



**Infectious Disease Epidemiology Section**  
**Office of Public Health, Louisiana Dept of Health & Hospitals**  
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**[www.oph.dhh.state.la.us](http://www.oph.dhh.state.la.us)**

# WEST NILE INFECTION

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## Epidemiology

West Nile virus (WNV) is a flavivirus belonging taxonomically to the Japanese encephalitis subgroup that includes the serologically closely related Saint Louis Encephalitis (SLE) virus, Japanese Encephalitis virus, Murray Valley encephalitis virus, and others. These viruses are commonly infecting birds in nature. Yellow Fever and Dengue viruses are also in the flavivirus group

The WNV infection is naturally spread from bird to bird by mosquito bites. Most reported WNV in the wild has occurred in crows but 150 species of birds have been found positive for WNV. The species more apt to be found positive among dead birds are crows, blue jays, grackles, house sparrows, cardinals, birds of prey and seagulls.

Like SLE virus, WNV is transmitted principally by Culex species mosquitoes, but may be transmitted by Aedes, Anopheles, and other species. Most of these are not very competent vectors.

In Louisiana, Culex quinquefasciatus also named the Southern House Mosquito is the main vector of WNV. The females lay single raft of 140-340 eggs on heavily polluted small water collection after each blood meal. The eggs hatch in 1-2 days and become adults in 8-12 days. Preferred breeding places are all types of large man-made containers, collections of ground water, storm sewer catch basin, ground pools, ditches, run off from sewage plants, small artificial containers, cesspits, drains, septic tanks, unused wells, storm water canals. The flying range of adult female Culex is limited, up to 3,600 feet (1,200m) /night. They prefer feeding on birds and poultry, however they also readily bite humans. They usually bite humans towards the middle of the night indoors and outdoors.

The role of other potential vectors such as Aedes albopictus also named the Asian Tiger Mosquito and other Culex is still to be determined. A lesser vector is Culex salinarius which lives mostly in Louisiana coastal areas, and breeds in fresh and brackish water in marshes, ponds, pools, ditches, barrels, bilge water from boats, and sometimes artificial containers around homes. They bite mostly outdoors, occasionally indoors and preferably at dusk, during the first hours of darkness.

Occasionally humans or other mammals are bitten by an infective mosquito and they get infected. Dogs, cats, cattle, horses and other domestic mammals get infected but their role in

transmission is minimal because of low viremia. Most of these animals do not present obvious illness except for horses which also suffer from WN encephalitis.

WNV was introduced in the USA, in New York in 1999. The first cases were diagnosed in Louisiana in 2001 among one human in Jefferson Parish, along with several birds (crows and blue jays) and a few horses. The year 2002 was marked by an epidemic of 204 cases of neuro-invasive disease (WN-NID). The total number of persons infected was estimated at 30,000 to 40,000. The disease was very unevenly distributed in foci appearing in successive waves.

The incubation period for West Nile virus invasive disease is 3 to 14 days.

Infectivity period: In birds the virus is present in blood for several days to a week. Humans will have viremia for a few days before onset of disease. Humans are not infectious for mosquitoes because of low viremia but may be infectious by transfusion, organ transplant, transmission in utero and breast milk.

### **Clinical Description**

The majority of those infected are completely asymptomatic (80-90%).

A small proportion have West Nile Fever (10 to 20%) presenting with febrile, influenza-like illness with abrupt onset of moderate to high fever, headache, sore throat, backache, myalgia, arthralgia, fatigue and a mild and transient rash and lymphadenopathy.

A minority of infected people have acute aseptic meningitis or encephalitis (0.2% below age 65, 2% above age 65). While some cases can easily be differentiated between encephalitis or meningitis, some are more difficult to classify. These cases should be classified as WNV Neuro-Invasive Disease (WNV-NID) and not as meningo-encephalitis which is a term reserved for those who have both meningeal and CNS cortical involvement. Encephalitis is diagnosed by the central nervous system (CNS) involvement, including altered mental status (altered level of consciousness, confusion, agitation, or lethargy) or other cortical signs (cranial nerve palsies, paresis or paralysis, parkinsonian signs, tremors, ataxia or convulsions).

Some individuals have severe muscle weakness or complete flaccid paralysis which is mostly due to axonal degeneration (poliomyelitis) rather than demyelinating syndromes like Guillain Barre syndrome.

Long term sequelae are very common. One year after illness, patients reported the following symptoms: Fatigue, (67%), memory loss (50%), difficulty walking (49%), muscle weakness (44%), and depression (38%).

The case fatality rate is elevated among the elderly, particularly among those 75 year and older.

### **Surveillance**

WNV fever, WNV Neuro-Invasive disease and West Nile Past or Present Infection are reportable conditions.

