DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Centers for Disease Control
and Prevention (CDC)

Memorandum

Date: May 26, 2000

From: Director, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC

Subject: National West Nile Virus Surveillance System, 2000: Final Plan

To: West Nile Virus Cooperative Agreement Recipients
Other West Nile Virus Research Partners

Objectives:

The objectives of the national West Nile (WN) virus surveillance system include:

- To monitor the geographic and temporal spread of WN virus over the eastern and southern United States.
- To further develop national public health strategies for WN virus surveillance, prevention, and control.
- To develop a more complete regional picture of the geographic distribution and incidence of the other clinically important arboviruses in the eastern and southern United States.
- To provide national and regional information to public health officials, elected government officials, and the public.
- To evaluate the use of cooperative agreement funds and the need for additional resources.

Scope:

During the workshop held in Fort Collins in November 1999[*], surveillance was identified as a high priority for those states[**] that were affected by WN virus in 1999 or that have a high potential for being

[*] See "CDC. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control", March 2000, available at: *http://www.cdc.gov/ncidod/dvbid/arbovirus_pubs.htm*

[**] For convenience, the term "state" will be used throughout this document to refer to not only the states themselves, but to New York City and the District of Columbia as well.

affected in the future because of bird migration patterns. These include states from Massachusetts to Texas along the Atlantic and Gulf Coasts. However, other states conducting surveillance for WN virus and other arboviruses are encouraged to participate in national data collection.

While the needs of individual jurisdictions vary, national WN virus surveillance will focus on collection of data from:

- Mosquito-based surveillance
- Sentinel chicken surveillance
- Avian morbidity/mortality surveillance
- Veterinary (non-avian) surveillance
- Human surveillance

State health departments are asked to coordinate the collection of surveillance data in their jurisdictions for submission to CDC.

Data from commercial laboratories may provide a crude measure of trends in the incidence of the clinical syndromes of viral encephalitis and meningitis (i.e., serve as a crude form of syndromic surveillance). CDC will encourage those commercial laboratories performing tests for arboviral infections to report positive test results to local and state health departments. Specifically, during the 2000 arbovirus transmission season, CDC will 1) formally notify all such laboratories of the need to report any positive laboratory results to the appropriate state or local health department, 2) provide them with a list of state health department contact persons, 3) regularly contact them to encourage reporting, 4) remind them of the need to have all positive screening tests for arboviral infections confirmed by state public health reference laboratories or CDC, and 5) request that they voluntarily report to CDC on a regular basis the number of patients tested for WN virus infection and other domestic arboviral infections by state.

In addition, CDC will provide a list of these commercial laboratories to its cooperative agreement partners, to facilitate their efforts to conduct active laboratory-based surveillance for arboviral infections.

Categories of Data to be Collected:

For each of the above surveillance subsystems, the national surveillance system will focus on the collection of two general categories of data:

- *"Denominator"* data

  Definition: Weekly totals of new individuals or groups of individuals reported to, sampled by, or tested by a state's WN virus surveillance system, by county (or similar jurisdiction).

- *"Numerator"* data

  Definition: More detailed information on individual mosquito pools, sentinel flocks, dead birds, and ill humans, horses, or other species with confirmed or suspected WN virus infections, as determined by laboratory-confirmatory, -probable, or -equivocal test results.

General Procedures:

Denominator data:

The reporting of denominator data will be done via secure file upload to the CDC fileserver on a weekly basis (see Appendix I).

Numerator data:

CDC strongly encourages prompt ("real-time") reporting by telephone of all laboratory results of potential public health importance, i.e., those indicating suspected, probable, or confirmed WN virus activity in an area, especially a *new* area (see Appendix II). CDC staff will collect such reports in a standardized manner, allowing them to monitor regional and national trends, and to facilitate prompt confirmatory testing when appropriate. As the arbovirus transmission season progresses, the need for immediate reporting of certain kinds of data to CDC could diminish, e.g., once numerous WN virus-positive mosquito pools have previously been documented in a given geographic area. In addition, if at any time the volume of such reports should become overwhelming (e.g., during an extensive epizootic or epidemic), adoption of an alternative system may be necessary.

WN virus laboratory and surveillance case criteria:

See Appendix III.

Submission of laboratory specimens to CDC for WN virus testing:

See Appendix IV.

Arboviruses other than WN virus:

It is anticipated that enhanced surveillance for WN virus will result in increased recognition of infections with other arboviruses, including eastern equine encephalitis (EEE), western equine encephalitis (WEE), St. Louis encephalitis (SLE) , and LaCrosse (LAC) viruses. Concerning these viruses, surveillance data should be reported to CDC in the same manner as for WN virus.

Data Security Issues:

General principles:

- State and local health authorities will retain control of the timing of data release.

