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CDC HEALTH ADVISORY

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West Nile Virus

There have been numerous questions about the process of testing and diagnosis of West Nile virus. The information below is intended as a resource for public health agencies, public information officers, and others who respond to specific questions that have been asked by the general public and news media. This information also will be made available through the CDC website at

http://www.cdc.gov/ncidod/dvbid/westnile/index.htm

Testing in 2002

Laboratory diagnosis of human WN virus infections 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 state (or local, as in the case of the cities and counties noted above) health department for diagnostic laboratory testing. Frequently, health departments will-in their own facilities-repeat the initial testing performed in the private commercial laboratory before reporting suspect human WNV cases to the Arbonet Surveillance System.

Currently the CDC laboratories function primarily to assist states in confirmation of cases when the states request such support from a reference laboratory. This usually occurs when:

- the state finds its initial case(s) of human WNV illness,
- the state results are not definitive due to equivocal laboratory testing results or insufficient specimens, or
- the patient might have been exposed to other closely related viruses - such as St. Louis encephalitis (SLE) virus - which may result in a "false" positive laboratory test for WNV.

This additional testing (*e.g.,* the plaque-reduction neutralization test, PRNT) may require growth of the virus and may take a week or longer (plus shipping time) to conduct. Results from the PRNT are often needed before CDC considers a human WNV infection to be confirmed.

Why laboratory diagnosis of WNV is critical

The clinical presentation of most patients with viral encephalitis is similar regardless of the cause. Infection by WN and SLE viruses, as well as many other encephalitic arboviruses usually does not result in clinical disease and may even be asymptomatic. A definitive diagnosis of WNV infection can only be made through appropriate laboratory testing. Additionally, appropriate diagnostic laboratory testing is critical for active human case surveillance.

Requirements for laboratories conducting WNV

Both WN and SLE viruses are classified as Biosafety Level 3 (BSL-3) agents, requiring that laboratory investigations involving handling of infectious virus be performed in either BSL-3 containment or BSL-2 containment using BSL-3 lab practices. The requirements for BSL-2 and BSL-3 containment and practices can be found in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual. Laboratories with appropriate biocontainment can conduct all routine diagnostic procedures, (*e.g.*, viral propagation for agent identification, typing, susceptibility testing etc.)

Testing procedures for human samples

Patient Information and index of suspicion: All specimens submitted for diagnostic testing from cases that are clinically compatible with viral encephalitis must be accompanied by appropriate patient information including date of onset of symptoms, date of sample acquisition, and the patient's travel and vaccination history.

Antibody tests: The frontline test for WNV diagnosis is the IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA). The MAC-ELISA 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. MAC-ELISA testing can be completed in 1 to 2 days from the time samples arrive at the laboratory. The CSF specimen is the preferred specimen for rapid diagnosis of human WNV infection using MAC-ELISA. A second test useful in identifying possible WNV infections is the IgG ELISA.

Virus detection: There are three approaches that can be used to detect virus in a clinical specimen: virus isolation in cell culture or laboratory animals, detection of viral proteins in antigen-detection assays, or detection of viral genomes in nucleic acid amplification tests using some variety of reverse transcriptase-polymerase chain reaction (RT-PCR) protocol. While these tests can be useful in diagnosis, they have low sensitivity for a variety of reasons (i.e., they are less likely to identify true-positives) for WNV, making them inappropriate as the sole test for laboratory diagnostic testing of possible human WNV infections.

Antibody cross-reaction among flavivirus: WNV is a flavivirus. Other flaviviruses include SLE and Japanese encephalitis (JE) viruses, both of which are closely related to WNV, and 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 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 DEN viruses is necessary.

Acute/convalescent samples: Antibodies from a previous infection with WNV may persist for years. This means that it may be necessary to get both acute- and convalescent-phase specimens from a patient living in an area where the virus is endemic in order to confirm the cause of the person's acute infection. Without testing paired samples, a positive test may actually be the result of an infection from a previous year. In these instances, the PRNT is used to identify the infecting virus by looking for a four-fold or greater rise in antibody titer between paired (acute- and convalescent-phase) serum specimens from the same individual.

Antigen detection assays: Assays to detect the presence of WNV or SLE virus antigen in clinical specimens are available. These tests were first developed in 96-well microplate format, but have since been adapted to a dipstick format. The dipstick

tests have only been shown to be practical and useful when testing either bird or mosquito tissues. This is due to the lower sensitivity (less ability to detect true positives) of the antigen-detection test when compared to RT-PCR assays.

For More Information about laboratory testing:

Please refer to "Epidemic/Epizootic West Nile Virus in the United States: Revised Guidelines for Surveillance, Prevention and Control," April 2001, http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-apr-2001.pdf

Petersen LR and Marfin AA, "West Nile Virus: A Primer for the Clinician [Review]" *Annals of Internal Medicine* (August 6) 2002:137:173-9.
http://www.annals.org/issues/v137n3/pdf/200208060-00009.pdf