Comparison of the Methods Available for Purification of *Brugia pahangi* Microfilariae in the Peritoneal Lavage of Jirds (*Meriones unguiculatus*)

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**Abstract:** The pros and cons of the methods available for the separation and purification of *Brugia pahangi* microfilariae in jirds peritoneal lavage was studied. The methods examined in the present study were agar-enclosing method described by Nogami et al. (1982) and Sephadex PD-10 column method described by Rathaur et al. (1987). Both methods allowed us to recover live microfilariae alone. The purity of microfilarial suspension was satisfactory in both methods; no peritoneal cells, dead microfilariae nor ovum. The PD-10 column method was superior in the percent recovery of microfilariae; 77–89% of microfilariae loaded were recovered by passage on PD-10 column, while 53–74% of microfilariae embedded were recovered by agar-enclosing method. Agar pads used for separation of microfilariae were fragile and frequently disrupted, resulting in the contamination of peritoneal cells in microfilarial suspension. In addition, the PD-10 method was superior in less time consuming and less laborious procedure. PD-10 column can be used repeatedly. Thus the PD-10 column method is, at present, the best available one for separation and purification of microfilariae from peritoneal lavage.

*Key words: Brugia pahangi, Microfilariae, Purification, Jird, Meriones unguiculatus, Peritoneal lavage*

**INTRODUCTION**

*Brugia pahangi* is a lymphatic dwelling filarial worm which can be easily maintained in the laboratory. Since McCall et al. (1973) reported that *B. pahangi* develops and produces microfilariae in the peritoneal cavity of jirds (*Meriones unguiculatus*), the recovery of all developmental stages of the worm in the final hosts becomes possible. Among the
various methods by which microfilariae can be purified from the peritoneal cells of jirds, the following two methods have been reported to yield microfilarial suspension with high purity. One is embedding microfilariae in agar pads and allowing them to migrate into the overlaid solution (Nogami et al., 1982), and the other is separation of microfilariae by passage on the Sephadex PD–10 column (Rathaur et al., 1987). The pros and cons of the methods, however, have not been examined thoroughly. In the present paper, we compare the two methods in terms of purity and percent recovery of microfilariae.

**Materials and Methods**

*B. pahangi* used in our experiments was one which had been maintained for a long time in our laboratory. The jirds were inoculated intraperitoneally with infective larvae obtained from *Aedes aegypti* which had fed on a microfilaremic jird 14 days previously. The animals were necropsied 4–8 months post-inoculation. The peritoneal cavity was washed with RPMI 1640. The lavage was centrifuged at 1200 rpm for 5 minutes. The sediment was again suspended in a given volume of RPMI 1640. An aliquot of microfilarial suspension was examined for microfilariae and peritoneal cells, and the total number of microfilariae and cells in the sample was calculated. The peritoneal cells were stained with Turck and counted in a hemocytometer.

**Separation on Sephadex PD–10 column:**

Microfilarial suspension of 0.5 ml was loaded on PD–10 column (Pharmacia AB Biotechnology, Sweden). The elution buffer used was RPMI 1640. The pressure was adjusted so that about 60 drops per minute was eluted. The elute of 8 ml each was collected in test tubes. Each sample was then fixed with formalin and later examined for microfilariae and cells. Samples were centrifuged and sediments were examined for microfilariae and cells when the numbers of microfilariae and cells were small.

**Separation by gel-enclosing method:**

The following method by Nogami *et al.* (1982) was used. Briefly, 1.2 ml of microfilarial suspension (30°C) was mixed with 0.8 ml of 1% agarose dissolved in saline (50°C). The mixture was poured into the Petri dishes with diameter of 60 cm (Falcon). After solidification of the gel at room temperature, 8 ml of RPMI 1640 was overlaid on the gel. After 2 hrs and 4 hrs incubation at 37°C, the RPMI 1640 was removed, the solution was centrifuged and sediment was examined for microfilariae and cells.

**Results**

**Separation by PD–10 column**

The elution pattern of microfilariae and peritoneal cells is shown in Fig. 1. A huge number of cells were eluted in the 1st tube and many cells were eluted in the 2nd tube.
Fig. 1. Elution pattern of microfilariae and peritoneal cells through PD-10 column
Microfilariae and cells loaded on the column were 600,000 and 380,000 respectively. Each tube contained 8 ml.

