

West Nile Virus Laboratory Testing at the California Department of Health Services' Viral and Rickettsial Disease Laboratory

Laboratory diagnosis of human West Nile virus (WNV) infection is a multi-step process. In some cases, physicians send specimens to private commercial laboratories for WNV diagnostic testing. More commonly, specimens are sent to the local or state health department for diagnostic laboratory testing.

The testing available at the California State Health Department includes:

Serologic tests

Enzyme Immunoassay (EIA) testing: The immunoglobulin M (IgM) EIA is the frontline test for WNV diagnosis. The EIA is the ideal test because it is both simple and sensitive (i.e., highly likely to find true-positives) and it can be used with both serum and cerebrospinal fluid (CSF) specimens. EIA testing can be completed in 1 to 2 days from the time samples arrive at the laboratory. Generally several specimens are done on each EIA run.

The immunoglobulin G (IgG) EIA test is used as an adjunct test—it cannot differentiate between old vs. new infection.

Immunofluorescence Assay (IFA) testing: IFA tests for WNV can also test for the IgM and IgG antibody. The advantages of these tests are that they are rapid and amenable to just a few samples.

Molecular tests

Molecular methods for WNV testing can be used as an adjunct to the serologic tests. For diagnosis of clinical disease, serological tests are more accurate than molecular tests. Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) is a process that uses nucleic acid amplification techniques. While these tests can be useful in diagnosis, they have low sensitivity for a variety of reasons for WNV, making them inappropriate as the sole test for laboratory diagnostic testing of possible human WNV infections. An advantage of this method is the relatively rapid turn around time. RT-PCRs may be useful for immunocompromised individuals that have a delay in antibody response and prolonged viremia. Additionally, VRDL uses molecular methods to rule out Enterovirus.

Confirmation of results

Plaque reduction neutralization test (PRNT)

Once the State has an initial positive result, further testing may be done to confirm that the infection detected is West Nile. WNV is a flavivirus, which can be problematic as far as cross-reactivity. The flaviviruses include St. Louis encephalitis (SLE) and Japanese encephalitis (JE) viruses, both of which are closely related to West Nile, yellow fever (YF) and dengue (DEN) viruses. People who have been recently vaccinated for JE or YF, or who have a recent exposure to JE, YF, SLE, or DEN viruses may have a positive MAC-ELISA for WNV, even though they have not actually been exposed to WNV.

Additional laboratory testing may be required to rule out the false-positive reactions that result from an exposure to a related flavivirus. The PRNT is the most specific test available for distinguishing between and among the arthropod-borne flaviviruses. Because exposure to other flaviviruses is possible in many areas of WNV activity, initial IgM-capture Enzyme Linked Immunosorbant Assays (MAC-ELISA) positive results should be confirmed by PRNT. As discussed earlier, the PRNT usually takes up to 8 days if testing for both WNV and SLE viruses is required. The process may take even longer if testing with YF or Dengue viruses is necessary. This additional testing (e.g., the PRNT) may require growth of

the virus and may take a week or more (plus shipping time) to conduct. Results from the PRNT are often needed before CDC considers a human WNV infection to be confirmed.

Tests in development

The VRDL is in the process of developing tests for more rapid confirmation of West Nile test—e.g. Western Blot. Additionally, VRDL is hoping to provide avidity testing in order to ascertain the binding strength of antibodies.

Reference: West Nile Virus in California: Guidelines for Human Testing and Surveillance, California Department of Health Services, April 2005.