should be appreciated that the 2 and 3 microfilariae which were detected in this test were those not collected on the filters whilst 304 microfilariae had been collected on the Millipore filter and 292 had been collected on the Nuclepore filter.

How many microfilariae are lost from the Nuclepore filter during staining?

A comparison was made with 20 Nuclepore filters on which counts were made before staining and after staining using blood containing either *B. pahangi* or *D. immitis*. Our suspicion was that some microfilariae might float off the filters during the staining - clearing process.

The mean count on the filter before staining for the *B. pahangi* blood was 31.85 (SE 2.41) and after staining it was 32.00 (SE 2.01). This difference is not significant, ($t = 0.0477$ with 39 degrees of freedom). Using *D. immitis* the pre-staining count was 14.35 (SE 0.80) and the post-staining count was 14.60 (SE 0.96).

DISCUSSION

Since their adaptation to field use by Chularerk and Desowitz (1970) and Dennis and Kean (1971) the membrane filtration techniques have enjoyed the reputation of being one of the most sensitive techniques for demonstrating microfilaria. In a comparative study between the membrane filtration technique and the Knott's technique, Desowitz (1974) reported that "membrane filtration also seemed more sensitive in detecting low-grade microfilaraemias than Knott's method". Many investigators notably Desowitz and Southgate (1973), Desowitz and Hitchcock (1974), Wang and Fan (1973), Southgate (1974) have used the technique in the field and found it to be simple and rapid.

In our study we were unable to find any difference in the number of microfilariae

detected by the two filtration techniques. We have rather more experience with the Nuclepore system and find this more convenient for routine use. Its major advantage is that unlysed blood can be used and that very large volumes may be processed through a single filter. We have, for example, filtered 75 ml of cat blood containing approximately 700 mf per 20 c.mm. This makes the Nuclepore system very useful for collecting large numbers of microfilariae for antigen (Ponnudurai *et al.*, 1974) and enables much more blood to be examined from cases of occult filariasis, such as tropical pulmonary eosinophilia.

It is difficult to account for the passage of microfilariae through the filters. However, in a field trail on patients infected with *Mansonella ozzardi*, which is much smaller than *B. pahangi* or *D. immitis*, Nelson (personal communication) found larger numbers of microfilariae in the filtrate but when he used 3 μ Nuclepore membranes all the microfilariae were retained on the filter.

Both of these filters have been widely used in field surveys.

SUMMARY

Using *Brugia pahangi* or *Dirofilaria immitis* as the test organisms no significant difference could be detected between Nuclepore and Millipore filters. It was found that 0.7% of microfilariae passed through the Millipore and 1% through the Nuclepore filters. No microfilariae were lost from the Nuclepore membrane during the staining process.

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