Overview

Useful For
Rapid testing for West Nile virus (WNV) RNA
As an adjunct in the diagnosis of early WNV virus infection

Testing Algorithm
See Mosquito-borne Disease Laboratory Testing in Special Instructions.

Special Instructions
- Mosquito-borne Disease Laboratory Testing

Method Name
Real-Time Polymerase Chain Reaction (PCR)/RNA Probe Hybridization

NY State Available
Yes

Specimen

Specimen Type
Plasma EDTA

Specimen Required
Collection Container/Tube: Lavender top (EDTA)
Submission Container/Tube: Screw-capped, sterile container
Specimen Volume: 1 mL

Collection Instructions: Spin down promptly

Forms
If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume
0.3 mL

Reject Due To
| Gross hemolysis | Reject |

Specimen Stability Information

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<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma EDTA</td>
<td>Refrigerated (preferred)</td>
<td>7 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>7 days</td>
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</table>
Clinical and Interpretive

Clinical Information

West Nile virus (WNV) is a mosquito-borne flavivirus (single stranded RNA virus) that primarily infects birds, but occasionally infects horses and humans. Until the virus infection was recognized in 1999 in birds in New York City, WNV was found only in the Eastern hemisphere, with a wide distribution in Africa, Asia, the Middle East, and Europe.(1-3) Most people who are infected with WNV do not experience symptoms. It is estimated that about 20% of those who become infected will develop West Nile fever with mild symptoms including headache, myalgia, and occasionally a skin rash on the trunk of the body. About 1 of 150 WNV infections (<1%) result in meningitis or encephalitis. Case fatality rates among patients hospitalized during recent outbreaks have ranged from 4% to 14%. Advanced age is the most important risk factor for death, and patients older than 70 years of age are at particularly high risk.

Laboratory diagnosis is best achieved by demonstration of specific IgG and IgM class antibodies in serum or cerebrospinal fluid (CSF) specimens (WNV / West Nile Virus [WNV] Antibody, IgG and IgM, Serum or WNVC / West Nile Virus [WNV] Antibody, IgG and IgM, Spinal Fluid).

The specific identification of WNV by detection of IgM in CSF is the recommended test to document central nervous system disease, but this test may be falsely negative in CSF collected <8 days after the onset of symptoms. Alternatively, experiences in nucleic acid testing for WNV RNA in blood prior to transfusion have indicated that PCR can detect viremic target RNA from patients with known West Nile infection when specific antibodies to the virus are not present (ie, from 2-8 days after onset of symptoms).(4,5)

Reference Values

Negative

Interpretation

The likelihood of detection of West Nile virus RNA by PCR is relatively low. In cerebrospinal fluid, the clinical sensitivity is approximately 55%, and in blood, about 10%. Specificity of the assay in either matrix is approximately 100%.(6)

Cautions

This assay should not be used for screening asymptomatic individuals, and should only be used to test patients with signs and symptoms of West Nile virus (WNV) disease.

The sensitivity of the assay is very dependent upon the quality of the specimen submitted.

A negative test does not exclude infection with WNV. Therefore, the results obtained should be used in conjunction with clinical findings to make an accurate diagnosis.

This assay detects both viable and nonviable virus. Test performance depends on viral load in the specimen and may not correlate with cell culture performed on the same specimen.

Possible cross-reactivity with other flaviviruses (eg, Dengue virus, St. Louis encephalitis virus, and Japanese encephalitis virus) may occur.

Supportive Data

The following validation data supports the use of this assay for clinical testing.
Accuracy/Diagnostic Sensitivity and Specificity:

To determine the ability of the assay to detect RNA from clinical specimens, 30 negative whole blood, serum, plasma, and cerebrospinal fluid (CSF) samples (120 total) were spiked with West Nile virus (WNV)-positive control material at the limit of detection (approximately 10 targets/microliter). The specimens were run in a blinded manner along with 30 negative (nonspiked) specimens for each matrix. 100% of the specimens were positive and 100% of the nonspared specimens were negative.

To supplement the above data, blinded proficiency panels were tested with this assay and demonstrated 100% concordance. In addition, 8 patient samples that were positive for WNV RNA by this assay were tested with an alternative reference lab assay. Results showed 75% concordance.

Analytical Sensitivity/Limit of Detection (LoD):

The LoD of this assay is approximately 10 targets/microliter.

Precision:

Interassay precision is 100% and intraassay precision is 100%.

Reference Range:

Fifty CSF samples from normal donors were tested and found to be negative for targeted WNV RNA.

Reportable Range:

This is a qualitative assay and the results are reported as either negative or positive for targeted WNV.

Clinical Reference

Performance

Method Description

This LightCycler PCR assay has been optimized to detect common conserved sequences in the nonstructural protein of West Nile virus (WNV). Viral nucleic acid is extracted by the MagNA Pure automated instrument (Roche Applied Science) from cerebrospinal fluid, or plasma. Primers directed to the nonstructural protein amplifies a specific sequence of the virus. For the test, WNV genomic RNA is transcribed to cDNA. The LightCycler instrument amplifies
and monitors the development of target nucleic acid sequences after the annealing step during PCR cycling by fluorescence assay. This automated PCR system utilizes stringent air-controlled temperature cycling and capillary cuvettes to rapidly detect (30-40 minutes) amplicon development. The detection of amplified products is based on the fluorescence resonance energy transfer (FRET) principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3'-end is excited by an external light source and emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5'-end. The acceptor fluorophore then emits a light of a different wavelength that can be measured with a signal that is proportional to the amount of specific PCR product. Analysis of the PCR amplification and probe melting curves are accomplished through the use of LightCycler software. (Cockerill FR III, Uhl JR: Applications and challenges of real-time PCR for the clinical microbiology laboratory. In Rapid Cycle Real-Time PCR Methods and Applications. Edited by U Reischel, C Wittwer, F Cockerill. Berlin, Germany, Springer-Verlag; 2002, pp 3-30)

PDF Report
No

Day(s) and Time(s) Test Performed
Monday through Saturday; Continuously 7 a.m.-8 p.m. (June through November)
Monday, Wednesday, Friday; 6 a.m. (December through May)

Analytic Time
Monday through Thursday: 2 days Friday, Saturday: 3 days

Maximum Laboratory Time
5 days

Specimen Retention Time
1 week

Performing Laboratory Location
Rochester

Fees and Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
87798

LOINC® Information

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<td>West Nile Virus PCR, P</td>
<td>34892-0</td>
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