An in-house-developed enzyme-linked immunosorbent assay detected West Nile virus (WNV) immunoglobulin A (IgA) in 65 of 68 sera from WNV-infected patients; 40 of 63 WNV IgM-positive, IgG-negative serum or plasma specimens; 65 of 67 WNV IgM-positive, IgG-positive specimens; 0 of 70 WNV IgM-negative, IgG-negative specimens; and 0 of 64 archived blood donation sera. WNV IgA is thus highly prevalent among WNV-infected patients and typically appears after WNV IgM but before WNV IgG.

**TABLE 1. Intra-assay and interassay variation data for the WNV IgA capture ELISA**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intra-assay comparison</th>
<th>Interassay comparison*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive control</td>
<td>Negative control</td>
</tr>
<tr>
<td>No. of determinations</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Range of index values</td>
<td>6.24–7.31</td>
<td>0.66–0.86</td>
</tr>
<tr>
<td>Mean index</td>
<td>6.66</td>
<td>0.73</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.31</td>
<td>0.07</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>5%</td>
<td>10%</td>
</tr>
</tbody>
</table>

* Assays were performed over a 22-day period by using the same lot of IgA capture microtiter well plates.
The net absorbance value was calculated for all samples by subtracting the absorbance value for the well receiving sample diluent from the absorbance value for the well receiving WNV antigen. Results were expressed as an index, calculated using the following formula: index = sample net absorbance value/calibrator net absorbance value. The calibrator was formulated to reflect the mean net absorbance plus three standard deviations for 31 WNV IgM-negative, IgG-negative specimens collected during a period of WNV inactivity (February 2004). An index of ≥1.00 was considered to indicate positivity.

**WNV IgA assay precision.** Intra-assay and interassay variation data for the WNV IgA assay positive and negative controls are shown in Table 1. Coefficient of variation values were <10% for the positive control and <15% for the negative control. Two lots of IgA capture wells were compared by testing 12 specimens (six WNV IgA negative, six WNV IgA positive) in parallel. Percent variance was calculated for each specimen by using the following formula: percent variance = [(lot 2 index – lot 1 index)/lot 1 index] × 100. The mean variance was 0% (range, –14 to +18%).

**Evaluation of serum and plasma panels.** Five serum or plasma panels were tested using the newly developed WNV IgA capture ELISA. Panels 1 to 3 were presumed to represent recent WNV infection, and panels 4 and 5 were included to assess assay specificity. Results for all five panels are summarized in Table 2. Panel 1 included 68 sera collected at the height of the 2002 WNV season (July to September) and confirmed as positive for WNV antibodies by Public Health Service Laboratories by using the plaque reduction neutralization test (PRNT) (10); 96% (65 of 68) of these samples were positive for WNV IgA. Panel 2 comprised 63 WNV IgM-positive, IgG-negative serum or plasma specimens collected from August to September 2003; due to the strong agreement between WNV IgM detection and positive PRNT results for samples from 2002 (10), we did not solicit Public Health Service Laboratories for PRNT results for WNV IgM-positive samples from 2003. The WNV IgM-positive, IgG-negative profile indicates that panel 2 samples were collected early in the WNV infectious process (4). WNV IgA was detected in 63% (40 of 63) of panel 2 samples; most WNV IgA-positive samples (26 of 40; 65%) had relatively low WNV IgA indexes (1.00 to 10.00). These findings are consistent with very recent infection and suggest that WNV IgA appears soon after WNV IgM but before WNV IgG. Panel 3 included 67 WNV IgM-positive, IgG-positive serum or plasma specimens also collected from August to September 2003. WNV IgA was present in 97% (65 of 67) of these samples, and most (44 of 67; 66%) had markedly elevated WNV IgA indexes (>10.00). Panel 4, containing 70 WNV IgM-negative, IgG-negative serum or plasma specimens collected during August 2004, was designed for assessment of the likelihood of WNV IgA detection in the absence of WNV IgM and IgG during a period of high WNV activity. All panel 4 samples were WNV IgA negative, indicating that detection of WNV IgA in the absence of WNV IgM and IgG is unlikely. Panel 5 included 64 sera collected from American Red Cross blood donors from the Los Angeles, Calif., area in 1999, well before WNV arrived in California; all panel 5 samples were WNV IgA negative.

Systematic studies assessing cross-reactivity in the WNV IgA assay due to St. Louis encephalitis virus (SLEV) infection, the other major flavivirus infection endemic in the United States, were not possible due to the unavailability of sufficient numbers of well-characterized specimens. WNV IgA was not detected in four sera with a profile of past SLEV infection (SLEV PRNT titers at least fourfold higher than the WNV PRNT titer; SLEV IgG positive, IgM negative by indirect immunofluorescence). Further studies using samples from recently infected SLEV patients are required to assess the reactivity of SLEV IgA with WNV antigens.

These studies demonstrate that an alpha-capture ELISA effectively measures WNV IgA in human serum and plasma specimens. This sensitive and reproducible assay, along with previously described WNV IgG and IgM ELISA systems (3), is currently being used to characterize the timeline of WNV antibody production and persistence in WNV-infected individuals. Such data will help determine whether WNV IgA can serve as an effective laboratory marker for distinguishing recent from past WNV infection.

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**TABLE 2. WNV IgA results for various serum and plasma panels**

<table>
<thead>
<tr>
<th>Panel no.</th>
<th>Description of samples</th>
<th>n</th>
<th>No. (%) with WNV IgA index of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WNV Ig+ by PRNT; collected July to September 2002</td>
<td>68</td>
<td>1.00 (41) 4.01–5.00 (21) 5.01–10.00 (9) 10.01–20.00 (16) 20.01–30.00 (24)</td>
</tr>
<tr>
<td>2</td>
<td>WNV IgM+, IgG+; collected August to September 2003</td>
<td>63</td>
<td>2.00 (37) 18.01–20.00 (19) 8.01–10.00 (13) 4.01–6.00 (6) 10.01–12.00 (16)</td>
</tr>
<tr>
<td>3</td>
<td>WNV IgM+, IgG+; collected August to September 2003</td>
<td>67</td>
<td>2.00 (37) 18.01–20.00 (19) 8.01–10.00 (13) 4.01–6.00 (6) 10.01–12.00 (16)</td>
</tr>
<tr>
<td>4</td>
<td>WNV IgA+, IgG+; collected August 2004</td>
<td>70</td>
<td>2.00 (100) 0.01–2.00 (0) 2.01–4.00 (0) 4.01–6.00 (0) 6.01–10.00 (0)</td>
</tr>
<tr>
<td>5</td>
<td>Los Angeles blood donor sera collected in 1999</td>
<td>64</td>
<td>2.00 (100) 0.01–2.00 (0) 2.01–4.00 (0) 4.01–6.00 (0) 6.01–10.00 (0)</td>
</tr>
</tbody>
</table>

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