- Via a secure web site, CDC will provide to submitting authorities early access to summary data from the surveillance system to ensure that error correction occurs before any data are made available to the public, and to provide time to prepare for public data release.

- Personal identifying or localizing (more specific than county) information will not be released unless agreed to by the cooperative agreement partner.

- Information of exceptional public health importance such as the identification of WN virus in a new area may require rapid release to the public health community, i.e., more quickly than indicated above. Such a release would occur only with the consent and collaboration of the authorities who reported the data to CDC.

Specific issues:

- Transmission of denominator data by states to CDC will utilize the CDC Secure Data Network, which will require users to apply for a digital certificate on the CDC certificate server and install the certificate on their web browser. This allows for the identification of the computer/browser that is accessing a secure web site and also provides for encryption of data transmitted via the internet.

- For users to receive a digital certificate and be approved to use the secure system, this Division's certificate authority (Mr. Jim Herrington, tel. [970] 221-6429, dvbid@cdc.gov) must approve the request and forward it to Atlanta. CDC requests that a maximum of 3 officials from each state be designated to receive digital certification. These should include those who will transmit denominator data to CDC, as well as those who will review and clear numerator and denominator data on the secure web site.

Summary Reports to be Produced by CDC and the National Atlas/U. S. Geological Survey (USGS):

A working list of basic summary reports (maps, tables, and graphs) is shown in Appendix V. The exact list and formats of these reports remain to be determined, and this should be viewed as a dynamic process. Modifications, additions, and deletions may take place over time, as dictated by feedback, experience, technical issues, and events.

Using only data contained in the master database of numerator and denominator data (i.e., data that have been approved and released by the states), these reports will be generated automatically each week. Maps will be generated by USGS/National Atlas Project staff and available on the National Atlas web site. (The National Atlas is a U. S. government-wide project directed by USGS. Further information is available at *http://www.nationalatlas.gov/federal.html*.) The basic set of dynamic maps and corresponding graphs and tables will be available on the National Atlas web site by each Friday evening. Additional maps will be available by the following Tuesday evening. The CDC web site and National West Nile Information Exchange (NWNIE; see below) will contain links to the appropriate page(s) of the National Atlas web site.

Communication Issues:

- A dedicated telephone line (970-266-3592) and fax machine (970-266-3599) will be available at DVBID (the Division of Vector-Borne Infectious Diseases, CDC/Fort Collins) 24 hours/day for reporting numerator data or other urgent WN virus-related business. During nights and weekends, calls to the dedicated phone line will be forwarded automatically to the cellular phone of an on-call DVBID staff scientist. *Because of potential delays in the receipt and reading of email and fax messages, in general please use the telephone for the initial reporting of numerator data or other time-sensitive business.*

- The National West Nile Information Exchange (NWNIE) is operational at: *http://wnv.forum.cdc.gov*

  NWNIE is a password-protected web-based forum consisting of a variety of "conferences" (listservers) on various WN virus-related topics. Some conferences will be accessible to all participants, while others will have restricted access. Participation in NWNIE must be approved by either a state epidemiologist or a state public health veterinarian, or for federal employees, by Dr. Duane Gubler of CDC/DVBID, Dr. Randy Crom of the U. S. Department of Agriculture, or Dr. Bob McLean of USGS. For further information, contact Mr. Jim Herrington, CDC/DVBID, at (970) 221-6429 or dvbid@cdc.gov.

Appendix I

Reporting of "Denominator" Data

Synopsis: CDC will collect aggregate denominator data each week via a secure file upload system using a simple MS-Access 97 database to be provided by CDC, or by other means (e.g., importation of delimited records in ASCII format). The following weekly schedule will be used:

Tuesday	• States report the previous week's "denominator" data (and any amendments of previous weeks' data) to CDC via secure file upload system • Data stored in "holding" database
Wednesday/Thursday	• Data examined by CDC staff • Any obvious data problems discussed with the reporting authorities
Thursday	• Final review and approval of data by reporting authorities via CDC Secure Data Network • Data incorporated into master database
Friday	• National Atlas/USGS and CDC prepare and release basic summary data (maps, graphs, tables, etc.) on the National Atlas web site (with additional maps available by the following Tuesday).

Details:

- For those state health departments that do not own a licensed copy of MS-Access 97 (or a later verison), CDC will provide a "run-time" version of MS-Access 97 to allow use of the database.

- CDC will attempt to accommodate state health departments with existing data collection systems, e.g., by arranging for uploads of delimited data in ASCII format.

- The data entry screens will be designed as a series of simple forms or tables, one each for mosquito, sentinel bird, avian morbidity/mortality, veterinary (non-avian), and human surveillance data.

- The system will accommodate updates and corrections of previously transmitted data by states.

