

Overview

Useful For

Rapid testing for West Nile virus (WNV) RNA

An adjunctive test to serology for detection of early WNV infection

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.

Testing Algorithm

The following algorithms are available in Special Instructions:

[-Meningitis/Encephalitis Panel Algorithm](#)

[-Mosquito-borne Disease Laboratory Testing](#)

Special Instructions

- [Meningitis/Encephalitis Panel Algorithm](#)
- [Mosquito-borne Disease Laboratory Testing](#)

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

CSF

Specimen Required

Container/Tube: Sterile vial

Specimen Volume: 0.5 mL

Collection Instructions: Do not centrifuge.

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

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
CSF	Refrigerated (preferred)	7 days	
	Frozen	7 days	

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 had been detected only in the Eastern hemisphere, with a wide distribution in Africa, Asia, the Middle East, and Europe. 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 specimens. PCR testing can detect WNV RNA in plasma specimens from patients with recent WNV infection (ie, 3-5 days following infection) when specific antibodies to the virus are not yet present. However, the likelihood of detection is relatively low as the sensitivity of PCR detection is approximately 55% in cerebrospinal fluid and approximately 10% in blood from patients with known WNV infection.

Reference Values

Negative

Interpretation

A positive result indicates the presence of West Nile virus (WNV) RNA and is consistent with early WNV infection.

Cautions

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 and serologic test results 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, 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 specimens per specimen type (120 total: whole blood, serum, plasma, and CSF specimens) were spiked with whole West Nile virus (WNV) near 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. All spiked specimens (100%) were positive and 100% of the nonspiked 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 West Nile virus RNA by this assay were tested with an alternative reference laboratory assay. Results showed 75% concordance.

Analytical Sensitivity/Limit of Detection (LoD):

The lower LoD of this assay is less than 10 targets/microliter.

Precision:

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

Reference Range:

A total of 50 CSF specimens 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

1. Petersen LR, Brault AC, Nasci RS: West Nile Virus: Review of the Literature. JAMA 2013;310(3):308-315
2. Busch MP, Tobler LH, Saldanha J, et al: Analytical and clinical sensitivity of West Nile virus RNA screening and supplemental assays available in 2003. Transfusion 2005;5(4):492-499
3. New York City Department of Health. West Nile surveillance and control: an update for healthcare providers in New York City. City Health Information. June 2001;20(2)

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, or plasma. Primers directed to the nonstructural protein amplify 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, pp3-30)

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

Test ID	Test Order Name	Order LOINC Value
LCWNV	West Nile Virus PCR	34461-4

Result ID	Test Result Name	Result LOINC Value
SS013	Specimen Source	31208-2
86197	West Nile Virus PCR	34461-4