## Report and Confirm Early Cases

Patients presenting with the following clinical syndromes should be suspected of having WVN illness particularly during the transmission season (May to November) and in transmission foci (Check the OPH website for recent data):

(1) Viral encephalitis, characterized by:

- Fever  $\geq 38$  °C or 100 °F, and
- CNS involvement, including altered mental status (altered level of consciousness, confusion, agitation, or lethargy) or other cortical signs (cranial nerve palsies, paresis or paralysis, parkinsonian signs, tremors, ataxia or convulsions), and
- An abnormal CSF profile suggesting a viral etiology (a negative bacterial stain and culture with pleocytosis [WBC between 5 and 1500 cells/mm<sup>3</sup>] and/or elevated protein level [ $\geq 40$  mg/dl]).

(2) Aseptic meningitis (among persons aged 12 years and up), characterized by:

- Fever  $\geq 38$  °C or 100 °F, and
- Headache, stiff neck and/or other meningeal signs, and
- An abnormal CSF profile suggesting a viral etiology (a negative bacterial stain and culture with pleocytosis [WBC between 5 and 1500 cells/mm<sup>3</sup>] and/or elevated protein level [ $\geq 40$  mg/dl]).

(3) Acute cases of Guillain-Barré syndrome, especially if associated with atypical features, such as fever, altered mental status and/or a pleocytosis

(4) Acute flaccid paralysis

(5) Rhabdomyolysis

To report mail a completed form, the Louisiana Office of Public Health's "*Lab submission form for Arboviral Testing in Humans*", (available below) with the specimen. If the specimen was sent to a diagnostic lab fax the same form to (504) 568-5006

## Case Definition

A case definition becomes important when it comes time to monitor progress of an outbreak. Without a case definition a migraine headache with antibodies to WNV may become a case. A case definition **is not** a diagnosis. It is important to explain to a clinician the difference between a case definition and a diagnosis. Case definitions are used for epidemiologic purposes to ensure consistency across jurisdictions and time, a case definition has to be somewhat rigid. Not defining a case as WNV illness does not mean that the case does not have actual WN infection.

### Serum

- Acute serum: collected within 8 days of onset
- Acute CSF: collected within 8 days of onset
- Convalescent serum: collected within 14 - 21 days of onset

### Clinical description

- Febrile illness of variable severity with neurologic symptoms ranging from headache to aseptic meningitis or encephalitis, nausea or vomiting
- Neurologic symptoms can include: headache, photophobia, confusion or other alteration of mental status.
- Neurologic signs: meningismus (stiff neck), cranial nerve palsies, paresis or paralysis, sensory deficits, altered reflexes, convulsions, abnormal movements and coma of varying degrees

### Meningitis

Clinical signs of meningeal inflammation: nuchal rigidity, Kernig or Brudzinski sign, or photophobia or phonophobia

And one of the following:

Fever  $\geq 38^{\circ}\text{C}$  or  $100^{\circ}\text{F}$ , or hypothermia  $<35^{\circ}\text{C}$

CSF pleocytosis  $\geq 5$  WBC

Peripheral WBC  $\geq 10,000$  WBC /mm<sup>3</sup>

### Viral encephalitis

CNS involvement, including altered mental status, altered level of consciousness, confusion, agitation, lethargy or personality change

And two or more of the following:

Fever  $\geq 38^{\circ}\text{C}$  or  $100^{\circ}\text{F}$ , or hypothermia  $<35^{\circ}\text{C}$

CSF pleocytosis  $\geq 5$  WBC

Peripheral WBC  $\geq 10,000$  WBC /mm<sup>3</sup>

Neuroimaging findings consistent with acute inflammation (with or without involvement of the meninges) or acute demyelination;

Focal neurologic deficit: cranial nerve palsies, paresis or paralysis, parkinsonian signs, tremors, ataxia

Electroencephalographic finding consistent with encephalitis

Seizures

### Acute flaccid paralysis

Acute onset of limb weakness with marked progression over 48 hours

And at least two of the following:

Asymmetry to weakness

Areflexia /hyporeflexia of affected limb(s)

Absence of pain, paresthesia or numbness in affected limb(s)

CSF pleocytosis  $\geq 5$  WBC and elevated protein level  $\geq 40$  mg/dL

Electrodiagnostic studies consistent with an anterior horn cell process

Spinal cord MRI documenting abnormal increased signal in the anterior gray matter

### Probable Case (CDC)

Clinical description +

- WNV EIA IgM positive in acute serum
- Or WNV IgG positive in convalescent serum with 4 fold elevation relative to acute serum + PRNT positive

