

**Specimen Requirements for West Nile Virus (WNV) Central Nervous System  
Disease by Polymerase Chain Reaction (PCR)**

- Methodology:** Real time (rti) Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)
- Performed:** Real time (rti) Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is the method used to detect West Nile Viral RNA from human and animal specimens. The sensitivity of the test is less than one (0.1) plaque forming unit (pfu) per ml. A positive detection by rti RT-PCR is considered presumptive positive until confirmed by running newly extracted RNA from the same specimen using a second set of primers.
- Only specimens meeting the case definition established by the Centers for Disease Control & Prevention (CDC) and the Disease Investigation Branch (DIB) of the Disease Outbreak and Control Division (DOCD) of the Department of Health will be tested.**
- Turn-Around-Time:** Results are reported 2-3 business days after specimen receipt and approval. Positive specimens will be sent to the Center for Disease Control and Prevention (CDC) for verification and final confirmation.
- Specimen required:** Cerebro-spinal fluid (CSF) collected within the first two (2) days from onset of clinical signs and symptoms.
- A minimum of one (1) ml of CSF is needed for PCR testing.
- Specimen storage, packing and transport:** Ship specimens with cold packs to keep the specimens at 4°C. If storage/transport will exceed 48 hours, freeze specimens at  $\leq -70^{\circ}\text{C}$  and ship on dry ice. Follow packing and shipping instructions of the U.S. Department of Transportation (U.S. DOT), and the International Air Transport Association (IATA) for Biological Substance, Category B.
- Specimen submission:** Submitters: Clinical Laboratories and the Disease Investigation Branch (DIB)

**Note:** It is the responsibility of the submitter to track the arrival of the specimens along with Form 81.3 and the West Nile Virus Initial Case and Lab Submission Form at the State Laboratories Division to ensure that these specimens are received at the respective testing laboratories.

**Unacceptable conditions:**

- Specimen is received in a container that is leaking. Specimen **will not be processed** if the safety of the laboratory worker is compromised;
- Specimen is not collected in a proper container or special handling instruction is not followed. Submitter will be asked to submit a repeat specimen. The quality of the test will be compromised if the specimen is not rejected;
- Specimen is not stored properly (should be at 4°C or packed in blue ice) in transit to the lab. Submitter will be asked to re-submit a repeat specimen;
- Specimen quantity is insufficient to perform the tests. Submitters will be notified to re-submit another specimen. If this is not possible, the specimen will be processed but the problem will be stated in the laboratory report.
- Unlabeled specimens;
- Incomplete specimen labeling and documentation;
- Specimen label does not match the requisition.

**Stability:**

All specimens must be refrigerated at 2-8°C immediately after collection. If storage/transport will exceed 48 hours, freeze specimens at  $\leq -70^{\circ}\text{C}$  and ship on dry ice. Avoid freezing and thawing specimens.

**Requisition Form:**

Each specimen submitted must have a completed Form 81.3. (<http://health.hawaii.gov/statelab/files/2013/05/sld-sld81-3.pdf>) and the West Nile Virus Initial Case & Lab Submission Form (<http://health.hawaii.gov/statelab/files/2013/05/sld-wnv-ics.pdf>) Submitter is responsible for completing both forms (including but not limited to the following information: patient identifier, date of onset of illness, signs and symptoms, travel history, immunization history, name and address of submitter).

Requisition forms shall be placed in a separate bag and shall not be packed with the specimen(s).

**Normal Value:** No West Nile Virus nucleic acid detected.

**Result Notification:** Laboratory results are reported to the submitters by electronic reporting system or via FAX. Laboratory reports for the Disease Investigation Branch (DIB) of the DOH Disease Outbreak Control Division (DOCD) will be posted to the DOCD SharePoint.

**Test performed at:** Biological Response Section  
Laboratory Preparedness and Response Program  
State Laboratories Division  
Department of Health  
2725 Waimano Home Road  
Pearl City, Hawaii 96782

**Contact:** Rebecca H. Sciulli, M.Sc., M.T. (AMT)  
808-368-3373; 453-5990  
or  
Remedios Gose  
808-453-5984

**Approved by:**



\_\_\_\_\_  
A. Christian Whelen, Ph.D.  
Administrator, State Laboratories Division



\_\_\_\_\_  
Date