- Following the entry of a week's data into the database at the state level, transmission of the data file via CDC Secure Data Network will involve a minimal number of keystrokes. Security will be insured by use of the sender's "digital signature". CDC will arrange for those who will be transmitting surveillance data to CDC to obtain digital signatures.

- Upon arrival at CDC, records from the specific reporting week of interest will automatically be captured and imported into a "holding" database on the CDC fileserver.

- Shortly thereafter, a state's data will be available for review as a series of tables via the CDC Secure Data Network (not on NWNIE). Authorized state personnel will have access to their state's data only. A positive checkoff system will be used to signify that state personnel have 1) proofread their data and 2) cleared it for release.

- Once a state has proofread and cleared its weekly data, these data will automatically be moved to a master database on the CDC fileserver and transmitted to National Atlas/USGS in Reston, Virginia.

- Using only data contained in the master database of numerator and denominator data (i.e., data that have been approved and released by the states), reports will be generated automatically each week. Maps will generated by USGS/National Atlas Project staff and available on the National Atlas web site. The basic set of dynamic maps and corresponding graphs and tables will be available by each Friday evening. Additional maps will be available by the following Tuesday evening. The CDC web site and NWNIE will contain links to the appropriate page(s) of the National Atlas web site.

Variable list:

Note: In all five surveillance categories, county-specific data will be requested.

- Common variables:

  State
  County
  MMWR Week (a look-up table will be available in the database)

- Mosquito surveillance:

  Number of mosquitoes collected that week, by species (pull-down list)
  Number of trap nights that week

- Sentinel chicken surveillance:

  Number of sentinel flocks in place that week
  Number of sentinel flocks bled that week

- Avian morbidity/mortality surveillance: (Categories: "crows" and "other spp.")

  Number of sick or dead birds reported to authorities that week (not necessarily received or submitted for laboratory testing)
  Number of birds from which specimens were received by authorities that week (not necessarily submitted for laboratory testing)
  Number of birds from which specimens were submitted for laboratory testing that week

- Veterinary (non-avian) surveillance:

  Number of new individuals from whom samples were submitted for laboratory testing that week, by species (canine, equine, feline, or "other", not including birds)

- Human surveillance:

  Number of new individuals from whom samples were submitted for laboratory testing that week

Appendix II

Reporting of "Numerator" Data

Synopsis: In "real time", states are urged to phone or fax to CDC reports of:

- all laboratory-confirmed, -probable, or -equivocal results (See Appendix III)
- all changes in the interpretation of previously reported laboratory results (e.g., confirmation or failure to confirm previously reported laboratory-probable or laboratory-equivocal results)
- all updates of vital status (e.g., fatalities) in human or veterinary cases

It is essential that each numerator data record include a unique identifier (UID) assigned by the reporting state. UIDs will be used by CDC staff to track and update individual numerator data records, and by states to proofread and clear data over the CDC Secure Data Network. They will not appear in output products for public release. Most states already have systems in place for generating UIDs, and they should continue to use them. CDC will design its numerator databases to accommodate UIDs that are either numeric or alphanumeric and up to 25 characters long. When practical, States are encouraged to begin their UIDs with their 2-letter postal code (or "NYC" for New York City).

CDC will collect numerator data in a standardized fashion. Once DVBID staff have been informed by authorized state personnel that individual numerator data records are cleared for uploading to Atlanta, DVBID staff will upload the "basic" fields of these records (see table below) to the "holding" database located on the CDC fileserver in Atlanta. Shortly thereafter, a state's numerator data will be available for state proofreading and clearance via the CDC Secure Data Network. Once numerator data records are proofread and cleared by states, they will be handled in the same manner as denominator data records (see Appendix I).

DVBID will not upload numerator data records associated with laboratory-equivocal results, pending the results of further laboratory tests.

The issue of numerator data records associated with laboratory-probable results deserves special mention. Although CDC strongly encourages attempts to confirm all laboratory-probable results, it is realized that under some circumstances some states may choose not to do so, depending on the epidemiologic situation, laboratory capacity and volume. For example, in the midst of a known WN viral epizootic, a mid-Atlantic state may decide that a crow brain associated with a single positive result for WN viral RNA by PCR will undergo no further testing, i.e., that the results for this bird will remain laboratory-probable (see table below). Furthermore, that state may decide to authorize DVBID staff to upload that bird's numerator data record to the CDC/Atlanta holding database, and subsequently authorize CDC to release it publicly.

In contrast, a southern state may delay the release of such results to the public until they have been laboratory-confirmed.

Therefore, CDC will rely on individual states to decide when and if to authorize the public release of numerator data records based on *laboratory-probable* results in mosquitoes, sentinel chickens, and dead birds.

