Centers for Disease Control and Prevention
Division of Vector-Borne Diseases
Promote health and quality of life by preventing and controlling vector-borne diseases

West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control

U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control and Prevention
National Center for Emerging and Zoonotic Infectious Diseases
Division of Vector-Borne Diseases
Fort Collins, Colorado

4th Revision
June 14, 2013

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Department of Health and Human Services
Centers for Disease Control and Prevention
National Center for Emerging and Infectious Diseases
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June 14, 2013

The following CDC, Division of Vector-Borne Diseases staff members formed the technical development group that prepared this report:

- Roger S. Nasci
- Marc. Fischer
- Nicole P. Lindsey
- Robert S. Lanciotti
- Harry M. Savage
- Nicholas Komar
- Janet C. McAllister
- John-Paul Mutebi
- Judy M. Lavelle
- Emily Zielinski-Gutierrez
- Lyle R. Petersen

We are grateful to representatives from the following organizations for their thoughtful review and contributions to this document:

- National Association of Vector-Borne Disease Control Officials (NAVCO)
- National Association of City and County Health Officials (NACCHO)
- Council of State and Territorial Epidemiologists (CSTE)
- Association of State and Territorial Health Officials (ASTHO)
- Association of Public Health Laboratories (APHL)
- American Mosquito Control Association (AMCA)

Foreword

As West Nile virus (WNV) spread and became established across the United States following its first identification in New York City in 1999, the responses of all levels of the public health system have resulted in a detailed understanding of WNV transmission ecology and epidemiology as well as development of systems and procedures to reduce human risk. This includes an expanded capacity to diagnose and monitor WNV infections in humans, measure WNV transmission activity in vector mosquitoes, and implement effective WNV control programs. These guidelines, which update the third revision released in 2003, incorporate this new knowledge with the goal of providing guidance to health departments and other public health entities in monitoring and mitigating WNV risk to humans.

Human disease surveillance provides an ongoing nationwide assessment of the human impact of WNV, and over the past decade has demonstrated where WNV incidence and total disease burden are greatest. However, human disease surveillance, by itself, is limited in its ability to predict the large focal outbreaks that have come to characterize this disease. These outbreaks typically intensify over as little as a couple of weeks; however, human case reports are lagging indicators of risk since case reports occur weeks after the time of infection. Thus, environmental surveillance – monitoring enzootic and epizootic WNV transmission in mosquitoes and birds – forms a timelier index of risk, and is an important cornerstone for implementing effective WNV risk reduction efforts. Research and operational experience shows that increases in WNV infection rates in mosquito populations can provide an indicator of developing outbreak conditions several weeks in advance of increases in human infections.

Communities that have a history of WNV, particularly metropolitan areas with large human populations at risk, should implement comprehensive, integrated vector management (IVM) programs that incorporate monitoring mosquito abundance and infection rates. Mosquito-based WNV surveillance programs should use strategies that assure data are comparable over time and space, and are designed to detect trends in WNV transmission levels. Programs should enlist quantitative indicators such as the WNV infection rate or vector index to represent WNV transmission activity in mosquito populations. Programs must be sustainable over the long term in order to provide sufficient information to link surveillance indicators with the degree of human risk. Consistency also requires that mosquito collections be repeated at regular (weekly) intervals over the course of the transmission season, and that collections are made at fixed collecting sites. Only through maintaining consistency can monitoring programs provide information useful in crafting thresholds to support decisions about vector control efforts and other interventions. Other surveillance modalities, such as sentinel chickens and dead bird surveillance, may be a valuable adjunct to mosquito-based surveillance in quantifying epizootic activity in some settings.

IVM programs must be proactive and make plans in advance for addressing increasing levels of WNV risk. The objective of IVM is to implement control measures sufficient to maintain mosquito abundance below levels that result in high risk of WNV transmission to humans. By establishing action thresholds based on the abundance of WNV-infected vector mosquitoes, IVM programs can monitor risk and the effectiveness of their programs, and implement more directed vector control efforts as needed. IVM implies that all of the tools available for managing mosquito populations should be considered for use as needed to maintain vector populations at low levels. Source reduction and larval control activities can be effective in maintaining low vector abundance, but adult mosquito control efforts through ground or aerially applied pesticides complement proactive vector management programs and should not be relegated to the status of a “last resort” measure to be used only during outbreaks. Aggressive and

timely efforts to reduce the number of infected adult mosquitoes will optimally impact human WNV case incidence when environmental surveillance indicates substantial WNV epizootic activity or when many human cases occur early in the season (e.g., June or July.)

This document provides guidance for communities and public health agencies developing new programs or enhancing existing WNV management programs. The CDC Division of Vector-Borne Diseases is available to provide additional consultation and technical assistance (by phone: 970-261-6400 or email: dvbid2@cdc.gov).

