

Division of Consolidated Laboratory Services (DCLS) - Arbovirus Testing

Testing Criteria for Encephalitis: Any adult or pediatric patient admitted to a hospital with a presumed diagnosis of viral encephalitis, or with focal CNS findings and fever should submit a whole blood or serum sample for diagnostic testing at DCLS following the guidelines listed below.

Recommended Criteria for Suspect Cases of WNV Infection:

Neuroinvasive disease requires presence of fever and at least one of the following, as documented by a physician and in the absence of a more likely clinical explanation: Acutely altered mental status (e.g., disorientation, obtundation, stupor, or coma), or Other acute signs of central or peripheral neurologic dysfunction (e.g., paresis or paralysis, nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, or abnormal movements), or Pleocytosis (increased white blood cell concentration in cerebrospinal fluid [CSF]) associated with illness clinically compatible with meningitis (e.g., headaches or stiff neck).

Non-neuroinvasive disease (West Nile Fever) requires, at minimum, the presence of documented fever, measured by the patient or clinician, the absence of neuroinvasive disease (above), and the absence of a more likely clinical explanation for the illness. Involvement of non-neurological organs (e.g., heart, pancreas, liver) should be documented using standard clinico-laboratory criteria.

Testing Systems Employed: Serological specimens submitted for arbovirus testing will be evaluated for West Nile virus (WNV) and St. Louis encephalitis virus (SLE) IgM specific antibody using a Microsphere Immuno Assay (IgM MIA). IgM Antibody Capture Enzyme-linked Immunosorbent Assay (MAC-ELISA) will be used to detect IgM specific for Eastern Equine Encephalomyelitis virus (EEE) and LaCrosse Encephalitis virus (LAC). IgG ELISA will be used on all IgM positive specimens to detect virus specific neutralizing antibody. Plaque Reduction Neutralization Testing (PRNT) will be performed at DCLS or the specimen will be submitted to the CDC for confirmation. Real-time RT-PCR will be performed on biopsy and postmortem tissue specimens. Viral isolation is available upon request.

Specimen Types and Amounts:

Specimen	Test	Specimen volume	Shipment
Paired acute-phase (0-8 days post-onset of illness) and convalescent-phase (14-21 days after acute specimen) sera	IgM MIA MAC-ELISA IgG ELISA	2 ml sera in plastic tube (preferred) or 5 ml whole coagulated blood	Refrigerated
Acute-phase CSF	IgM MIA MAC-ELISA Viral Isolation Real-time RT-PCR	1.0 ml in plastic tube	Refrigerated
Brain, biopsy or postmortem tissue	Viral Isolation Real-time RT-PCR	1 gram	Fresh Frozen

Submission Form: Include an Arboviral Infection Case Report Form for each patient to be tested. Fill out the form as completely as possible. Accurate interpretation of serologic findings requires knowledge of the specimen. It is imperative that the following data accompany specimens submitted: 1) symptom onset date; 2) date of sample collection; 3) unusual immunological status of patient; 4) current address and travel history; 5) history of prior vaccination against flavivirus disease; and 6) brief clinical summary including suspected diagnosis.

Packaging for Shipment:

Serum, whole coagulated blood samples, and CSF: If you are requesting serology, the specimen should be kept cool but there is no need to keep it frozen. Wrap your specimen in absorbent material, pack it in a sealable plastic bag, place the bag in a rigid container (styrofoam box or cardboard mailing container), place the container and a cold pack in a styrofoam cooler for shipment. **Tissue:** If you are requesting virus isolation or real-time RT-PCR for WNV on tissue, the specimen should be frozen in a sterile container prior to shipment and it should be sent on enough dry ice to insure it will remain frozen until receipt. These specimens should be wrapped in absorbent material, packed in a sealable plastic bag, placed in a second sealable bag, and then placed in a cooler with dry ice for shipment. Coolers should be shipped 24 hour/overnight delivery.

Sample Shipment: Package specimens as indicated above and send to DCLS using the following address:

Non-Courier Shipment	Courier Shipment
Division of Consolidated Laboratory Services 600 North 5th Street Richmond, Virginia 23219-3691	Package specimens as indicated above and send via DCLS courier.

Reporting of Test Results: Test results are normally available 8 days after the receipt of specimens. During periods of heavy submission, turn around times may be longer. Hard copy results will be sent to the submitter, LHD, and DEE. If initial screening tests are positive, results will be communicated to DEE prior to the availability of confirmatory test results. DEE will notify the appropriate District Health Department who will in turn assure that the patient and his/her physician are notified.

Please contact the DCLS Virology/Immunology Group Manager (804-648-4480 Ext. 270) if you have questions regarding sample collection or shipment.