In terms of human surveillance, the national surveillance case definition of arboviral encephalitis in humans, adapted for use in WN encephalitis cases (see Appendix III), includes only two official case-status categories, i.e., confirmed and probable. In terms of national arboviral encephalitis surveillance in humans, CDC has traditionally lumped these two case-status categories together in its annual summary maps and other graphics, and will continue this practice within the national WN virus surveillance system. Thus, states are encouraged to promptly report to DVBID staff by telephone both laboratory-confirmed and laboratory-probable human WN encephalitis cases as numerator data records. In addition, states are encouraged to report "laboratory-equivocal" human cases (as defined in the table in Appendix III) in the same manner, *although DVBID staff will not upload such records to the CDC/Atlanta fileserver until and unless they are reclassified as laboratory-confirmed or -probable cases.*

Pending the appearance of surveillance case definitions for veterinary (non-avian) disease due to WN viral infection, the national surveillance case definition of arboviral encephalitis in humans should be used.

Events Appropriate for Immediate Reporting to CDC:

Mosquito surveillance data:

- Any laboratory test-confirmed, -probable, or -equivocal results (see Appendix III)

Sentinel chicken surveillance data:

- Any laboratory test-confirmed, -probable, or -equivocal results

Avian morbidity/mortality surveillance data:

- Any laboratory test-confirmed, -probable, or -equivocal results
- Any epizootics or die-offs possibly due to WN virus infection

Veterinary (non-avian) surveillance data:

- Any laboratory test-confirmed, -probable, or -equivocal results
- Any suspicious clinical cases or apparent epizootics possibly due to WN virus infection

Human surveillance data:

- Any laboratory test-confirmed, -probable, or -equivocal results
- Any suspicious clinical cases or apparent case clusters possibly due to WN virus infection

Numerator Data Variable List:

Surveillance Type	Basic numerator data to be collected by DVBID, for eventual uploading to master database in Atlanta (for eventual public release)	Additional information to be collected by DVBID (for internal use only)	Additional information to be collected by DVBID only if laboratory testing at CDC is requested (for internal use only)
Mosquito	• state • county • pool UID • week of collection • species	• contact information • mosquito pool information (collection date, collection site below county level *[optional]*, collection method, species, sex, pool size, collecting agency, phone #) • available laboratory results (facility, phone #, date tested, test type, results)	• available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)
Sentinel chicken	• state • county • flock UID • week of collection	• contact information • flock information (collection site below county level *[optional]*, flock size, length of time flock in place, evidence of illness in infected birds) • available laboratory results (facility, phone #, bleed date, date tested, test type, results)	• available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)
Avian morbidity/ mortality	• state • county • bird UID • week bird found sick or dead • species (crow or "other")	• contact information • bird information (exact species *[optional*], date bird found sick or dead, collection site below county level *[optional]*) • available necropsy results (facility, phone #, date of necropsy, results) • available laboratory test results (facility, phone #, specimen type[s], collection date, date tested, test type, results)	• available necropsy results (list of specimens available) • available laboratory test results (list of specimens available) • sample disposition (arrangements for shipment of specimens)
Veterinary (non-avian)	• state • county • animal UID • week of onset • species (canine, equine, feline, or "other")	• contact information • patient information (exact species *[optional*], age, residence location below county level *[optional]*, date of onset, clinical manifestations, fatal?, recent travel history, referring veterinarian, phone), • available necropsy results (facility, phone #, date of necropsy, results) • available laboratory results (facility, phone #, specimen type[s], collection date, date tested, test type, results)	• available necropsy results (list of specimens available) • available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)

Surveillance Type	Basic numerator data to be collected by DVBID, for eventual uploading to master database in Atlanta (for eventual public release)	Additional information to be collected by DVBID (for internal use only)	Additional information to be collected by DVBID only if laboratory testing at CDC is requested (for internal use only)
Human	• state • county • patient UID • week of onset	• contact information • patient information (age, sex, residence location below county level *[optional]*, date of onset, clinical manifestations, fatal?) • available autopsy results (facility, phone #, date of autopsy, results) • available laboratory results (facility[s], phone #, specimen type[s], collection date, date tested, test type, results)	• patient information (name, recent travel history, flavivirus vaccination history), • available autopsy results (list of specimens available) • available laboratory results (list of specimens available) • sample disposition (arrangements for shipment of specimens)