No cells, however, were eluted in the 3rd tube and thereafter. The rate of cells passed through PD-10 column in 3 experiments were 20.3%, 8.9% and 23.6% of the cells loaded on the column respectively. Microfilariae were eluted in the 1st and 2nd tubes, but the number was small. The number of microfilariae eluted increased in the 3rd tube and thereafter, and the peak number was found around the 10th tube. A few microfilariae were eluted in the 25th tube at which point the experiment was stopped. The separation of microfilariae by PD-10 column, therefore, is identified as a highly efficient method by which microfilariae can be easily purified from peritoneal lavage. Since the tubes which contained the peritoneal cells should be discarded, the percent recovery of microfilariae by the PD-10 column was calculated as follow: %recovery = (total number of microfilariae eluted in all tubes but 1st and 2nd tubes/number of microfilariae loaded on column) × 100. All our experiments showed high %recovery and complete separation of microfilariae from the peritoneal cells (Table 1).

Separation by gel-enclosing method

In our study, the gels in which microfilariae were embedded were incubated for 2 and 4 hours, and overlaid RPMI 1640 was recovered as completely as possible. The percent recovery is described in Table 1. Although the long incubation yielded high recovery rate by gel-enclosing method, the rate was much less than that obtained by PD-10 col-
Table 1. Number of microfilariae and peritoneal cells in microfilarial suspension separated by PD-10 column and agar-enclosing method

<table>
<thead>
<tr>
<th>Separation method</th>
<th>No. of microfilariae(^1) loaded on/embedded in agar</th>
<th>No. of microfilariae recovered (^2)</th>
<th>(% recovery)</th>
<th>No. of cells in concentrate (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-10</td>
<td>600,000</td>
<td>478,000(^2)</td>
<td>(80.0%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>609,150</td>
<td>471,400(^2)</td>
<td>(77.4%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>625,000</td>
<td>559,000(^2)</td>
<td>(89.4%)</td>
<td>0</td>
</tr>
<tr>
<td>agar 2 hrs</td>
<td>600,000</td>
<td>309,000</td>
<td>(51.5%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>609,150</td>
<td>319,400</td>
<td>(52.4%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>625,000</td>
<td>295,000</td>
<td>(47.2%)</td>
<td>1,000,000(^3)</td>
</tr>
<tr>
<td>agar 4 hrs</td>
<td>600,000</td>
<td>315,000</td>
<td>(52.5%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>609,000</td>
<td>448,100</td>
<td>(73.6%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>625,000</td>
<td>435,000</td>
<td>(69.6%)</td>
<td>2,500,000(^3)</td>
</tr>
</tbody>
</table>

1. Microfilariae used were those in fresh peritoneal lavage.
2. Total number of microfilariae in all tubes but 1st and 2nd.
3. Gel was partially disrupted when fluid was collected.

Table 2. Recovery of live and dead microfilariae through PD-10 column.

<table>
<thead>
<tr>
<th>microfilariae</th>
<th>Total No. of microfilariae loaded</th>
<th>No. of microfilariae recovered</th>
<th>(% recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>exclusively live(^1)</td>
<td>920,000</td>
<td>708,800</td>
<td>77%</td>
</tr>
<tr>
<td>killed by formalin(^2)</td>
<td>920,000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>mixture(^3) of motile and non-motile</td>
<td>724,000</td>
<td>292,400</td>
<td>40%</td>
</tr>
<tr>
<td>(live 414,000)</td>
<td>(live 271,600)</td>
<td>(66%)</td>
<td></td>
</tr>
<tr>
<td>(non-motile 310,000)</td>
<td>(non-motile 20,800)</td>
<td>(7%)</td>
<td></td>
</tr>
</tbody>
</table>

1. microfilariae in fresh peritoneal lavage of jird infected with *B. pahangi*
2. microfilariae fixed by formalin and washed several times with RPMI 1640
3. microfilariae in peritoneal lavage kept at 4°C for 7 day

umn. The overlaid fluid recovered did not contain any peritoneal cells in two experiments. In another experiment, however, where the gel was partially disrupted, numerous cells were present in the fluid recovered.

Separation of live and dead microfilariae by PD-10 column

Since the agar-enclosing method was reported to have the advantage that live microfilariae alone were collected (Nogami et al., 1982), we examined what type of microfilariae is eluted through PD-10 column. In this experiment, we loaded three different microfilariae samples; 1) fresh peritoneal lavage which included exclusively live microfilariae, 2) peritoneal lavage fixed by formalin, which included killed microfilariae, and 3) the peritoneal lavage kept at 4°C for 7 days which included both active and non-motile microfilariae. The results obtained are presented in Table 2. When the microfilariae
fixed by formalin were loaded, no microfilariae were eluted. When the peritoneal lavage kept at 4 °C for 7 days which included active microfilariae (57%) and non-motile microfilariae (43%), many active microfilariae (66% of microfilariae loaded) were recovered, but the recovery of non-motile microfilariae was low (7% of microfilariae loaded). The percent recovery of fresh microfilariae in this experiment was as high as 77%.