Introduction

Ten years have passed since the 2003 publication of the 3rd edition of "West Nile virus in the United States: Guidelines for surveillance prevention and control". At that time, only 4 years since West Nile virus (WNV) was first detected in New York City, the virus had already established itself across approximately the eastern half of the country and produced the largest epidemic of arboviral encephalitis ever experienced in the United States. Knowledge about WNV epidemiology and transmission ecology was expanding rapidly, but numerous gaps remained in our understanding of how this relatively new exotic disease would affect public health, what monitoring practices would provide the best indicators of human risk, and what interventions would be most effective in reducing human infections. Thus, large portions of the WNV Guidelines 3rd edition were predicated on relatively limited research and operational experience, and were dedicated to identifying and prioritizing specific basic and operational research directions.

Since that time, WNV has expanded to the point that it can now be found in all 48 contiguous states and has produced two additional, large nationwide epidemics in 2003 and 2012. Also, considerable new information about WNV epidemiology, ecology and control has been generated since 2003. The objective of this 4th edition of the WNV guidelines is to consolidate and describe this information and describe how these new findings can be used to better monitor WNV and mitigate its public health impact.

This document was produced through a comprehensive review of the published literature related to WNV epidemiology, diagnostics, transmission ecology, environmental surveillance, and vector control. The publications were reviewed for relevance to developing operational surveillance and control programs, and selected for inclusion in a draft document by a technical development group of CDC subject matter experts. Numerous stakeholder groups were requested to review the document. Comments and additional material provided by National Association of Vector-Borne Disease Control Officials (NAVCO), National Association of City and County Health Officials (NACCHO), Council of State and Territorial Epidemiologists (CSTE), Association of State and Territorial Health Officials (ASTHO), Association of Public Health Laboratories (APHL), and American Mosquito Control Association (AMCA) were incorporated to produce this document.

We view the recommendations contained in these guidelines as the best that can be derived from the currently available information, and will provide updates as new information about WNV epidemiology, ecology, or intervention becomes available.

West Nile Virus Epidemiology and Ecology

WNV, a mosquito-transmitted member of the genus Flavivirus in the family *Flaviviridae*, was discovered in northwest Uganda in 1937 (Smithburn et al. 1940) but was not viewed as a potentially important public health threat until it was associated with epidemics of fever and encephalitis in the Middle East in the 1950's (Taylor et al. 1956, Paz 2006). In the following years, WNV was associated with sporadic outbreaks of human disease across portions of Africa, the Middle East, India, Europe and Asia (Hubalek and Halouzka 1999). In the mid to late 1990's outbreaks occurred more frequently in the Mediterranean Basin and large outbreaks occurred in Romania and the Volga delta in southern Russia (Hayes et al. 2005).

The first domestically-acquired human cases of WNV disease in the Western Hemisphere were detected in New York City in 1999 (Nash et al. 2001). WNV rapidly spread during the following years, and by 2005 had established sustained transmission foci in much of the hemisphere with an overall distribution that extended from central Canada to southern Argentina (Gubler 2007). WNV transmission persists across this large, ecologically-diverse expanse, and as a result this virus is recognized as the most widely distributed arbovirus in the world (Kramer et al. 2008).

WNV has become enzootic in all 48 contiguous United States and evidence of transmission in the form of infected humans, mosquitoes, birds, horses, or other mammals has been reported from 96% of U.S. counties. This extensive distribution is due to the ability of WNV to establish and persist in the wide variety of ecosystems present across the country. WNV has been detected in 65 different mosquito species in the U.S. (CDC 2012), though it appears that only a few *Culex* species drive epizootic and epidemic transmission. The most important vectors are *Cx. pipiens* in the northern half of the country, *Cx. quinquefasciatus* in the southern states, and *Cx. tarsalis* in the western states where it overlaps with the *Cx. pipiens and quinquefasciatus* (Fig 1.) (Andreadis et al. 2004, Kilpatrick et al. 2006a, Godsey et al. 2010). However, the population structure of *Culex pipiens* and *Cx. quinquefasciatus* is more complex than indicated in Fig. 1 as these species readily hybridize and produce a stable hybrid zone across the United States. Barr (1957) set the limits of the hybrid zone at 36^{O} N and 39^{O} N based on measurements of the male genitalia. Subsequent work using microsatellites (Huang et al. 2008, Edillo et al. 2009, Kothera et al. 2009, Kothera et al. 2013, Savage and Kothera 2012) and other molecular markers (Huang et al. 2011) indicates that the hybrid zone extends farther north and south than suggested by Barr (1957). In the middle latitudes of the US, both nominal species and hybrids may be present and are commonly reported as *Cx. pipiens* complex mosquitoes (Savage et al. 2007, Savage and Kothera 2012). The implications of *Cx. pipiens – Cx. quinquefasciatus* population genetics any hybridization patterns for WNV transmission are not well understood.