### Confirmed Case (CDC)

Clinical description + WNV EIA IgM positive in acute CSF

OR

Clinical description +

- WNV EIA IgM positive + WNV EIA IgG positive + PRNT positive
- Or 4fold change in PRNT antibody titer to WNV in paired, appropriately times acute and convalescent serum samples + PRNT positive
- Or WNV virus isolation in blood, CSF, other body fluid or tissue
- Or WNV genomic sequence in blood, CSF, other body fluid or tissue
- Or WNV antigen in blood, CSF, other body fluid or tissue

## **Laboratory Tests**

### Test Methods

- **Screening EIA Assay:** This ELISA test is used as a screening tool. It is a more rapid method than the CDC Antigen capture EIA. There are very few false negatives but many false positives. Therefore it is a good screening tool to rapidly identify positive tests but all positives must be confirmed by a more specific method.

- **Antigen Capture Enzyme Immuno Assay** following CDC protocols. This test requires a 24-hour incubation period. Depending on the timing of receipt of specimens, results will take from 48 to 72 hours to be reported.

- The bottom of the tube is coated with an Anti-Human IgM. Then the serum of the patient is added, then the antigen (extract of cell culture infected with WNV), then an anti-WNV antibody tagged with an enzyme, then a substrate that will change color in the presence of the enzyme.

- If the serum contains anti-WNV antibodies, the sandwich is complete and the substrate will change color: this is a positive reaction.

- If the serum does NOT contain anti-WNV antibodies, one of the layers of the sandwich is missing, and the upper layers of the sandwich do not stick. When the substrate is added, there is no change in color. This is a negative reaction.

- For each serum several tests are done:

- 1-Test with patient serum and WNV antigen. This the “test antigen”.

- 2-Test with patient serum and material on which WNV grew but free of WNV. This is the “normal antigen” . The ratio of patient/test antigen over patient/normal antigen must meet certain criteria to be acceptable.

- 3-Test with negative control

The ratio of patient/test antigen over negative control must meet certain criteria to be acceptable.

These sets of reactions are performed in triplicate and an average of the 3 are done.

Tests are done with both WNV and SLE antigens. Those with WNV infections are higher results with the WNV antigen than with the SLE antigen.

The optical density of the reaction measures the intensity of the reaction. The numeric result presented is **not a titer**, but the ratio of optical density of the patient test over the control test (extract of cell culture infected without WNV). A **ratio of 3.0 is a minimum to interpret as positive**. A WNV positive serum will also show positive with the same technique using a SLE antigen. To be interpreted as WNV positive, the ratio using WNV antigen should be at least twice higher than that using SLE antigen.

- Testing is also performed for Saint Louis Encephalitis (SLE), Eastern Equine Encephalitis (EEE) and California Virus encephalitis using an **immunofluorescence technique**.

- **Reverse transcriptase polymerase chain reaction (RT-PCR)** is used to detect viral RNA. The sensitivity of Taqman RT-PCR in the acute phase is 57% in CSF and 14% in serum. Because of low sensitivity, these tests will not be routinely used for the diagnosis of WNV neuro-invasive disease.

- **A Plaque Reduction Neutralization Test (PRNT)** will also be used by OPH lab as soon as viral material will become available (2004). The serum of the suspect is incubated with the live WN virus then added to a cell culture. If there are antibodies against the virus in the test serum, there is reduction in virus damage compared to control with no antibodies (hence the term “Plaque Reduction”). This is the best test for differentiating WNV from SLE, dengue or Yellow Fever. But it requires handling cell cultures and live virus and it takes several days to evaluate plaque reduction. OPH will use a live horse vaccine virus strain instead of the wild live virus.

#### Indications for testing:

Testing for WNV at the State Public Health Laboratory is being prioritized for hospitalized patients with viral encephalitis, aseptic meningitis, Guillain-Barré syndrome, acute flaccid paralysis or rhabdomyolysis.

In order to keep the number of lab tests manageable, avoid testing asymptomatic patients bitten by mosquitoes, the worried well, those who have a viral infection, and those who are suspected of West Nile Fever (fever and headache without any cerebral or meningeal involvement).

There is no charge for arboviral encephalitis testing.

#### Specimens to obtain:

**Acute phase** (collected within 8 days of illness onset): 2 mL serum in labeled red top tube and CSF (if collected): 2 mL without preservatives

**Convalescent phase** (collected within 14-21 days of illness onset) At least 2 mL serum in labeled red top tube

#### Specimen labeling, packaging and mailing

-Label: Label with patient's name, date of birth, medical record number, and date of specimen collection. **All specimens should be accompanied by the appropriate form:** "Lab submission form for Arboviral Testing in Humans"

Unless there is an emergency, avoid sending samples over the weekend or on holidays. Hold the samples for delivery until the next business day. In case of emergency, make prior arrangements with the laboratory (Virology Section 504-568-4039 or Infectious Disease Epidemiology Section 504-568-5005).

