Solid-Phase Radioimmunossay for Rapid Detection and Identification of Western Equine Encephalomyelitis Virus

Neil H. Levitt, Helen Y. Miller, and Gerald A. Eddy
United States Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21701

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A solid-phase radioimmunossay technique was adapted for the rapid detection and identification of western equine encephalomyelitis virus in clinical specimens.

Radioimmunossay (RIA) procedures for detection of viral antigens and antibodies have been found to be more sensitive than conventional serological techniques (2, 3, 6, 9). In addition, it has been shown that 125I-labeled antibodies can be used to detect herpesvirus antigens on the surface of infected cells (7, 8). Applying this finding, Forghani et al. (2) developed a solid-phase RIA procedure for the type-specific identification of herpesvirus types 1 and 2 in clinical specimens. We have recently adapted this procedure for the rapid identification of western equine encephalomyelitis (WEE) virus.

A schematic diagram illustrating the procedure developed in this study is shown in Fig. 1. Glass vials (1 by 3 cm; A. H. Thomas, Co., Philadelphia, Pa.) containing confluent monolayers of primary duck embryo cells (DEC) were inoculated with 0.1 ml of DEC-grown WEE virus (strain 628). After a 2-h adsorption period, the specimens were removed and 1.0 ml of medium 199 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 2% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) was added to each vial. The vials were then incubated overnight at 37°C in a 5% CO2 atmosphere. After incubation, the medium was removed and the monolayers were rinsed with a balanced salt solution and fixed with cold acetone for 10 min to expose any intracellular viral antigen present. Once the vials were dry, 0.05 ml of rabbit hyperimmune WEE antiserum was added; vials were incubated at 37°C for 1 h. The optimal antiserum concentration used in these experiments was determined previously by block titration. The serum was then removed and the vials were rinsed three times to remove residual, unabsorbed antibody. Goat anti-rabbit gamma globulin was obtained from Cappel Laboratories, Downington, Pa. The antiglobulin was labeled by the lactoperoxidase technique of Marchal.

FIG. 1. Flow sheet illustrating the solid-phase RIA procedure for detection and identification of WEE virus.
VOL. 4, 1976

mean counts per minute in corresponding uninfected cultures. Elapsed time from specimen inoculation to final counting was less than 24 h.

The results from a typical experiment demonstrate the sensitivity and specificity of this identification procedure (Fig. 2). Replicate cell cultures were infected with virus concentrations ranging from 1 to 6 logs of either WEE or Venezuelan equine encephalomyelitis (VEE) virus. After overnight incubation, the cultures were examined for virus content by RIA. These same suspensions were centrifuged and used as inocula for virus isolation by plaque assay on DEC monolayers. These same suspensions were examined for virus content by RIA. Optic nerve

![Table 1. Radioimmunoassay of WEE virus-infected tissues](https://example.com/table1)

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Plaque assay titer (PFU/ml)</th>
<th>IC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic nerve</td>
<td>$1.5 \times 10^6$</td>
<td>3.9</td>
</tr>
<tr>
<td>Eye</td>
<td>$5.0 \times 10^6$</td>
<td>3.0</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>$5.0 \times 10^6$</td>
<td>4.2</td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>$6.5 \times 10^6$</td>
<td>4.2</td>
</tr>
<tr>
<td>Spinal fluid</td>
<td>$8.0 \times 10^6$</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Supernatants of tissue homogenates from infected (I) and uninfected (C) monkey fetuses. Virus titer of supernatants titrated on DEC monolayers; all uninfected tissues were negative for infectious virus. PFU, plaque-forming units.

Binding ratio as defined in text.

We feel that this procedure can be utilized in clinical laboratories for the rapid and specific identification of WEE virus and can be readily adapted to several other group A arboviruses.

**LITERATURE CITED**