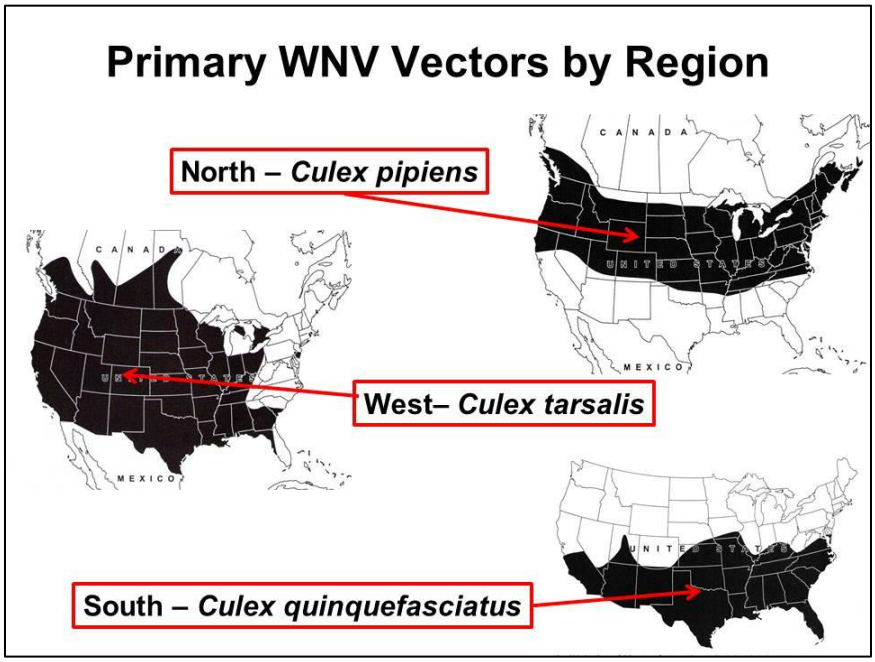

Figure 1. Approximate geographic distribution of the primary WNV vectors, *Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. tarsalis* (modified from Darsie and Ward 2005).

Culex salinarius has been identified as an important enzootic and epidemic vector in the northeastern U.S. (Anderson et al. 2004, 2012, Molaei et al. 2006). Other mosquito species including *Cx. restuans, Cx. nigripalpus* and *Cx. stigmatosoma* may contribute to early season amplification or serve as accessory bridge vectors in certain regions, but their role is less well understood (Kilpatrick et al. 2005). WNV has been detected in hundreds of bird species in the United States (CDC 2012). However, relatively few species function as primary amplifiers of the virus, and a small subset of bird species may significantly influence WNV transmission dynamics locally (Hamer et al. 2009). For example, the American robin (*Turdus migratorious*) can play a key role as amplifier host, even in locations where it is present in relatively low abundance (Kilpatrick et al. 2006b).

As a result of this extensive distribution in the U.S., WNV is now the most frequent cause of arboviral disease in the country. Since 1999, WNV disease cases have been reported from all 48 contiguous states and two-thirds of all U.S. counties. Though widely distributed, WNV transmission is temporally and spatially heterogeneous. Human WNV disease cases have been reported during every month of the year in the United States, but as is characteristic of zoonotic arboviruses in temperate climates, intense transmission is limited to the summer and early fall months; 94% of human cases have been reported from July through September (CDC 2010) and approximately two-thirds of reported cases occurred during a 6-week period from mid-July through the end of August. At a national level, the annual incidence and number of cases reported have varied dramatically since 1999 (CDC 2010, CDC 2010a, CDC 2011, CDC 2012). Weather, especially temperature, is an important modifier of WNV transmission, and has been correlated with increased incidence of human disease at regional and national scales (Soverow et al. 2009), and likely drives the annual fluctuations in numbers of cases reported at the national level. However, WNV epidemiology is characterized by focal and sometimes intense outbreaks (CDC 2010). Epidemiological data gathered since 1999 demonstrate regions in the United States with recurring high levels of WNV transmission and risk to humans (Fig. 2). High average annual incidence of WNV disease occurs in the West Central and Mountain regions (CDC 2010), with the highest cumulative incidence of infection occurring in the central plains states (i.e., South Dakota, Wyoming and North Dakota) (Petersen et al. 2012). The greatest disease burden occurs where areas of moderate to high incidence intersect metropolitan counties with correspondingly high human population densities.