#### -Storage:

- **CSF:** Keep specimens refrigerated. Do not send or store at room temperature.
- **Sera:** Centrifuge, separate from clots, dispense into two sterile tubes (at least 2 cc each) for transport, and refrigerate (do not freeze).

-Packaging: Package CSF and sera in separate bags for transport to OPH. Pack blue ice or other coolants along with serum sample. Do not freeze. Do not use dry ice.

Ship to the following address: *Office of Public Health Virology Laboratory, 325 Loyola Avenue, Room 709, New Orleans, LA 70112*

#### Reporting test results:

Negative test results of WNV testing will be mailed to the submitter (physician, hospital laboratories) by OPH, positive test results will be faxed as soon as they are made available.

### **Interpretation of Lab Test Results for the Clinician**

To correctly interpret a test result it is absolutely necessary to have the following information (requested in the lab submission form)

- Delay between symptom onset (Onset Date) and specimen collection
- Signs and symptoms (those listed in the lab submission form are essential for an accurate interpretation)

Interpreting the results of an antigen capture EIA test:

OPH will be using an antigen capture enzyme immuno-assay (EIA) techniques detecting IgM and /or IgG antibodies to West Nile and Saint Louis encephalitis viruses following CDC protocols. This test requires a 16-hr incubation period and results may take 48 to 72 hours to be reported.

Positive test results are those with a numeric ratio of 3.0 or higher

This number is not a titer but a ratio of the optical density of the patient test over a control test. (See Testing Handout for more detailed explanations). Patients are likely to have a positive ratio for both Saint Louis and West Nile viruses. In order to be considered WN positive, the WNV ratio should be at least twice that of SLE ratio.

Criteria for classification and collection of convalescent sera:

Category	NID Clinical Criteria &	Delay Onset /Collection	Interpretation	Fup needed
<b>CSF</b>				
Early Neg CSF	WNV IgM EIA Neg	< 8 days	Probably Not WN-NID (1)	None
Late Neg CSF	WNV IgM EIA Neg	≥ 8 days	Probably Not WN-NID	None
Pos CSF	WNV IgM EIA Pos	anytime	WN-NID	Convalescent
<b>Serum</b>				
Early Neg Serum	WNV IgM EIA Neg	< 8 days	Not WN-NID (1)	None
Late Neg Serum	WNV IgM EIA Neg	≥ 8 days	Not A Case	None
Pos IgM /IgG	WNV IgM EIA Pos and WNV IgG EIA Pos	anytime	WN-NID	Convalescent
Old Flaviviral infection	WNV IgM EIA Neg and WNV IgG EIA Pos	≥ 8 days	Flavi Old	None
Old Flaviviral infection	WNV IgM EIA Neg and WNV IgG EIA Pos	< 8 days	Old infection or New Inf in Old case	Convalescent

CSF = CerebroSpinal Fluid

Convalescent = Patients who need convalescent serum

PRNT\* = If processed for PRNT wait for results, if not get serum for PRNT

IgG\* = If processed for PRNT wait for results, if not get serum for PRNT

WN-NID: West Nile Neuro Invasive Disease

(1) In the USA and in Louisiana in 2003, 99% of cases had IgM positive test results at onset.

Cross-Reactivity between Flaviviruses

West Nile Encephalitis, Saint Louis Encephalitis, Japanese Encephalitis, and Murray Valley Fever all belong to the same encephalitis virus complex, along with Yellow Fever and Dengue Fever, all these viruses are in the same family of Flaviviruses. They all cross-react in serologic testing. Therefore it is important to obtain a history of Yellow Fever or Japanese Encephalitis vaccine or history of a trip to a dengue endemic area that would explain a positive test, particularly an IgG positive result.

Make sure to differentiate from IgG and IgM

IgG for any of the flaviviral infection or vaccine will last for years, even a lifetime. Therefore interpretation of an IgG positive test for flavivirus with IgM negative result reflect an old infection and is not useful for the diagnosis of a recent clinical infection. **WNV IgM may persist over a year** therefore a person infected in 2002 may still harbor IgM antibodies in 2003 (Up to 42% of patients were IgM positive 500 days after infection).

IgM antibodies do not cross the blood brain barrier therefore IgM antibodies in CSF strongly suggest central nervous system involvement.

Interpretation of a positive test result in a fever case:

Since WN IgM antibodies may last for over a year (42% positive after 500 days), a positive test in a patient with fever and headache will not mean automatically that the patient has West Nile Fever.

