Comparison of a modified shell vial culture procedure with conventional mouse inoculation for rabies virus isolation

María de los Angeles Ribas Antúnez1/+, Blanca Girón1, Iraima Monsalvez1, Luis Morier1, Gretel Acosta1, Yahisel Tejero1, Yanislet Cordero1, Dainelyd Piedra1

1Pedro Kouri Institute, Havana, Cuba 2Rafael Rangel National Institute of Hygiene, Caracas, Venezuela

Rabies is an acute viral disease that causes encephalitis and is often lethal. The early diagnosis of rabies infection is important and requires methods that allow for the isolation of the virus from animals and humans. The present study compared a modified shell vial (MSV) procedure using 24-well tissue culture plates with the mouse inoculation test (MIT), which is considered the gold standard for rabies virus isolation. Thirty brain samples (25 positive and 5 negative by the fluorescent antibody test) obtained from different animal species at the National Institute of Hygiene Rafael Rangel in Caracas, Venezuela, were studied by the MIT and MSV assays. Nine samples (36%) were positive at 24 h, 10 (40%) were positive at 48 h and six (24%) were positive at 72 h by the MSV assay. With the MIT assay, 76% were positive at six days post inoculation and 12% were positive at 12 and 18 days post inoculation. One sample that was negative according to the MSV assay was positive with MIT on the 12th day. The MSV procedure exhibited a sensitivity of 96.2%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value 80%. This procedure allowed for rapid rabies virus detection. MIT can be employed as an alternative method in laboratories without tissue culture facilities.

Key words: rabies - modified shell vial procedure - mouse inoculation
and a negative predictive value of 80% (95% CI: 45-100). The kappa value was 0.86. A false-negative result was obtained by the MSV assay for one specimen that was positive by MIT on the 12th day post inoculation (Table I).

Several comparative studies of CC and the MIT have shown that CC is as sensitive as the MIT; however, other studies have found that CC is less sensitive than the MIT (Rudd & Trimarchi 1987, Barrat et al. 1988).

In this study, the MSV assay was less sensitive, but more specific than the MIT and was also faster (positivity at 24 h post inoculation), more economical with respect to human resources and animals and inexpensive. In this assay, low-speed centrifugation is used to increase the rate of viral infection of susceptible cells. Low-speed centrifugation causes a low level of damage to the surface of the cells, thus increasing the rate of viral entry into the cells and decreasing the time necessary for infection (Jayakaerthi et al. 2006).

Table II summarises the distribution of results for rabies obtained using the MSV assay and the MIT at different times post inoculation. The mice were observed daily for 21 days and all dead mice were examined by FAT. Any deaths occurring during the first five days were regarded as non-specific due to stress, bacterial infection or other causes.

The kappa value was 0.86. A false-negative result was obtained by the MSV assay for one specimen that was positive by MIT on the 12th day post inoculation (Table I). The MSV assay had a positivity of 36% at 24 h post inoculation, 40% at 48 h and 24% at 72 h. For the MIT, 73% positivity was obtained at six days after inoculation and a positivity of 11.5% was observed at 12 (15.4%) and 18 days. Approximately one-third of the samples assessed using the MSV assay were positive between 24-48 h post inoculation. The average MIT incubation period was five days (range 8-28 days).

According to the 1984 guidelines of the World Health Organization Experts Committee, mice should be sacrificed after the fourth day post inoculation because the symptoms observed during the first 24-48 h could be attributed to other causes, such as trauma, poor inoculation technique and bacterial contamination (Bourhy et al. 1990).

When the treatment of a patient exposed to rabies is necessary, it is not practical to wait for the MIT results. Delays in diagnosis increase the number of persons potentially exposed to the rabies virus; moreover, early diagnosis can reduce the cost of treatment by eliminating unnecessary vaccination and allow for the implementation of potentially useful emerging therapeutic strategies.

This report is believed to be the first study on rabies virus isolation using a modified shell vial technique. This assay can make a major contribution to rabies diagnosis and the MIT can be used when ambiguous or negative results are obtained by FAT and cell culture.

### TABLE I

Comparison of the results of the modified shell vial (MSV) procedure and the mouse inoculation test

<table>
<thead>
<tr>
<th></th>
<th>MSV</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25</td>
<td>0</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>4</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26</td>
<td>4</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

Negative predictive value: 80%; positive predictive value: 100%; sensitivity: 96.2%; specificity: 100%.

### TABLE II

Distribution of the results for rabies detection using the modified shell vial (MSV) technique and the mouse inoculation test (MIT) (days/h post inoculation)

<table>
<thead>
<tr>
<th>h</th>
<th>n (%)</th>
<th>Days</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>9 (36)</td>
<td>6</td>
<td>19 (73)</td>
</tr>
<tr>
<td>48</td>
<td>10 (40)</td>
<td>12</td>
<td>4 (15.4)</td>
</tr>
<tr>
<td>72</td>
<td>6 (24)</td>
<td>18</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>-</td>
<td>- (+)</td>
<td>21</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>256 (100)</strong></td>
<td><strong>26 (100)</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: one sample that was negative according to the MSV was positive by the MIT on the 12th day.

### REFERENCES