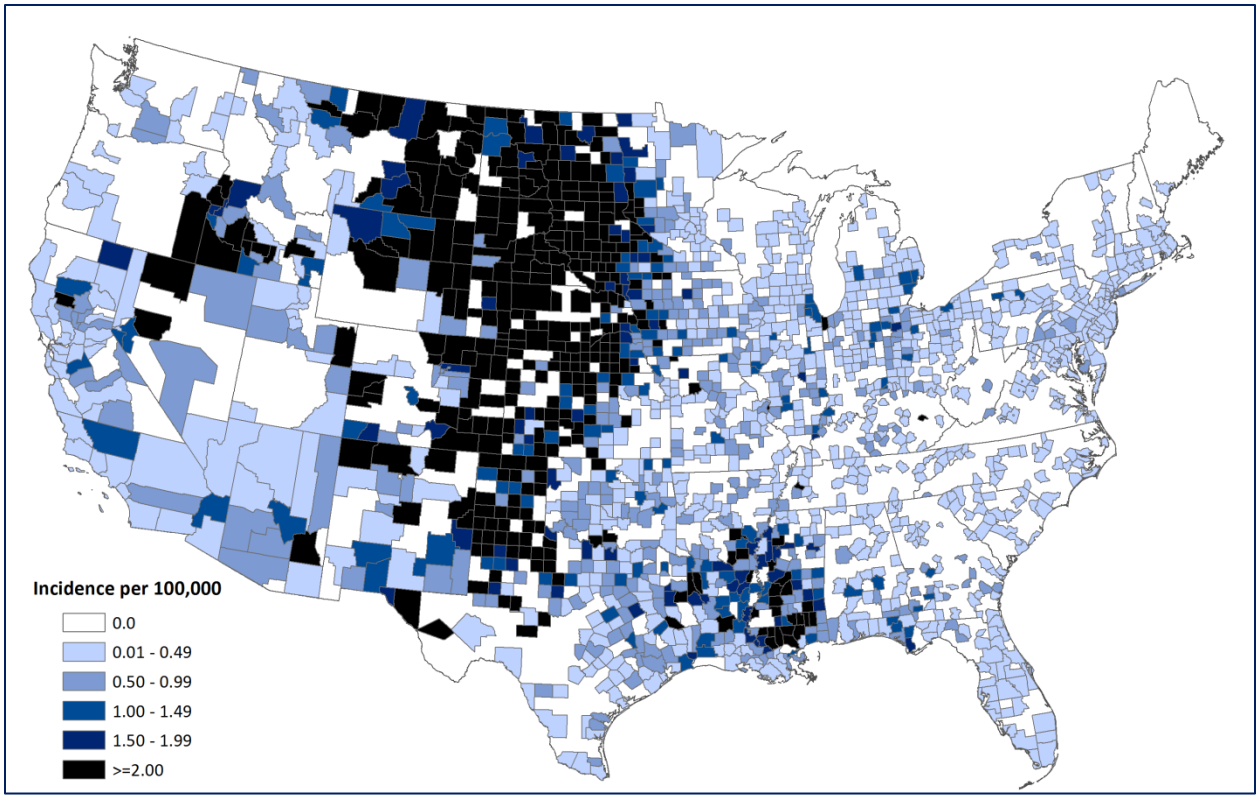

Figure 2. Average annual incidence of West Nile Virus neuroinvasive disease, 1999-2012.

Limits to Prediction – The Need for Surveillance

WNV outbreaks have been associated on a local level with a variety of parameters including urban habitats in the Northeast and agricultural habitats in the western United States (Bowden et al. 2011), rural irrigated landscapes (DeGroote and Sugumaran 2012), increased temperature (Hartley et al. 2012), specific precipitation patterns, several socioeconomic factors such as housing age and community drainage patterns (Ruiz et al. 2007), per capita income (DeGroote and Sugumaran 2012), and neglected swimming pool density (Reisen et al. 2008, Harrigan et al. 2010). Despite these documented associations with a variety of biotic and abiotic factors, and recognition that certain regions experience more frequent outbreaks and higher levels of human disease risk, no models have been developed to provide long-term predictions of how and where these factors will combine to produce outbreaks. The unpredictable nature of WNV outbreaks necessitates the establishment and maintenance of surveillance systems capable of detecting increases in WNV transmission activity and the ability to respond to the surveillance data with effective, disease-reducing interventions. Such surveillance and control programs can be costly to maintain. However it is important that communities with large human populations in areas with documented WNV risk establish and maintain surveillance for human cases and effective integrated vector management programs that incorporate environmental surveillance components capable of providing indicators predictive of human risk.

This document provides guidance for developing systems to 1) Monitor WNV enzootic and epizootic transmission activity as indicators of human risk; 2) Maintain surveillance for human infections and

disease to monitor trends in WNV disease burden and clinical presentation; and 3) Implement prevention and control programs that reduce community level risk by managing vector mosquito populations and that reduce individual risk by promoting effective personal protection measures.

Surveillance

Objectives of WNV Surveillance

WNV surveillance consists of two distinct, but complementary activities. Epidemiological surveillance measures WNV human disease to quantify disease burden and identify seasonal, geographic, and demographic patterns in human morbidity and mortality (Lindsey et al. 2008, CDC 2010). Environmental surveillance monitors local WNV activity in vectors and non-human vertebrate hosts in advance of epidemic activity affecting humans.

In addition to monitoring disease burden and distribution, epidemiological surveillance has been instrumental in characterizing clinical disease presentation and disease outcome, as well as identifying high risk populations and factors associated with serious WNV disease (Lindsey et al. 2012). Epidemiological surveillance has also detected and quantified alternative routes of WNV transmission to humans, such as contaminated blood donations and organ transplantation (Pealer et al. 2003, Nett et al. 2012). Epidemiological and environmental surveillance for WNV was facilitated by development and implementation of ArboNET, the national arbovirus surveillance system (Lindsey et al. 2012). ArboNET was developed in 2000 as a comprehensive surveillance data capture platform to monitor WNV infections in humans, mosquitoes, birds, and other animals as the virus spread and became established across the country. This comprehensive approach was essential to tracking the progression of WNV activity and remains a significant source of data adding to our understanding of the epidemiology and ecology of WNV.