## West Nile Viral Fatal Encephalitis Cases

Fatal viral encephalitis cases of unknown etiology must be reported to the Infectious Disease Epidemiology Section (IDES). Tissue samples, including brain, brainstem, and spinal cord will be examined by the Centers for Disease Control and Prevention (CDC) for viral testing and immunohistochemical staining (contact IDDES to receive the “How to collect and submit” instruction form). These samples should be accompanied by an appropriate laboratory form and then submitted to the OPH Central Laboratory to be forwarded to CDC.

### All specimens should be accompanied by:

1. Copy of preliminary or final autopsy report, and pertinent clinical information if available.
2. Case identification on each specimen.

### A complete collection of tissue specimens should include the following:

#### 1- 10% buffered formalin – for routine H & E, special stains.

- Formalin-fixed tissues taken at time of autopsy is the first preference. Formalin-fixed tissues may be taken at a later date (preferably within two weeks of initial autopsy examination).
- Paraffin embedded tissue may be submitted if formalin-fixed tissues are not available or if period of fixation exceeds four weeks.

The proper and adequate sampling of lesions must be tailored to fit the case. Tissues routinely fixed in formalin should include: lungs, heart, liver, spleen, kidney, adrenals, lymph nodes, bone marrow, skin, gastrointestinal, and central nervous tissues (including cortex, cerebellum, brain stem, spinal cord, and meninges). Various tissues can be submitted in a single container of 10% buffered formalin.

Tissues should be shipped at room temperature. (Do Not Freeze this part of collection)

#### 2- 2.5% glutaraldehyde and EM buffer – for EM study

- The tissues to be fixed for EM examination must be tailored to fit the case. Suggested list includes lung, spleen, liver, lymph node, and bone marrow.
- The tissue should be minced, preferably into 1-2mm cubes.
- Store at 4°C. (Do Not Freeze)

#### 3- Fresh frozen tissues in sterile container – for PCR

Tissue of interest should be frozen at -20°C or preferably -70°C. The procedure should be as sterile as possible.

#### 4- Others:

- At least 5 cc of whole blood and 5 cc of serum may be frozen and held until decisions are made as to what specimens and tests are needed for further testing.
  - Freezing at or below -20 degrees C is sufficient for short-term storage.
- For specimen collection questions: During business hours, please call 504-568-5005 and ask to speak the State Epidemiologist, State Public Health Veterinarian, or EIS Officer.
- After hours, call (800) 256-2748 or 504-568-5005 and request to speak to the physician /veterinarian on call.



## Case investigation / Follow up

Category	NID Clinical Criteria &	Delay Onset /Collection	Interpretation	Fup needed
<b>CSF</b>				
Early Neg CSF	WNV IgM EIA Neg	<8 days	Probably Not WN-NID(1)	None
Late Neg CSF	WNV IgM EIA Neg	≥ 8 days	Probably Not WN-NID	None
Pos CSF	WNV IgM EIA Pos	anytime	WN-NID	Convalescent
<b>Serum</b>				
Private Lab	WNV IgM EIA Pos	anytime	Suspect	Convalescent
Early Neg Serum	WNV IgM EIA Neg	< 8 days	Suspect(1)	Convalescent
Late Neg Serum	WNV IgM EIA Neg	≥ 8 days	NotACase	None
Pos IgM /IgG	WNV IgM EIA Pos and WNV IgG EIA Pos	anytime	WNV Probable	Convalescent
IgM Pos early	WNV IgM EIA Pos W>S	< 8 days	WNV Probable	PRNT*
IgM Pos late	WNV IgM EIA Pos W>S	≥ 8 days	WNV Probable	PRNT*, IgG*
Old Flaviviral infection	WNV IgM EIA Neg and WNV IgG EIA Pos	< 8 days	Old infection or New Inf in Old case	Convalescent
Flavi Old	WNV IgM EIA Neg and WNV IgG EIA Pos	≥ 8 days	Flavi Old	None
PRNT Pos	WNV IgM EIA Pos WNV IgG EIA Pos Neutralization Pos	anytime	WNV Confirmed	None
Fuzzy Neut	acute serum Pos for WNV IgM but Pos WNV neut not clearly distinguishable from the titers to other flaviviruses used in tests	<20days		Convalescent

CSF = CerebroSpinal Fluid

Convalescent= Patients who need convalescent serum

PRNT\* = If processed for PRNT wait for results, if not get serum for PRNT

IgG\* = If processed for PRNT wait for results, if not get serum for PRNT

WN-NID: West Nile Neuro Invasive Disease

(1)In the USA and in Louisiana in 2003, 99% of cases had IgM positive test results at onset. Negative serums and CSF obtained after 8 days do not need to be followed up. Follow up of negative specimens collected early (before 8 days after onset) yielded very few positive in 2002 (3 additional cases out of 329 cases and 4,500 lab tests).

