Simplified Slide Preparation in the Circumoval Precipitin (COP) Test for Field Survey

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Summary: A simplified slide preparation for the circumoval precipitin (COP) test for field use is described. The test makes use of an insulating tape and cellulose tape in place of coverslips and also makes use of Schistosoma eggs dried on slides. S. japonicum was used in this study, and dried eggs gave satisfactory COP reaction up to 2 month storage. Use of tapes was found to be more suitable for field use and, at the same time, cheaper than coverslips.

Key words: Blood fluke — Schistosoma japonicum — egg — Circumoval precipitin test — Sero-diagnosis

Introduction

The circumoval precipitin (COP) test, first reported by Oliver-Gonzalez (1954), has been evaluated as one of those principal methods for use in the immunodiagnosis of schistosomiasis (Kagan and Pellegrino, 1961). Although the technique is relatively simple, further simplification has been made through the use of lyophilized eggs (Revera de Sala et al. 1962; Yogore et al. 1968). These improvements were assessed to be very practical and suitable for survey in rural areas (Nosenas et al. 1975; Tanaka et al. 1975; Matsuda et al. 1977). Recent report on availability of air-dried S. japonicum eggs also makes field COP test more practical (Kamiya, 1983).

In addition, there has been a notable report on the use of microscope slides with perforated Dubl-stik tape in which reaction of lyophilized eggs with test serum was observed through microscope coverslips (Lewert and Yogore, 1969). The present paper describes a modification of this technique in which electrical insulating and cellulose tapes have been used instead of Dubl-stik tape and coverslips.

Materials and Methods

Slide preparation

A roll of PVC electrical insulating tape (19 mm width, 10 m length, 0.2 mm thick, Nichiban Co. Ltd, Japan) was drilled with heated 11 mm and 5 mm tubular cork bors. The two holes were positioned such that they had a common edge which opened to each other as shown in Fig. 1.

A strip of perforated tape was adhered onto a microscope slide previously cleaned with alcohol. In order to avoid air bubbles from being trapped, the tape was pressed
starting on the slide from the centre outwards. The slide was left in a room at least 2 days to ensure tight adhesion of the tape, otherwise, leakage would occur through minute gaps. Ten μl of S. japonicum egg suspension containing approximately 100 eggs obtained from an 8-week-infected rabbit (Yokogawa et al. 1966), was placed in the large hole using a micropipette, spread evenly and allowed to dry according to Kamiya (1983).

One drop (25 μl) of serum was placed in the large hole at the edge bordering the small hole. A piece of cellulose tape (24 mm width, 35 m length, 0.02 mm thick, Sekisui Co. Ltd, Japan) was then adhered onto the insulating tape beginning from the side of the large hole toward the small hole, with continuous gentle pressing with the index and the middle finger. This process caused the serum to enter into the space between the tape and the slide by the capillary phenomenon.

Preliminary studies had indicated that the use of one hole only would be inadequate because in situations where there was excess of serum applied, adhesiveness of the tape was reduced. A smaller hole was therefore necessary as a reservoir for any overflow. The completed slide (Fig. 2) was incubated at room temperature for 48 hours after which the microscopic examination of COP reaction through cellulose tape was made (Fig. 3). A further test was done to compare COP reaction utilizing lyophilized and dried eggs following this procedure.

**Results and Discussion**

It was not possible to completely avoid evaporation using cellulose tape. The degrees of evaporation were tested under different conditions. In ordinary circumstances (15 to 28°C, 40 to 80% humidity), no noticeable change in the morphology of eggs occurred with an incubation time of 2 days. On day 3, a portion of eggs began to destroy with advancement of evaporation. Higher incubation temperature (28 to 36°C) and low humidity (20 to 30%) caused significant evaporation. However, the eggs were safe on day 1 and the results of COP reaction was no different from those obtained on day 2, as has been shown that almost the reaction completes within 24 hours in room temperature (28°C) (Garcia et al. 1981).

It has been reported that the sensitivity of the COP test differed with egg number
TABLE 1

Comparison of COP reactivity between lyophilized eggs and eggs dried on slides

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Lyophilized eggs</th>
<th></th>
<th>dried eggs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% positive</td>
<td>COP Index</td>
<td>% positive</td>
<td>COP Index</td>
</tr>
<tr>
<td>1</td>
<td>2.6</td>
<td>1.2</td>
<td>5.3</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>3.7</td>
<td>1.6</td>
<td>9.5</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>15.0</td>
<td>6.0</td>
<td>32.9</td>
<td>12.6</td>
</tr>
<tr>
<td>4</td>
<td>16.8</td>
<td>7.8</td>
<td>32.1</td>
<td>18.5</td>
</tr>
<tr>
<td>5</td>
<td>19.7</td>
<td>7.3</td>
<td>24.4</td>
<td>9.3</td>
</tr>
<tr>
<td>6</td>
<td>23.0</td>
<td>8.6</td>
<td>15.7</td>
<td>6.5</td>
</tr>
<tr>
<td>7</td>
<td>28.1</td>
<td>14.0</td>
<td>18.2</td>
<td>10.9</td>
</tr>
<tr>
<td>8</td>
<td>33.0</td>
<td>12.0</td>
<td>39.4</td>
<td>19.2</td>
</tr>
<tr>
<td>9</td>
<td>33.7</td>
<td>13.9</td>
<td>25.7</td>
<td>16.2</td>
</tr>
<tr>
<td>10</td>
<td>37.3</td>
<td>17.9</td>
<td>35.1</td>
<td>17.1</td>
</tr>
<tr>
<td>Mean</td>
<td>21.3</td>
<td>9.0</td>
<td>23.8</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Index was calculated according to Tanaka (1976) as follows:

\[
\text{Total of grades of precipitation} \times \frac{100}{\text{No. of eggs observed}} \times 3
\]

The grades were determined by classification proposed by Yokogawa et al. (1967).

employed (Garcia et al. 1981). Thus, it is desirable to adjust egg number to be placed on a slide. In our experience, taking out a constant number of eggs with a tool from a vial was accompanied by difficulty as has been found by other workers (Lewert and Yogore, 1969; Matsuda et al. 1977). To overcome this, air-dried eggs on slides were employed (Kamiya, 1983). This method enabled us to adjust the egg number and also made it possible for it to be applied in the field.

The antigenicity of lyophilized eggs has been reported to be highly stable in a refrigerator for up to 3 years (Yogore et al. 1968) and at elevated temperature (60°C) for 8 months (Kamiya, 1980). It has been also reported that the air-dried eggs could be preserved without changes in a refrigerator with silica gel, at 4°C for about 1 year (Kamiya, 1983). In our study, egg-sticked slides were left in a room (15 to 28°C, 40 to 80% humidity) without use any special equipment, for 2 months and bioactivity of the eggs was compared to that of lyophilized eggs of the same batch kept in a refrigerator; in the latter case, slides were covered with vaselin-rimmed coverslip. Table 1 indicates no significant difference in the activity of the two groups of eggs. The results also indicate that cellulose tape was satisfactory for the microscopical observation. For prolonged storage, egg-sticked slides should be kept in a desiccator under conditions which limit the growth of mold.

The procedure for the COP test described in this paper is different from the methods previously described in that use of coverslips was omitted and substituted with electrical insulating tape and cellulose tape. Each roll of tape could be sufficient for about 250 slides which cost about one fifth of the required of coverslip (22 × 22 mm). The tapes are also more readily available which make the COP test routinely possible especially in areas where *S. haematobium* is endemic.
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References