Appendix III

West Nile Virus Laboratory and Surveillance Case Criteria

Surveillance Type	Laboratory-confirmed WN virus infection*	Laboratory-probable WN virus infection	Laboratory-equivocal WN virus infection
Mosquito	• WN virus isolation (identity of virus established by IFA using specific monoclonal antibodies, cross-neutralization, RT-PCR, or gene sequencing) • Positive RT-PCR test for WN viral RNA with validation by 1) repeated positive RT-PCR using different primers, 2) positive PCR result using another system (e.g., TaqMan), or 3) virus isolation. • Capture of WN viral antigen validated by results of inhibition test	• Positive RT-PCR test for WN viral RNA in a single test	• Flavivirus isolation
Sentinel chicken	• WN virus isolation (identity of virus established by IFA using specific monoclonal antibodies, cross-neutralization, RT-PCR, or gene sequencing) • Seroconversion to WN virus in serially collected serum specimens, by plaque-reduction neutralization** • Detection of IgM antibody to WN virus, validated by demonstration of neutralizing antibody to WN virus**	• Detection of IgM antibody to WN virus	• Flavivirus isolation • Seroconversion to WN virus in serially collected serum specimens, by hemagglutination-inhibition
Avian morbidity/mortality	• WN virus isolation (identity of virus established by IFA using specific monoclonal antibodies, cross-neutralization, RT-PCR, or gene sequencing) • Positive RT-PCR test for WN viral RNA with validation by 1) repeated positive RT-PCR using different primers, 2) positive PCR result using another system (e.g., TaqMan), or 3) virus isolation. • Detection of specific WN viral antigen in tissues (e.g., by immunohistochemistry)	• Positive RT-PCR test for WN viral RNA in a single test • Detection of flaviviral antigen in tissues (e.g., by immunohistochemistry)	• Flavivirus isolation • Gross pathologic or histopathologic findings suggestive of WN viral infection

Surveillance Type	Laboratory-confirmed WN virus infection*	Laboratory-probable WN virus infection	Laboratory-equivocal WN virus infection
Veterinary (non-avian)	• As for humans (see below)	• As for humans (see below)	• Flavivirus isolation • Any serologically equivocal results (see below)
Human	• See below	• See below	• Flavivirus isolation • Any serologically equivocal results (see below)

* CDC strongly encourages attempts to confirm <u>all</u> laboratory-probable and -equivocal results.

** SLE virus infection should be ruled-out by cross-neutralization.

<u>Humans:</u>

> *Note:* The following is modified from "CDC. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control", which is available at: *http://www.cdc.gov/ncidod/dvbid/arbovirus_pubs.htm*

The following working surveillance case definition of WN encephalitis was used in the 1999 New York epidemic and is an adaptation of the national arboviral encephalitis surveillance case definition [CDC 1997]. As such, it is a public health tool intended only for the surveillance of health events in populations. It is neither 100% specific nor 100% sensitive, and it is not intended for use in clinical diagnosis or management decisions in individual cases. It should also be emphasized that the current national arboviral encephalitis surveillance case definition [CDC 1997] was approved and implemented by the Council of State and Territorial Epidemiologists – in consultation with CDC -- at a time when SLE virus was the only neurotropic flavivirus with epidemic potential known to occur in the United States. However, it is now conceivable that WN and SLE viruses coexist in this country. Antibodies to these closely related neurotropic flaviviruses and dengue viruses, which are increasingly imported, cross-react extensively in enzyme immunoassays (EIA) and hemagglutination-inhibition (HI) tests, to a lesser extent, in neutralization tests. (To an even lesser extent, serologic cross-reactivity also occurs between these two viruses and Powassan virus, a tick-borne flavivirus endemic to the northeastern United States and eastern Canada and which causes rare, sporadic, encephalitis cases in humans.) Thus, in future epidemics and sporadic viral encephalitis cases alike, the potential for initial misclassification of SLE cases as WN encephalitis cases -- and vice versa -- must be recognized and addressed, mainly by the use of cross-neutralization tests of serum or cerebrospinal fluid (CSF) or both, by virus isolation, or by detection of viral genome or antigens. Once WN virus (or SLE virus) has been determined to be the cause of an epidemic/epizootic (e.g., by cross-neutralization tests and/or virus isolation from, or direct virus detection in, humans, birds, or mosquitoes), further cross-neutralization tests generally may be unnecessary to classify human cases for surveillance purposes.

Confirmed case

A confirmed case of WN encephalitis is defined as a febrile illness associated with neurologic manifestations ranging from headache to aseptic meningitis or encephalitis, plus at least one of the following:

- Isolation of WN virus from, or demonstration of WN viral antigen or genomic sequences in, tissue, blood, CSF, or other body fluid;[1]

- Demonstration of IgM antibody to WN virus in CSF by IgM-capture EIA;[2-4]

- A ≥4-fold serial change in plaque-reduction neutralizing (PRNT) antibody titer to WN virus in paired, appropriately timed serum or CSF samples;[2, 3, 5]

- Demonstration of both WN virus-specific IgM (by EIA) and IgG (screened by EIA or HI and confirmed by PRNT) antibody in a single serum specimen.[2, 4-6]

Probable case

A probable case is defined as a compatible illness (as above) that does not meet any of the above laboratory criteria, plus at least one of the following:

- Demonstration of serum IgM antibody against WN virus (by EIA);[3, 4]

- Demonstration of an elevated titer of WN virus-specific IgG antibody in convalescent-phase serum (screened by EIA or HI and confirmed by PRNT).[3-6]

Non-Case

A non-case is defined as an illness that does not meet any of the above laboratory criteria, plus:

- A negative test for IgM antibody to WN virus (by EIA) in serum or CSF collected 8-21 days after onset of illness;[3, 4]

  and/or

- A negative test for IgG antibody to WN virus (by EIA, HI, or PRNT) in serum collected ≥22 days after onset of illness.[3-5]

Notes:

1. Although tests of tissues or fluids by PCR, antigen detection, or virus isolation can be used to confirm WN encephalitis cases, they cannot be used to rule-out cases because the negative predictive values of these test methods in this disease are unknown.