## Case management

Each case (positive lab, suspect needing follow up) is assigned to an Infectious Disease Epidemiologist / Surveillance Specialist based on residence or hospital who takes the lead to follow up the person and does the following:

1-Collect basic Demographic information (Name, age, gender, address, parish of residence, family contacts) if not collected on the form “*Laboratory Submission Form for Arboviral Testing in Humans*” which includes:

Clinical History: Try to standardize by using the following terms: Fever, headache, stiff neck, back pain, photophobia, flaccid paralysis, meningitis, altered mental status (confusion, disorientation, coma), slurred speech, hearing or vision disturbance, tremor, ataxia and seizures.

**Important: Get an accurate date for onset of symptoms.**

Note if the patient if the patient has history of having a surgery, receiving a transfusion, donating blood, being pregnant, giving birth or breastfeeding in the four weeks prior to onset of first symptoms.

Deceased patients: attempt to get information on course of treatment.

Hospitalization: Name of hospital, admission and discharge date, type of unit (ICU or regular ward).

Travel: Record dates and location of any recent travel. If travel is to a region endemic for Dengue or Yellow Fever, make sure to ask if patient received a vaccine for either.

2-Update hospitalization date and disposition

3-Obtain copies of any lab tests made in other laboratories

4-Update information in the central office database (LaArbo)

5- Reporting results to health care provider and regional staff.

Note that reporting of confidential information (identifiers) need to follow strict privacy rules. In general do not report name and personal identifiers; report address (to the block level), date of blood collection, date of onset. Check with State Epidemiologist or one of the Assistant State Epidemiologists when in doubt.

In communication with media or public: Report age, gender, city and parish. Do not report hospital name. For patient condition, report if ICU, regular ward or home. All public reports are in the Excel file named Handout.

#### 5.1-Cases reported from a private laboratory

- Enter in LaArbo database
- Call to obtain the blood submitted to the laboratory or request to submit another blood sample
- If there is no address, call the reporting entity to obtain residence information as well as additional clinical information
- Report as suspect to mosquito control if address is known
- Do not count as cases

#### 5.2-Cases accepted by OPH:

Report and discuss significance of results with ICP or clinician: This is very important and should be completed before reporting new cases to the press.

#### 5.3-Statistics:

- Report to Communication and Center Directors
- Prior to report to Regional Medical Directors, Regional Epidemiologists, Regional DSS, Sanitarian Services/Vector Control Program and Mosquito Control
- Report to Parish Officials is the responsibility of regional staff (Regional Medical Directors or assignee).

## 5.4- Report to CDC

### **Database (Central Office staff)**

This is the main tool to track down suspects, cases, lab tests, questionnaires, produce statistics. The fields in RED are important. If left blank some of the queries, reports, forms will not function properly. Do Not Enter Lab Results. Lab results from OPH and CDC are uploaded through a series of queries. But you can edit lab results that are repeats. Report positives with numeric ratios. We prefer to use ratios (12.3) than have “Positive” for example.

### Main Patient Form

#### DateCollection:

Date first positive blood result or if negative, date first blood collected.

#### Lab Subform:

Source: Blood or CSF

LabName: OPH (Not state lab) or Private lab name

Lab #: Enter OPH lab number as AR03-000000

Access Label    Lab report label

EE\_M            Alphavirus IFA IgM

EE\_G            Alphavirus IFA IgG

CE\_M            California virus IFA IgM

CE\_G            California virus IFA IgG

Fl\_If\_M         Flavi virus IFA IgM

Fl\_If\_G         Flavi virus IFA IgG

SL\_M\_EI        SLE EIA IgM (Enter numeric data)

SL\_G\_EI        SLE EIA IgG (Enter numeric data)

WN\_M\_EI        WNV EIA IgM (Enter numeric data)

WN\_G\_EI        WNVEIA IgG (Enter numeric data)

SL\_Neut        SLE Neutralization

WN\_Neut        WNV Neutralization

Suspects considered NOT to have WNV infection: (lab tests not consistent with arboviral infection):  
Check “NotaCase” in Final diagnosis field and ZNO in Case ID field.

Suspects with some significant lab tests: Enter final diagnosis choices from combo box.