In the absence of effective human WNV vaccines, preventing disease in humans depends on application of measures to keep infected mosquitoes from biting people. A principal objective of WNV environmental surveillance is to quantify the intensity of virus transmission in a region in order to provide a predictive index of human infection risk. This risk prediction, along with information about the local conditions and habitats that produce WNV vector mosquitoes, can be used to inform an integrated vector management program and the associated decisions about implementing prevention and control interventions (Nasci 2013).

Though epidemiological surveillance is essential for understanding WNV disease burden, utilizing human case surveillance by itself is insufficient for predicting outbreaks (Reisen and Brault 2007). WNV outbreaks can develop quickly, with the majority of human cases occurring over a few weeks during the peak of transmission (CDC 2010). The time from human infection to onset of symptoms, to diagnosis and reporting can be several weeks or longer. As a result, human WNV case reports lag well behind the transmission from mosquitoes that initiated the infection. By monitoring WNV infection prevalence in mosquito vectors and incidence in non-human vertebrate hosts, and comparing these indices to historical environmental and epidemiological surveillance data, conditions associated with increasing human risk can be detected 2-4 weeks in advance of human disease onset (Kwan et al. 2012a). This provides additional lead time for critical vector control interventions and public education programs to be put in place. The following sections describe the elements of epidemiological and environmental WNV surveillance and how they may be used to monitor and predict risk and to trigger interventions.

Human Surveillance

Routes of Transmission

WNV is transmitted to humans primarily through the bite of infected mosquitoes (Campbell et al. 2001). However, person-to-person transmission can occur through transfusion of infected blood products or solid organ transplantation (Pealer et al. 2003, Iwamoto et al. 2003). Intrauterine transmission and probable transmission via human milk also have been described but appear to be uncommon (O'Leary et al. 2006, Hinckley et al. 2007). Percutaneous infection and aerosol infection have occurred in laboratory workers, and an outbreak of WNV infection among turkey handlers also raised the possibility of aerosol transmission (CDC 2002, CDC 2003a).

Since 2003, the U.S. blood supply has been routinely screened for WNV RNA; as a result, transfusion-associated WNV infection is rare (CDC 2003b). The Food and Drug Administration recommends that blood collection agencies perform WNV nucleic acid amplification test (NAAT) year-round on all blood donations, either in minipools of six or sixteen donations (depending on test specifications) or as individual donations. Organ and tissue donors are not routinely screened for WNV infection (Nett et al. 2012).

Clinical Presentation

An estimated 70-80% of human WNV infections are subclinical or asymptomatic (Mostashari et al. 2001, Zou et al. 2010). Most symptomatic persons experience an acute systemic febrile illness that often includes headache, myalgia, or arthralgia; gastrointestinal symptoms and a transient maculopapular rash also are commonly reported (Watson et al. 2004, Hayes et al. 2005b, Zou et al. 2010). Less than 1% of infected persons develop neuroinvasive disease, which typically manifests as meningitis, encephalitis, or acute flaccid paralysis (Hayes et al. 2005b). WNV meningitis is clinically indistinguishable from aseptic meningitis due to most other viruses (Sejvar and Marfin 2006). Patients with WNV encephalitis usually present with seizures, mental status changes, focal neurologic deficits, or movement disorders (Sejvar and Marfin 2006). WNV acute flaccid paralysis is often clinically and pathologically identical to poliovirus-associated poliomyelitis, with damage of anterior horn cells, and may progress to respiratory paralysis requiring mechanical ventilation (Sejvar and Marfin 2006). WNV-associated Guillain-Barré syndrome has also been reported and can be distinguished from WNV poliomyelitis by clinical manifestations and electrophysiologic testing (Sejvar and Marfin 2006). Cardiac dysrhythmias, myocarditis, rhabdomyolysis, optic neuritis, uveitis, chorioretinitis, orchitis, pancreatitis, and hepatitis have been described rarely with WNV infection (Hayes et al. 2005b).

Clinical Evaluation and Diagnosis

WNV disease should be considered in the differential diagnosis of febrile or acute neurologic illnesses associated with recent exposure to mosquitoes, blood transfusion or organ transplantation, and of illnesses in neonates whose mothers were infected with WNV during pregnancy or while breastfeeding. In addition to other more common causes of encephalitis and aseptic meningitis (e.g., herpes simplex virus and enteroviruses), other arboviruses (e.g., La Crosse, St. Louis encephalitis, Eastern equine encephalitis, Western equine encephalitis and Powassan viruses) should also be considered in the differential etiology of suspected WNV illness.

WNV infections are most frequently confirmed by detection of anti-WNV immunoglobulin (Ig) M antibodies in serum or cerebrospinal fluid (CSF). The presence of anti-WNV IgM is usually good evidence of recent WNV infection, but may indicate infection with another closely related flavivirus (e.g., St. Louis encephalitis). Because anti-WNV IgM can persist in some patients for >1 year, a positive test result occasionally may reflect past infection unrelated to current disease manifestations. Serum collected within 8 days of illness onset may lack detectable IgM, and the test should be repeated on a convalescent-phase sample. IgG antibody generally is detectable shortly after the appearance of IgM and persists for years. Plaque-reduction neutralization tests (PRNT) can be performed to measure specific virus-neutralizing antibodies. A fourfold or greater rise in neutralizing antibody titer between acute- and convalescent-phase serum specimens collected 2 to 3 weeks apart may be used to confirm recent WNV infection and to discriminate between cross-reacting antibodies from closely related flaviviruses.