2. See the above discussion concerning serologic cross-reactivity between WN and SLE viruses. Prior to a more definitive demonstration of WN virus as the cause of an epidemic or a sporadic viral encephalitis case, this serologic criterion should be used to classify human cases as *probable* only, pending definitive identification of the circulating flavivirus type (see discussion above).

3. Although the antibody response to human infection with WN virus has not been thoroughly or systematically studied, the following are reasonable assumptions, based on extensive experience with other flaviviruses, or preliminary conclusions based on empirical observations made during the 1999 New York epidemic of WN encephalitis:

   - IgM antibody in serum: By the eighth day of illness, a large majority of infected persons will have detectable serum IgM antibody to WN virus; in most cases it will be detectable for at least 1-2 months after illness onset; in some cases it will reach undetectable levels prior to 1 month after illness onset; in some cases it will be detectable for 6 months or longer.

   - IgG antibody in serum: By 3 weeks post-infection (and often earlier), virtually all infected persons should demonstrate long-lived serum IgG antibody to WN virus by EIA, HI, and PRNT.

   - IgM antibody in CSF: In WN encephalitis cases, IgM antibody will virtually always be detectable in CSF by the eighth day of illness and sometimes as early as the day of onset; the duration of WN virus-specific IgM antibody in CSF has not been studied.

   - IgG antibody in CSF: IgG antibody in CSF often does not reach detectable levels and thus is a relatively insensitive indicator of infection.

   - Specificity of IgM-capture EIA: Serum (and CSF) from recently WN virus-infected persons will cross-react in IgM-capture EIAs when either WN virus or any closely related flavivirus is used as antigen. The homologous (infecting) serotype should be determined by cross-neutralization.

   - Specificity of IgG EIA: WN viral IgG antibody detectable by EIA (or HI) is broadly cross-reactive with all closely related flaviviruses, and this usually cannot be resolved with comparative EIAs (or HIs) using various flavivirus antigens. The homologous serotype should be determined by cross-neutralization.

   - Specificity of PRNT: In previously WN virus-infected persons *without* an antecedent history of infection with another flavivirus (e.g., yellow fever vaccine virus or dengue), serum cross-neutralization tests against a battery of flaviviruses will usually implicate WN virus as the homologous virus. Serum from previously WN virus-infected persons *with* an antecedent history of infection with another flavivirus is often broadly cross-reactive by PRNT against a variety of other flaviviruses (due to "original antigenic sin"), and comparative titers are often insufficiently different to implicate the homologous virus.

   Based on these assumptions or preliminary conclusions:

- Persons whose acute-phase serum or CSF specimens (collected 0-7 days after illness onset) test negative for IgM antibody to WN virus should have convalescent-phase serum specimens submitted for testing. Generally, convalescent-phase specimens should be drawn at least 2 weeks after acute-phase specimens. These intervals are arbitrary and not part of the national arboviral encephalitis surveillance case definition. In some cases, for example, seroconversion to WN virus can be demonstrated in specimens collected only a few days apart during the late acute or early convalescent phase of the illness.

- Negative tests for IgM antibody to WN virus in serum specimens collected more than 3 weeks after illness onset could be due to rapid waning of antibody; these results should be considered as potential false-negatives, pending IgG antibody testing.

- The EIA (or HI) for serum IgG antibody is a sensitive but relatively nonspecific test for previous WN virus infection. Positive results should be confirmed by PRNT.

- CSF should generally not be tested by WN viral IgG EIA (or HI). Instead, it should usually be reserved for testing by IgM-capture EIA and possibly by other means, including virus isolation, PCR, and neutralization.

4. At CDC, EIA results are based on "P/N ratios", which are optical density (OD) ratios or signal-to-noise ratios, not titers. A P/N ratio is calculated by dividing the OD of the test sample [P] by the OD of a normal [N] human antibody control. At CDC, serum specimens are routinely tested at a dilution of 1:400 and CSF specimens are tested undiluted. Empirically, CSF P/N ratios of greater than or equal to 3 are considered positive for flavivirus IgM antibody at CDC, and serum IgM P/N ratios of 2.00-2.99 are considered to be equivocal pending further serologic testing (e.g., EIA endpoint titration), and ratios <2 are is considered uninterpretable if the OD of the test sample with viral antigen is less than or equal to 2 times the OD of the test serum with normal mouse brain antigen. Because of the potential for interlaboratory variability in P/N ratios generated for identical serum samples, appropriate positive, negative, and equivocal ranges of P/N ratios must be empirically determined by each laboratory.

