Frequency of missed cases of probable acute West Nile virus (WNV) infection when testing for WNV RNA alone or WNV Immunoglobulin M alone

Harry E. Prince*, Jan Calma, Tiffany Pham, and Brent L. Seaton.
Focus Diagnostics, Inc., Cypress, CA 90630

*Corresponding author:
Focus Diagnostics, Inc.
5785 Corporate Avenue
Cypress, CA 90630
Telephone: 714 822-2457
FAX: 714 821-3364
E-mail: hprince@focusdx.com

Running title: WNV RNA and IgM detection
To estimate the frequency of missed cases of acute West Nile virus (WNV) infection if only WNV RNA or Immunoglobulin M (IgM) testing is requested, we measured IgM in specimens negative for RNA, and vice versa. Whereas 6/110 (5.5%) RNA-negative sera were IgM-positive, only 3/299 (1.0%) IgM-negative sera were RNA-positive (P<0.05). Similarly, 11/141 (7.8%) RNA-negative cerebrospinal fluids (CSF) were IgM-positive, but 0/118 (0%) IgM-negative CSF were RNA-positive (P<0.05). WNV infections may be missed if only RNA or IgM testing is requested, with a higher frequency of missed cases if only RNA testing is requested.

Acute West Nile virus (WNV) infection remains a serious public health issue in the United States, with >1300 cases reported to the Centers for Disease Control and Prevention (CDC) in 2008 (2). As recommended by the CDC (3), WNV IgM detection in serum or cerebrospinal fluid (CSF) is the major laboratory tool used to identify symptomatic individuals with acute WNV infection; the vast majority of these individuals are positive for WNV IgM at the time they first seek medical attention (4, 10). In addition, WNV RNA detection has emerged as another useful laboratory tool for identifying patients with acute WNV infection; although of limited utility due to the short viremic phase and low viral load (6, 7), the RNA assay may be the only test with a positive result among WNV-infected patients seeking medical attention very soon after symptom onset (6, 11). Individuals presenting with acute WNV infection may thus be
positive for WNV IgM and RNA, WNV IgM only, or WNV RNA only. This finding raises concerns about the frequency of missed cases of acute WNV infection if only one of these tests is requested, and the result is negative; in this situation, WNV infection may be incorrectly ruled out. We therefore sought to estimate the frequency of missed probable WNV cases if only WNV IgM testing or only WNV RNA testing is requested.

The serum and CSF specimens utilized for this study were submitted to our facility by other laboratories for WNV RNA or WNV IgM testing during the 2008 North American WNV season; clinical information (e.g., time since symptom onset) was not provided for any of the samples. Specimens included 110 sera and 141 CSF samples submitted for RNA testing and found to be RNA-negative, as well as 299 sera and 118 CSF samples submitted for IgM testing and found to be IgM-negative. After the requested test was performed, specimens were de-identified and stored at ≤-20°C up to 2 weeks before further testing was performed.

WNV IgM was assayed using enzyme-linked immunosorbent assay (ELISA) kits manufactured by Focus Diagnostics (5, 8), per the manufacturer’s instructions. This kit is FDA-cleared for testing serum specimens only; in-house studies validated the kit for CSF testing (9). Index values >1.1 were considered positive.

Nucleic acid extraction was performed using the MagNA Pure™ Total Nucleic Acid Isolation kit (Roche Applied Science, Indianapolis, IN) on the MagNA Pure LC (Roche Applied Science) automated extraction platform. A starting specimen volume of 200 uL
was extracted and eluted into a final volume of 50 μL. All eluates were assayed using 10 μL of extracted DNA/RNA as template. TaqMan® real-time reverse transcription-PCR (6) was used to amplify and detect a 121 nucleotide sequence of the WNV genome that flanks the NS1 and NS2a genes.

Our findings are summarized in Table 1. Of 110 serum samples submitted for WNV RNA testing and found to be RNA-negative, 6 (5.5%) were positive for WNV IgM. In contrast, of 299 serum samples submitted for WNV IgM testing and found to be IgM-negative, only 3 (1.0%) were positive for WNV RNA. This difference in proportions was statistically significant, with a P-value of 0.019 (significance defined as P<0.05). Similar findings were observed for CSF; 11 of 141 (7.8%) CSF samples submitted for RNA testing and found to be RNA-negative were positive for WNV IgM, whereas 0 of 118 (0.0%) CSF samples submitted for IgM testing and found to be IgM-negative were positive for WNV RNA (P=0.005).

These results demonstrate that probable cases of acute WNV infection may be missed if either WNV RNA testing alone or WNV IgM testing alone is requested. Further, the likelihood of missing acute WNV cases is higher if only RNA testing is requested, particularly for CSF. These findings are consistent with our understanding of the timelines for WNV viremia and antibody production (1, 4, 7, 10). Serum levels of WNV RNA typically peak before symptoms appear and then rapidly decline over several days as antibody production begins (1). By the time patients seek medical attention, RNA levels are often below detectable levels, whereas IgM (and often IgG) are present at
easily-detectable levels (4, 7, 10). However, some patients, particularly those seeking medical attention within a week of symptom onset, may still be in the RNA-positive/antibody-negative window (11). Thus, to avoid missing cases of acute WNV infection, it may be appropriate to request both WNV RNA testing and WNV IgM testing, depending on the history the patient relates at presentation and the specimen type(s) selected for testing.

References


Table 1. Frequency of detection of WNV RNA or IgM in samples submitted for measurement of the other analyte

<table>
<thead>
<tr>
<th>Sample description</th>
<th>N</th>
<th>Additional analyte assayed</th>
<th># positive for additional analyte (% of N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum submitted for, and negative for, WNV RNA</td>
<td>110</td>
<td>WNV IgM</td>
<td>6 (5.5)</td>
</tr>
<tr>
<td>Serum submitted for, and negative for, WNV IgM</td>
<td>299</td>
<td>WNV RNA</td>
<td>3 (1.0)*</td>
</tr>
<tr>
<td>CSF submitted for, and negative for, WNV RNA</td>
<td>141</td>
<td>WNV IgM</td>
<td>11 (7.8)</td>
</tr>
<tr>
<td>CSF submitted for, and negative for, WNV IgM</td>
<td>118</td>
<td>WNV RNA</td>
<td>0 (0.0)*</td>
</tr>
</tbody>
</table>

*The percentage is significantly different from the percentage immediately above it in the table (P<0.05, comparison of proportions as determined using Medcalc® software).