Viral culture and WNV NAAT can be performed on acute-phase serum, CSF, or tissue specimens. However, by the time most immunocompetent patients present with clinical symptoms, WNV RNA may not be detectable, thus negative results should not be used to rule out an infection. NAAT may have utility in certain clinical settings as an adjunct to detection of IgM. Among patients with West Nile fever, combining NAAT and IgM detection identified more cases than either procedure alone (Tilley et al. 2006). NAAT may prove useful in immunocompromised patients, when antibody development is delayed or absent. Immunohistochemical staining can detect WNV antigens in fixed tissue, but negative results are not definitive. See the Laboratory Diagnosis and Testing chapter for additional information.

Passive Surveillance and Case Investigation

WNV disease is a nationally-notifiable condition and is reportable in most, if not all, states and territories. Most disease cases are reported to public health authorities from public health or commercial laboratories; healthcare providers also submit reports of suspected cases. State and local health departments are responsible for ensuring that reported human disease cases meet the national case definitions. The most recent case definitions for confirmed and probable neuroinvasive and non-neuroinvasive domestic arboviral diseases were approved by the CSTE in 2011 (Appendix 1). Presumptive WNV-viremic donors are identified through universal screening of the blood supply; case definitions and reporting practices for viremic donors vary by jurisdiction and blood services agency.

All identified WNV disease cases and presumptive viremic blood donors should be investigated promptly. Jurisdictions may choose to interview the patient's health care provider, the patient, or both depending on information needs and resources. Whenever possible, the following information should be gathered:

- Basic demographic information (age, sex, race/ethnicity, state and county of residence)

- Clinical syndrome (e.g., asymptomatic blood donor, uncomplicated fever, meningitis, encephalitis, acute flaccid paralysis)
- Illness onset date and/or date of blood donation
- If the patient was hospitalized and if he/she survived or died
- Travel history in the four weeks prior to onset
- If the patient was an organ donor or a transplant recipient in the 4 weeks prior to onset
- If the patient was a blood donor or blood transfusion recipient in the 4 weeks prior to onset
- If the patient was pregnant at illness onset
- If the patient is an infant, was he/she breastfed before illness onset

If the patient donated blood, tissues or organs in the four weeks prior to illness onset, immediately inform the blood or tissue bank and public health authorities. Similarly, any WNV infections temporally-associated with blood transfusion or organ transplantation should be reported. Prompt reporting of these cases will facilitate the identification and quarantine of any remaining infected products and the identification of any other exposed recipients so they may be managed appropriately.

Passive surveillance systems are dependent on clinicians considering the diagnosis of an arboviral disease and obtaining the appropriate diagnostic test, and reporting of laboratory-confirmed cases to public health authorities. Because of incomplete diagnosis and reporting, the incidence of WNV disease is underestimated. Reported neuroinvasive disease cases are considered the most accurate indicator of WNV activity in humans because of the substantial associated morbidity. In contrast, reported cases of non-neuroinvasive disease are more likely to be affected by disease awareness and healthcare-seeking behavior in different communities and by the availability and specificity of laboratory tests performed. Surveillance data for non-neuroinvasive disease should be interpreted with caution and generally should not be used to make comparisons between geographic areas or over time.

Enhanced Surveillance Activities

Enhanced surveillance for human disease cases should be considered, particularly when environmental or human surveillance suggests that an outbreak is suspected or anticipated. Educating healthcare providers and infection control nurses about the need for arbovirus testing, and reporting of all suspected cases could increase the sensitivity of the surveillance system. This may be accomplished through distribution of print materials, participation in local hospital meetings and grand rounds, and providing lectures/seminars. Public health agencies should also work to establish guidelines and protocols with local blood collection agencies for reporting WNV viremic blood donors.

At the end of the year, an active review of medical records and laboratory results from local hospitals and associated commercial laboratories should be conducted to identify any previously unreported cases. In addition, an active review of appropriate records from blood collection agencies should be conducted to identify any positive donors that were not reported.

Environmental Surveillance

Mosquito-Based WNV Surveillance

Mosquito-based surveillance consists of the systematic collection of mosquito samples and screening them for arboviruses. Mosquitoes become infected with WNV primarily through taking blood meals from infected birds. However, WNV may be passed from infected female mosquitoes to their eggs, resulting in infected offspring (i.e., vertical transmission) (Anderson et al. 2006). Vertical transmission is likely responsible for virus maintenance over the winter in northern parts of the country, but the extent of its contribution to virus amplification and human risk during the peak transmission season is not well understood.