5. At CDC, a serum PRNT titer of 10 (i.e., a 1:10 dilution of serum neutralizes at least 90% of the test virus dose) or greater is considered positive.

6. As discussed above, in some previously infected persons, detectable IgM antibody to WN virus can persist for 6 months or longer. Thus, in a person with a viral syndrome (e.g., viral encephalitis) with onset in 2000, who coincidentally had been previously infected with WN virus in 1999, false-positive test results for both IgM and neutralizing antibodies to WN virus in a single serum sample collected in 2000 could occur, although this is very unlikely. In such cases, tests of serially collected serum specimens for evidence of seroconversion in neutralization tests, attempts at virus detection in CSF or tissues, or both, may be needed.

References

CDC. Case definitions for infectious conditions under public health surveillance. *MMWR* 1997;46[RR-10]:12-3 (available at *http://www.cdc.gov/epo/dphsi/casedef/enceph97.htm*).

Appendix IV

Instructions for Submitting Laboratory Specimens to CDC for West Nile Virus Testing

Arrangements for Testing:

Mosquito specimens: Specimens will be accepted for confirmatory testing at CDC when requested by a state health department vector surveillance coordinator. For specimens considered by a state health department vector surveillance coordinator to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Roger Nasci, tel. 970-221-6432, RNasci@cdc.gov; if Dr. Nasci cannot be reached, please phone 970-266-3592.

Sentinel chicken specimens: Serum specimens will be accepted for confirmatory testing at CDC when requested by a state health department vector or vertebrate surveillance coordinator. For specimens considered by a state health department vector or vertebrate surveillance coordinator to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

Avian morbidity/mortality specimens: On a case-by-case basis, special arrangements can by made for CDC to conduct initial and/or confirmatory tests of tissues specimens (especially brain, heart, kidney, and spleen) from dead birds that cannot otherwise be tested in state health department laboratories or by the National Wildlife Health Center, USGS. For further information, please contact Dr. Nick Komar, tel. 970-221-6496, NKomar@cdc.gov; if Dr. Komar cannot be reached, please call 970-266-3592.

Veterinary (non-avian) specimens: Specimens will be accepted for confirmatory testing at CDC when requested by a state health department laboratory director. For specimens considered by a state health department laboratory director to be of high priority and beyond the capacity of the state public health laboratory, National Veterinary Services Laboratory, USDA, or other collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

Human specimens: Specimens will be accepted for confirmatory testing at CDC when requested by a state health department laboratory director. For specimens considered by a state health department laboratory director to be of high priority and beyond the capacity of the state public health laboratory or collaborating laboratory, initial and confirmatory testing can be obtained at CDC by special arrangement, depending on CDC laboratory capacity. For further information, please contact Dr. Rob Lanciotti, tel. 970-221-6440, RSLanciotti@cdc.gov; if Dr. Lanciotti cannot be reached, please call 970-266-3592.

General Shipping Instructions:

All shippers should adhere to International Air Transport Association regulations (*http://www.iata.org*).

Specimens should be shipped by overnight courier to arrive at CDC on Tuesday-Friday. *Always notify CDC staff in advance of an impending shipment* (tel. 970-221-6445; if no answer, phone 970-266-3592). Do not ship specimens on Friday unless special arrangements have been made.

Shipping address: CDC/DVBID
1300 Rampart Road
CSU Foothills Campus
Fort Collins, CO 80521
ATTENTION: Arbovirus Diagnostic Laboratory (tel. 970-221-6445)

Shipping containers: Use only durable containers. Seal specimen containers tightly. Wrap specimen containers in absorbent material and pack them into two different plastic containers to insure that any leakage is contained. Specimens for virus isolation must be sent on enough dry ice to insure that they remain frozen until receipt. Specimens for serologic testing can be shipped on gel-ice and need not remain frozen. Hand-carrying specimens is not recommended but if specimens are hand-carried, the above packing instructions are applicable.

Minimal Information to Accompany Specimens Shipped to CDC:

See information in columns 2, 3, and 4 of the table in Appendix II. Please read carefully and supply all available information. Use CDC Form 5034 (the "DASH" form) or comparable form. Form 5034 is available electronically at: *http://www.cdc.gov/ncidod/dvbid/arbovirus_pubs.htm.*

Some circulating versions of Form 5034 lack spaces for a patient's name. Nevertheless, please always include the patient's name when using any version of Form 5034 or other submission form.