### “Clinical” field

#### WNV NID:

Fever with 1 or more neurologic sign

Or 1 or more neurologic symptom + 1 or more neurologic sign

Or 1 or more neurologic symptom + CSF collected

Or abnormal CSF with pleiocytosis and high proteinemia

Or pos IgM EIA in CSF

AND Laboratory criteria met for probable or confirmed

#### WNV Fever (WNF):

Fever, no neurologic symptoms, no neurologic signs

Or Headache, no other neurologic symptom , no neurologic signs, no fever, no collection of CSF

AND Laboratory criteria met

#### “Current Status”

Field name: [FinalDiag]

This field is used for internal classification

- WNV: Cases reported to the public include probable and confirmed cases of both WN-NID and WNF
- WNVSuspect: Cases coming from active surveillance with CNS involvement
- WNFSuspect: Cases coming from active surveillance with NO CNS involvement
- NotACase:
  - No clinical info, no CSF, serum negative
  - Or Serum & CSF negative, Follow up serum neg

#### “Case ID”

This field is ONLY used for ranking cases and suspect in queries.

Cases have a number starting at 001, NotACase are ZNO and Suspects are AA

#### “GET”

Use the Table to tag those who need follow up serum

Click “GET” on the labtestFUp line

#### “Outcome/Status”

Check Home, Regular Ward, ICU, Rehabilitation, Deceased (WN associated) or DeadOther.

#### “Open/Closed”

Enter Closed when case reports are complete.

#### “OutbreakAssociated”

Click the OutbreakAssociated field only if we identify a specific outbreak. Do not use routinely.

### **West Nile Surveillance in Birds, Mosquitoes and Other Animals**

#### 1. Sentinel Chickens

- Location of sentinel chickens must be based on entomological data to maximize benefits. Sentinel chickens may be used throughout the year.
- Chickens should be at least 9 weeks old. Maintain a supply of chickens in a mosquito free environment to replace infected chickens.
- Detection of antibodies by serologic tests is the method of choice. The low viremia in chickens preclude the use of cloacal swabs.
- Chickens should preferably be bled every week.
- Chickens should be replaced after sero-conversion (positive for antibodies to any other arboviruses).
- Test method:
  - IgM capture ELISA for Eastern equine encephalitis (EEE), St. Louis encephalitis (SLE), and West Nile virus (WNV).
  - Plaque Reduction Neutralization Test (PRNT) will be performed when necessary.
  - Lab tests done at the LSU Veterinary Lab. Results should be available within 5 days of sample receipt at the lab.

#### 2. Mosquito Pools

- Pools should consist of 5-50 mosquitoes of the same species, some should be sorted to genus only.
- Test method:

- VecTest (Medical Analysis Systems, Inc.) for *Culex quinquefasciatus* during the peak transmission season. The test can be done as a field test by the Mosquito Control Programs after training. The test is very specific (no false positives). Its sensitivity is similar to EIA tests. Results are obtained in 15 mn. The test can be safely performed in the Mosquito Control District laboratories: Female mosquitoes are placed in a plastic culture tube, then a 2.5 mL of grinding solution is added which inactivates the virus and makes the rest of the test safe.
- Reverse transcriptase - polymerase chain reaction (RT-PCR) TaqMan for all other species of mosquitoes and outside the peak transmission season. Lab tests done at the LSU Veterinary Lab. Results should be available within 5 days of sample receipt at the lab.
- Viral isolation: Because it is very sensitive it is the method of choice off-season. It is done at the LSU Veterinary Lab

### 3. Dead Birds

- Birds to be tested for WNV by the laboratory include blue jays, crows, cardinals, grackles, house sparrows, birds of prey and seagulls.
- Birds should be collected fresh, double bagged, kept frozen, and transported to the lab for testing with the proper submission form. The submission should be placed in a bag separate from the bird. Decomposed birds and birds of species other than listed above will not be tested.
- Oral swabs will be collected from the frozen bird sent to laboratory.
- Test method - VecTest and/or RT-PCR.
- Birds are tested year round.

Transportation of samples to the laboratory will be through the public health truck route system. These samples must be shipped in a timely manner in order to ensure their value in surveillance for disease transmission.

### 4. Horse Surveillance

Horses infected with West Nile Virus may also be valuable sentinels for human disease. Although OPH has no direct role in surveillance of disease in horses, the agency requests that veterinarians promptly report all cases of encephalitis in horses to the State Veterinarian's office (225-925-3980). Veterinarians should also report the precise location of the horse as well as the date of onset of clinical signs.

Signs of West Nile virus infection in horses include stumbling or tripping, muscle weakness, twitching, partial paralysis, appetite loss, depression, head tilt or head pressing, circling, difficulty swallowing, recumbency, fever, convulsions, and coma. West Nile virus infection should be considered in the differential diagnosis for any horse exhibiting these signs. Serum should be submitted for analysis to the Louisiana Veterinary Medical Diagnostic Laboratory in Baton Rouge (225-578-9777). All horse testing is conducted on a fee for services basis.