Repeated use of PD-10 column for separation of microfilariae

After the peritoneal lavage was separated, if the column was washed by RPMI 1640 thoroughly for 1 hour and kept at 4 °C, the column could be used repeatedly for separation of microfilariae. The efficiency in recovering was compared between new and used column. When we separated the peritoneal lavage by using a column which had been used 7 times, the percent recovery was as high as that obtained with a new column (Table 3).

Table 3. Comparison of percent recovery of microfilariae by new and used column

<table>
<thead>
<tr>
<th></th>
<th>No. of microfilariae</th>
<th>No. of microfilariae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>loaded</td>
<td>recovered # (%) recovery</td>
</tr>
<tr>
<td>new column</td>
<td>4,604,100</td>
<td>3,637,300 (79%)</td>
</tr>
<tr>
<td>used column##</td>
<td>4,604,100</td>
<td>3,412,266 (74%)</td>
</tr>
</tbody>
</table>

# Total number of microfilariae in all tubes but 1st and 2nd tubes which contained 8 ml each
## The column had been used for separation of microfilariae 7 times.

DISCUSSION

So far, 4 methods are available for isolation and purification of microfilariae from jirds peritoneal lavage. Ah et al. (1974) incubated the peritoneal lavage in petri dishes for 2 hours and allowed the peritoneal macrophages to adhere to the bottom of the dishes. Then, for further elimination of the remaining macrophages and other leukocytes, washing and centrifugation of the microfilarial suspension were repeated. Their method, however, is not satisfactory, because the purity of microfilarial suspension depended on repeated washing, and the preparation contained ovum and dead microfilariae. Longworth et al. (1987) used Ficoll–Paque for isolation and purification of microfilariae. In their microfilarial suspension, however, jirds peritoneal cells numbered 1 per 2500 microfilariae. Nogami et al. (1982) embedded microfilariae in agar and succeeded in obtaining microfilarial suspension without any peritoneal cells. Their method is a modification of the method by Green and Schiller (1979) which was developed for purification of *Onchocerca volvulus* microfilariae from tissues. Recently, Rathaur et al. (1987) introduced Sephadex PD–10 column separation of microfilariae in jirds peritoneal lavage. The advantages of this method were, however, not examined in detail.

The aim of this study was to examine the pros and cons of the latter two methods, which seem to yield higher percent recovery and least amount of contaminating peritoneal cells. Since Nogami et al. (1982) reported that live microfilariae alone were recovered
when agar pads were used, we examined what type of microfilariae was recovered by the passage using Sephadex PD–10 column. When the fresh peritoneal lavage was loaded, live microfilariae were exclusively eluted, whilst no microfilariae were eluted when microfilariae killed by formalin were loaded. When peritoneal lavage kept at 4 °C for 1 week was loaded, a low percentage of non-motile microfilariae were eluted. However, these may have been alive because it is impossible to distinguish the live from dead microfilariae by short microscopic observations for motility. The PD–10 column method, therefore, can be described as one of the best available methods by which successful recovery of live microfilariae alone is possible.

Evaluation of microfilarial suspension produced by these methods was based on the contaminating amount of peritoneal cells and the microfilarial recovery percentage as the principal parameters. PD–10 column method, when first 16 ml of elute was discarded, yielded no contaminating cells in microfilarial suspension. The agar method also yielded satisfactory purity, since no cells were counted in the overlaid fluid on agar. However, when the agar is partially disrupted, many cells become contaminated. Since the agar pad used for recovery of microfilariae is fragile, frequent disruption of the agar pads is inevitable.

The sephadex PD–10 column is superior as regards percent recovery of microfilariae. In all our experiments, more than 77% of microfilariae loaded were recovered, and the separation was completed within 40 minutes. In addition, repeated used of the column did not affect microfilarial yield. In our experiments with agar pads, percent recovery was low (52.5–69.6% for 4 hour incubation), though Nogami et al. (1982) showed that when agar pads were allowed to float in the incubation medium, percent recovery of microfilariae increased. This method, however, causes frequent unwanted breaks or disruption of the agar pads, and the procedure for isolating microfilariae using agar is laborious and time consuming. In conclusion the separation by Sephadex PD–10 column introduced by Rathaur et al. (1987), is taken as the best available method for the separation and purification of microfilariae in jirds peritoneal lavage.

ACKNOWLEDGMENTS

This work was supported by Japan International Cooperation Agency (JICA). We would like to express our sincere thanks to all the staff of the Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan.

REFERENCES