Tubes, cryovials, and other specimen containers should be clearly labeled with – at minimum – the specimen's UID, patient's name (human), state, date of collection, and specimen type.

Special Collection, Shipping, and Handling Instructions:

Mosquitoes:

Ship on dry ice.

Serum:

Store in externally threaded plastic tubes. Ship at least 0.5 mL per specimen. Whenever possible, acute and convalescent specimens should be shipped together.

CSF:

Store in externally threaded plastic tubes. Ship at least 1.0 mL per specimen.

Whole blood:

In general, send only if requested for virus isolation attempts in fatal cases (heart blood).

Human tissues:

In suspected cases of arboviral encephalitis in which an autopsy is performed, both fresh-frozen and formalin-fixed tissues can be tested, including brain (multiple areas of cortex, midbrain, brainstem, and spinal cord), other solid organs (liver, spleen, pancreas, heart, kidney, etc.), CSF (collected from ventricles), and heart blood (for virus isolation attempts).

Fresh-frozen material should be shipped on dry ice to CDC/Fort Collins at the above address.

After consulting with Dr. Sherif Zaki or other CDC/Atlanta pathology staff member (tel. 404-639-3133), tissue samples suspended in formalin should be sent to:

Infectious Disease Pathology Activity
DVRD/NCID/CDC
Building 1, Room 2301
1600 Clifton Road, N. E.
Atlanta, GA 30333

Veterinary (non-avian) tissues:

As for human specimens.

Avian tissues:

Submit fresh-frozen brain, heart, kidney, and spleen samples.

Appendix V

Working List of Basic Weekly Summary Reports to be Produced by CDC

NOTE: The exact list and formats of these reports remain to be determined, and this should be viewed as a dynamic process. Modifications, additions, and deletions may take place over time, as dictated by feedback, experience, technical issues, and events.

A. National map: United States map with state boundaries; action buttons will allow the selection of each of the following categories (two maps or tables for each category, one reflecting the current week's data and the other reflecting cumulative data):

1. Mosquito surveillance:
   a. Map showing each state as WN virus-positive, WN virus-negative, or blank (no data)

2. Sentinel chicken surveillance:
   a. Map showing each state as WN virus-positive, WN virus-negative, or blank (no data)

3. Avian morbidity/mortality surveillance:
   a. Map showing each state as WN virus-positive, WN virus-negative, or blank (no data)
   b. Graph showing number of cases by week of onset (cumulative national data)

4. Veterinary (non-avian) surveillance:
   a. Map showing each state as WN virus-positive (# cases), WN virus-negative, or blank (no data)
   b. Graph showing number of cases by week of onset (cumulative national data)

5. Human surveillance:
   a. Map showing each state as WN virus-positive (# cases), WN virus-negative, or blank (no data)
   b. Graph showing number of cases by week of onset (cumulative national data)

B. State Maps: Selecting an individual state from the national map will produce a map of that state with its county boundaries; action buttons will allow the selection of each of the following categories (two maps or tables for each category, one reflecting the current week's data and the other reflecting cumulative data):

1. Mosquito surveillance:
   a. Map showing each county as WN virus-positive, WN virus-negative, or blank (no data)
   b. Summary table (see below)

2. Sentinel chicken surveillance:
   a. Map showing each county as WN virus-positive, WN virus-negative, or blank (no data)
   b. Summary table (see below)

3. Avian morbidity/mortality surveillance:
   a. Map showing each county as WN virus-positive, WN virus-negative, or blank (no data)
   b. Summary table (see below)
   c. Graph showing number of cases by week of onset (cumulative state data)

4. Veterinary (non-avian) surveillance:
   a. Map showing each county as WN virus-positive (# cases), WN virus-negative, or blank (no data)
   b. Graph showing number of cases by week of onset, by species (cumulative state data)
   c. Summary table (see below)

5. Human surveillance:
   a. Map showing each county as WN virus-positive (#cases), WN virus-negative, or blank (no data)
   b. Graph showing number of cases by week of onset (cumulative state data)
   c. Summary table (see below)

Working headers for tables*:

Surveil-lance system \ Column	A	B	C	D	E	F	G
Mosquito	Week #	County	Species	# test-positive	# collected	# trap nights	
Sentinel chicken	Week #	County	# flocks test-positive	# flocks bled	# flocks in place		
Avian morbidity/mortality	Week #	County	Species ("crow" or "other")	# test-positive	# reported to authorities	# received by authorities	# submitted for testing
Veterinary (non-avian)	Week #	County	Species (canine, equine, feline, or "other")	# confirmed cases	# new individuals from which specimens submitted		
Human	Week #	County	# confirmed cases	# new individuals from which specimens submitted			

* Two tables for each header: one reflecting current week's data and the other reflecting cumulative data.