

## Laboratory Diagnosis of West Nile Virus Encephalitis Virus Infection

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West Nile Virus (WNV) is a member of the flavivirus genus and belongs to the Japanese encephalitis antigenic complex, which includes other neurotropic viruses such as Murray Valley encephalitis virus (MVEV), St. Louis encephalitis virus (SLEV) and Usutu virus (USUV). WNV is an arbovirus transmitted in a zoonotic cycle between birds and ornithophilic mosquitoes, primarily of the *Culex* species. Horses and humans can also be incidentally infected. The majority of WNV infections are asymptomatic or cause mild illness. However, WNV can infect the central nervous system and cause a neurological disease that is usually severe.

### Type of samples and laboratory procedures

Handling of suspected samples requires containment at Biosafety Level 2 (BSL2); however, for purposes of viral isolation and viral cultures, BSL3 containment is necessary.

#### ***Molecular diagnosis:***

Specimen type: serum or cerebrospinal fluid (CSF)(5 to 7 cc in dry tube) taken during the first 3 days after the onset of symptoms.

Although molecular methods such as the Polymerase Chain Reaction (PCR) are very sensitive and can be used for laboratory diagnosis, the performance of these techniques may be limited for the confirmation of cases of neuroinvasive disease associated with WNV, due to the low-level, transient viremia present at the time of clinical presentation (in immunocompetent patients).

**For this reason, a negative PCR result does not rule out WNV infection.**

#### ***Serological diagnosis (detection of antibodies):***

Specimen type: serum or CSF (5 to 7 cc in dry tube) taken in the acute phase (ideally 1-10 days after onset of symptom) .

**CSF is the preferred sample to collect because antibody in the CSF confirms the virus has infected the nervous system. Antibody in the serum only, could simply reflect recent coincidental asymptomatic WNV infection.**

The detection of WNV-specific IgM antibodies demonstrates recent infection and the IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) can be used. Antibodies are detected generally by the 3rd day after clinical onset and may persist for more than 90 days.

Considering that the detection of antibodies in a serum from the acute phase is presumptive, it is recommended that a second sample be obtained between one and two weeks after the first sample to demonstrate seroconversion (negative to positive) or increase up to four times the titer of antibodies (quantitative assay).

**The level of antibodies during the acute phase of the disease is very low and IgM may not be detected. Therefore, a negative result in a sample taken within the first 8 days of symptom onset does not rule out WNV infection.**

## Interpretation of serology results

Cross-reactivity in secondary flavivirus infections should be considered in areas where co-circulation of other flaviviruses (St. Louis encephalitis, yellow fever, Zika virus, dengue, and others from the Japanese encephalitis complex) has been documented and the population has likely been previously infected.

A positive result for WNV IgM may indicate previous infection by other related flaviviruses. Therefore, carrying out parallel detection by MAC-ELISA to other flaviviruses and carefully interpreting the results, taking into account available clinical and epidemiological information, is recommended.

In general, the plaque reduction neutralization test (PRNT) technique offers greater specificity for the detection of flavivirus neutralizing antibodies (IgG), but is useful only in primary infections in paired samples or against several flaviviruses. Cross-reactivity in secondary flavivirus infections has also been documented for the neutralization assays; therefore, specific confirmation may be limited in these cases.

## Sample Storage

- Keep refrigerated (2 - 8 °C) if samples will be processed (or sent to a reference laboratory) within 48 hours.
- Keep frozen (-10 to -20 °C) if sample will be processed after the first 48 hours or for a period of no more than 7 days.
- Keep frozen (-70 °C) if sample will be stored for more than a week before processing. Sample storage at -70 °C can be adequately preserved for extended periods of time.
- Avoid multiple freeze-thaw cycles.

## Shipping of clinical specimen by air to the reference laboratory

- Use dry ice (if possible) or refrigerant gels to guarantee cold chain is maintained. Always use triple packaging.
- Send within the first 48 hours.
- The original samples must be packaged, appropriately labeled (if dry ice is used), and documented as **category B**.
- The complete clinical and epidemiological record should accompany the shipment.