The principal enzootic and epidemic vectors vary regionally across the United States. In the northern states, *Culex pipiens* mosquitoes are the primary vectors (Savage et al 2007) with *Cx. salinarius* serving as an important vector in portions of the Northeast (Anderson et al. 2004, 2012, Molaei et al. 2006). *Culex quinquefasciatus* is the main vector in the southern states, and *Culex tarsalis* is an important vector in western states where it overlaps the distribution of *Cx. pipiens* and *Cx. quinquefasciatus* and likely enhances transmission in these areas (Reisen et al. 2005, Andreadis 2012). Therefore, mosquito-based surveillance programs for WNV in the United States primarily target these *Culex* species and may include other species suspected of contributing to transmission and human risk in local areas.

Mosquito-based surveillance is an integral component of an integrated vector management program and is the primary tool for quantifying WNV virus transmission and human risk (Moore et al. 1993). The principal functions of a mosquito-based surveillance program are to:

- Collect data on mosquito population abundance and virus infection rates in those populations.
- Provide indicators of the threat of human infection and disease and identify geographic areas of high-risk.
- Support decisions regarding the need for and timing of intervention activities (i.e., enhanced vector control efforts and public education programs).
- Monitor the effectiveness of vector control efforts.

Mosquito-based WNV monitoring has several positive attributes that contribute to its value in surveillance programs. These include:

- Quick turn-around of results. Mosquito samples can be processed quickly, usually within a few days. Some programs maintain local, in-house laboratories where samples are processed daily leading to rapid results.
- Collecting adult mosquitoes provides information about vector species community composition, relative abundance, and infection rates. This provides the data needed for rapid computation of infection indices and timely risk assessment.
- Maintaining consistent programs over the long-term provides a baseline of historical data that can be used to evaluate risk and guide control operations.

However, there are some limitations to mosquito-based surveillance. Virus may not be detected in the mosquito population if the infection rates are very low (i.e., early in the transmission season) or if only small sample sizes are tested. In addition, WNV transmission ecology varies regionally, and surveillance practices vary among programs (e.g., number and type of traps, testing procedures), which limits the

degree to which surveillance data can be compared across regions. This prohibits setting national thresholds for assessing risk and implementing interventions. Developing useful thresholds requires consistent effort across seasons to assure the surveillance indices and their association to human risk is comparable over time, and may require mosquito surveillance and human disease incidence data from several transmission seasons.

Specimen Collection and Types of Traps

Adult mosquitoes are collected using a variety of trapping techniques. Adequate sampling requires regular (weekly) trapping at fixed sites throughout the community that are representative of the habitat types present in the area. The commonly used types of mosquito traps for WNV surveillance sample host-seeking mosquitoes or gravid mosquitoes (carrying eggs) seeking a place to lay eggs (oviposition site).

Traps used to sample host-seeking mosquitoes are available in several configurations. The most commonly used are based on the CDC miniature light trap (Sudia and Chamberlain 1962). The CDC miniature light trap and similar configurations are lightweight and use batteries to provide power to a light source and fan motor. CO_2 (usually dry ice) is frequently used as an additional attractant. In some programs, the light sources are removed to minimize the capture of other nocturnal insects that are attracted to light, such as moths and beetles. In those cases CO_2 is the only attractant used. The advantage of light traps is that they collect a wide range of mosquito species (McCardle et al. 2004), which provides information about both primary and secondary vectors and a better understanding of the species composition in an area. A limitation of light traps is that the collections in certain locations and times may consist largely of unfed, nulliparous individuals (McCardle et al. 2004), which greatly reduces the likelihood of detecting WNV and other arboviruses. Also, not all mosquito species are attracted to light traps (Miller et al. 1969) and the numbers captured may not reflect the population size of a particular species (Bidlingmayer 1967). Light traps are of little use in sampling day-active time mosquitoes such as *Ae. albopictus* (Haufe and Burgess 1960, Unlu and Farajollah 2012), though these species can be collected in other traps such as the BG Sentinel (Krokel et al. 2006). However, the role of these species in WNV transmission is not well understood. The three major WNV vectors (*Cx. pipiens*, *Cx. quinquefasciatus* and *Cx. tarsalis)* can be collected in light traps, and some surveillance programs rely on light traps alone. This should be done with the understanding that, while effective in collecting large numbers of *Cx. tarsalis*, light traps typically collect relatively few *Cx. pipiens* or *Cx. quinquefasciatus* and the resulting small sample sizes may reduce the ability to accurately estimate WNV infection rates. Gravid traps are more effective in collecting *Cx. pipiens* and *Cx. quinquefasciatus* in urban areas (Andreadis and Armstrong 2007, Reisen et al. 1999).

Gravid traps target gravid females (i.e., those carrying mature eggs) of the *Cx. pipiens* complex (Reiter et al. 1986). The strength of gravid traps is that gravid females have previously taken a blood meal, which greatly increases the likelihood of capturing WNV infected individuals and thus detecting virus. Gravid traps can be baited attractants such as fresh or dry grass clippings infusions, rabbit chow infusions, cow manure, fish oil, or other materials that mimic the stagnant water in habitats where these species lay eggs. The different infusions vary in attractiveness (Burkett et al 2004, Lampman et al. 1996). It is advisable that infusion preparations are consistent within a surveillance program because variations may lead to changes in number and/or type of species captured. One limitation of gravid traps is that they selectively capture mosquitoes in the *Cx. pipiens* complex, and therefore provide limited information on species composition within a region (Reiter et al. 1986).

