Kinetics of the Vaccinia Virus Plaque Neutralization Test

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Neutralization of vaccinia virus with immune rabbit serum occurred optimally when incubated at 37 °C for 16 to 24 hr in the plaque neutralization test employing the MA-104 embryonic rhesus monkey kidney cell line.

A variety of techniques have been employed to measure neutralizing activity to vaccinia virus. Virus-serum mixtures have been inoculated into scarified rabbit skin (3, 4, 14) and into the chorioallantoic membrane of 12-day-old chicken embryos (5, 12, 15, 16). The measurement of neutralizing activity by these methods has been shown to be unsatisfactory because the small amount of residual unneutralized virus (5%) could multiply and obscure the results (7, 12, 22). Noyes (22) explored the use of fluid-fed primary chicken embryo cell cultures, and Falchetti and Merieux (11) utilized HeLa monolayer and suspension cultures to measure vaccinia virus neutralization. Cutchins and Warren (6), in a comparative study, showed that plaque titrations of vaccinia virus in primary rhesus monkey kidney cell cultures to be the most sensitive, simple, and reproducible assay method for this virus. Cutchins et al. (7) further demonstrated that a plaque neutralization test, employing primary rhesus monkey kidney cells, could detect minute amounts of antibody. In this system, any residual active virus would be immobilized by the agar overlay and not interfere with the test.

Although the vaccinia assay system in rhesus monkey kidney monolayer cell cultures is now well established, there is considerable confusion in the literature as to the optimum temperature-time period for vaccinia virus neutralization: 4 °C for 1 hr (11); room temperature for 1 hr (9, 14, 15, 16, 21); room temperature for 24 hr (4); room temperature for 4 days (3); 37 °C for 30 min (12); 37 °C for 1 hr followed by 4 °C overnight (13); 37 °C for 70 min plus 37 °C for 10 min with goat anti-rabbit gamma-globulin (25); 37 °C for 2 hr (2, 8, 17, 26); 37 °C for 2 hr followed by 4 °C overnight (1, 7); 37 °C for 4 hr (18); and 37 °C for 24 hr (2, 3, 20). We were confronted with this confusion when we attempted to select the ideal vaccinia virus neutralization test for use in our laboratory. Consequently, we were prompted to study the kinetics of vaccinia virus neutralization in a plaque assay system to determine the optimal temperature and length of incubation of the virus-antibody mixture.

### TABLE 1. Optimum temperature for vaccinia virus plaque formation

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>23°</td>
<td>≤1.0</td>
<td>1.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>33</td>
<td>1.3</td>
<td>5.1</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>36</td>
<td>5.3</td>
<td>5.8</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>
| 39         | 5.1 | 5.3 | 5.4 | 5.5 | where a Plaque were smaller and less distinct when cultures were maintained at 23 °C.

### TABLE 2. Thermal inactivation of vaccinia virus

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>32</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6.1°</td>
<td>6.1</td>
<td>6.1</td>
<td>6.0</td>
<td>6.1</td>
<td>6.0</td>
<td>5.9</td>
<td>5.6</td>
</tr>
<tr>
<td>23</td>
<td>6.2</td>
<td>6.1</td>
<td>6.0</td>
<td>5.8</td>
<td>5.9</td>
<td>6.0</td>
<td>5.7</td>
<td>5.7</td>
</tr>
<tr>
<td>37</td>
<td>6.2</td>
<td>6.0</td>
<td>6.1</td>
<td>6.1</td>
<td>5.3</td>
<td>5.4</td>
<td>5.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

* Titer expressed as log_{10} plaque-forming units per 1.0 ml.

The plaque neutralization (PN) test was performed in the following manner. All reagents were kept in an ice-water bath before and during mixing; unheated immune rabbit serum (CDC reagent lot #4) diluted 1:2.5 in phosphate-buffered saline (PBS; reference 10) with 0.5% bovine plasma albumin (BPA), pH 7.2, was added to
equal amounts of serial 10-fold dilutions of virus in the same diluent. Identical virus dilutions were prepared with an equal volume of the PBS-BPA diluent as a thermostability control. Virus-serum and control virus-diluent mixtures were incubated at 4°C, 23 to 25°C (room temperature), and 37°C; at various times, 0.2 ml of each mixture was inoculated into each of two 2-oz (0.059 liter) bottle cultures of the MA-104 embryonic rhesus monkey kidney cell line (23). After adsorption for 1.5 hr at 36°C, the cultures were overlaid once with 5 ml of an agar medium (24). Plaques were counted after incubation at 36°C for 6 days because this was shown to be the optimal temperature and time for the development of vaccinia virus plaques (Table 1). End points were usually determined from dilutions containing 10 to 50 plaque-forming units (PFU). The difference in titer between the virus-diluent and the virus-serum mixtures represents the neutralizing capacity of the serum and is expressed as the log₁₀ neutralization index.

Table 2 shows the thermal inactivation of vaccinia virus-diluent mixture at different incubation temperatures and time intervals. Inactivation at 37°C began to occur after 8 to 16 hr of incubation. Figure 1 shows the rapid decrease in log₁₀ PFU with increasing time only when the virus-serum mixture was incubated at 37°C. Figure 2 illustrates the kinetics of vaccinia virus PN. The optimum temperature and time of incubation of the virus-serum mixture occurred at 37°C after 16 to 32 hr.

Neutralization of vaccinia virus with immune rabbit serum occurred optimally at 37°C for 16 to 24 hr, and the optimum temperature and time for the development of vaccinia virus plaques were at 36°C and 6 to 7 days, respectively.

**Fig. 1. Kinetics of immune rabbit serum and vaccinia virus plaque neutralization.**

**Fig. 2. Kinetics of vaccinia virus plaque neutralization.**

**LITERATURE CITED**