### **Dead Bird Sightings /Reports to Public Health**

As part of the Louisiana Office of Public Health's efforts to detect West Nile Virus in Louisiana, the Louisiana Office of Public Health is asking citizens to report any sightings of dead birds. In addition, citizens may be asked to collect any crows, blue jays, grackles, house sparrows, birds of prey and seagulls and bring them to their local or parish health units for testing, as explained below.

Because of the impact of WNV on these birds, an early indication of WNV in an area is sightings of a higher than usual number of dead blue jays, crows, cardinals, grackles, house sparrows, birds of prey and seagulls. This has been the experience in Louisiana during the 2002 epidemic. West Nile Virus (WNV), which can be transmitted between birds or from birds to mammals by mosquitoes, can cause encephalitis in humans and horses. The disease is not transmissible between positive humans and horses. While less

than 1 percent of humans who become infected with WNV demonstrate any symptoms of disease, the virus seriously affects some species of birds, mainly blue jays, crows, cardinals, grackles, house sparrows, and birds of prey causing death in the majority of infected birds.

#### Call to report location and species of dead bird

##### --During working hours

Citizens who encounter dead birds are requested to immediately **call their Parish Health Unit** and report the location, as well as the numbers and types of birds. If the identification of the bird species is not known, a description such as size, coloration, and any other distinguishing features would be helpful.

##### --After hours

For a prompt response, we recommend waiting until the following business day to report the bird directly to the Parish Health Unit Call. Reports can also be called into Infectious Disease Epidemiology at 1-800-256-2748 (but may cause some delay).

800-Number answering system options:

Press 3 to report a dead bird: Leave name, phone number and parish of caller

Press 0 or call the CDC Hotline at 1-888-246-2675 to obtain more information on West Nile

#### Collect the bird for lab testing

If blue jays, crows, cardinals, grackles, house sparrows, birds of prey, and seagulls are found dead for less than 24 hrs:

Citizens are requested to collect the birds and bring them to their local Parish Health Unit. If the dead bird cannot be delivered immediately, freeze the bird and deliver it when it is convenient. Birds can safely be kept in the freezer or can be kept in a cooler on ice.

-To properly handle the dead bird, the recommended method of collection is to turn a plastic bag inside-out, pick up the bird with the hands protected by the bag, then turn the bag right-side-out. The bag should then be tied or sealed and placed inside another plastic bag. Another method is to wear disposable gloves or small plastic bags to protect the hands while placing the bird in a double plastic bag. Birds can be disposed off in household trash. It is a good hygienic practice to wash hands after any activities that may soil hands.

-The U. S. Centers for Disease Control advises that there is no danger of contracting WNV from handling dead birds.

### **Protocol for Handling Dead Bird Reports**

Parish Health Units receiving a dead bird sighting call will fill the “2003 Dead Bird Surveillance Log”, an Excel file available on request (call 504-568-5005 ask for WNV Surveillance Coordinator).

The file requires the following information: Date of sighting, Address, City, Zip, Phone (only if the dead bird was submitted), species, was the bird collected, was the bird shipped, and specimen number for those that were shipped to the State Lab.

Species: Cardinal, crow, grackle, blue jay, sparrow, sea gull, raptor and other.

Submit the form weekly by e-mail to [charliea@dhh.state.la.us](mailto:charliea@dhh.state.la.us)

## **Protocol for Notification of Dead Bird Test Results**

- In order to standardize the notification procedures and to assure that all affected parishes are notified, the following protocol will be used to communicate laboratory results on all dead birds submitted through the Office of Public Health's Arboviral Surveillance System:
- The Louisiana Veterinary Medical Diagnostic Laboratory (LVMDL) provides positive results daily and negative results weekly. The results will be reported to OPH in an Excel ® file by e-mail.
- Those results are analyzed and entered into the Infectious Disease Epidemiology database.
- Results on all positive dead birds will be forwarded regularly from Infectious Disease Epidemiology (via e-mail) to each Regional Office (Regional Medical Director, Regional Administrator, and Regional Sanitarian). A follow-up line listing containing all dead bird results (both positive and negative) will be forwarded to each regional office as well.
- It is the responsibility of each regional office to notify the local parish president/police jury offices, and/or other parish authorities (on request to, and determination of need to know by the regional medical director) of the positive test results. Information provided should include the location of the bird(s). It becomes the responsibility of the designated parish government office to notify the proper parish officials (i.e. mosquito control, public works, etc.).
- The Regional office staff will forward the information to the parish sanitarians' offices and any other OPH staff member as warranted. For those birds that tested positive for arboviruses, the local parish sanitarian will notify the person who submitted the dead bird for testing. It is not necessary for the sanitarian to notify individuals when the test results are negative.