Several other traps may be used to collect mosquitoes for WNV monitoring. Collecting resting mosquitoes provides a good representation of vector population structure since un-fed, gravid and blood-fed females (as well as males) may be collected (Service 1992). Since resting populations typically provide samples that are representative of the population they can also provide more representative WNV infection rates. Resting mosquitoes can be collected using suction traps such as the CDC resting trap (Panella et al. 2011), and by using handheld or backpack mechanical aspirators (Nasci 1981) to remove mosquitoes from natural resting harborage or artificial resting structures (e.g., wooden resting boxes, red boxes, fiber pots and other similar containers) (Service 1992). Because of the wide variety of resting sites and the low density of resting mosquitoes in most locations, sampling resting populations is labor intensive and sufficient sample sizes are often difficult to obtain.

Host-baited traps, usually employing chickens or pigeons as bait, can collect mosquito vectors of interest in large numbers. However, these methods require use of live animals and adherence to animal use requirements and permitting. In addition, they are similar to light traps in that collections in certain locations and times may consist largely of unfed nulliparous individuals. Human landing collections have been used to accurately measure the population of human-feeding mosquitoes in an area and can be quite valuable in monitoring the effectiveness of adult mosquito control efforts. However, human landing collections may expose collectors to infected mosquitoes and is not recommended as a sampling procedure in areas where WNV transmission is occurring.

Specimen Handling and Processing

Since mosquito-based WNV surveillance relies on identifying WNV in the collected mosquitoes through detection of viral proteins, viral RNA, or live virus (see Diagnostics section), efforts should be made to handle and process the specimens in a way that minimizes exposure to conditions (e.g., heat, successive freeze-thaw cycles) that would degrade the virus. Optimally, a cold chain should be maintained from the time mosquitoes are removed from the traps to the time they are delivered to the processing laboratory, and through any short-term storage and processing. Transport mosquitoes from the field in a cooler either with cold packs or on dry ice. Sort and identify the mosquitoes to species on a chill-table if available. This is particularly important if the specimens will be tested for infectious virus or viral antigen. However, lack of a cold chain does not appear to reduce the ability to detect WNV RNA by RT-PCR (Turell et al. 2002).

Mosquitoes are generally tested in pools of 50 to 100 specimens grouped by species, date, and location of collection. Larger pool sizes may result in a loss of sensitivity (Sutherland and Nasci 2007). Follow package instructions that may indicate smaller pool sizes if using a commercial WNV assay – see Diagnostics section. Usually only female mosquitoes are tested in routine WNV surveillance programs. If WNV screening is not done immediately after mosquito identification and pooling, the pooled samples should be stored frozen, optimally at -70^{O}C, but temperatures below freezing may suffice for short-term storage.

Mosquito-Based Surveillance Indicators

Data derived from mosquito surveillance include estimates of mosquito species abundance and WNV infection rate in those mosquito populations. The indices derived from those data vary in information content, ability to be compared over time and space, and association with WNV transmission levels and levels of human risk. The five indicators that have commonly been used are:

- Vector abundance
- Number of positive pools
- Percent of pools positive
- Infection rate
- Vector index

Vector abundance provides a measure of the relative number of mosquitoes in an area during a particular sampling period. It is simply the total number of mosquitoes of a particular species collected, divided by the number of trapping nights conducted during a specified sampling period, and is expressed as the number/trap night. High mosquito densities have been associated with arboviral disease outbreaks (Olson et al. 1979, Eldridge 2004); thus, risk assessments involve estimating mosquito abundance (Tonn et al. 1969, Sheppard et al. 1969, Bang et al. 1981). In the WNV outbreak in Maricopa County, AZ, 2010, *Cx. quinquefasciatus* densities were higher in outbreak areas compared to non-outbreak areas (Godsey et al. 2012, Colborn et al. 2013 in press). Vector abundance can provide measures of population abundance that are useful as thresholds in conducting pro-active integrated vector management and in monitoring the outcome of mosquito control efforts. However, high mosquito abundance may occur in the absence of virus or detectable virus amplification, and WNV outbreaks often occur when abundance is low but the mosquito population is older and the infection rate is high. As with all environmental surveillance efforts, numerous trapping locations and regular collecting are required to obtain spatially and temporally representative data.

Number of positive pools is the total of the number of WNV positive mosquito pools detected in a given surveillance location and period. It is sometimes separated by species, or may be a tally of the total number of positive pools for all species tested. While detection of positive pools provides evidence of WNV activity in an area, expression of the total number of pools without a denominator (e.g., number of pools tested) limits the comparative value and the ability of this index to convey relative levels of WNV transmission activity. Expressing WNV activity as the number of positive pools is not recommended since the same data can be used to produce more informative indices.

Percent of pools positive is calculated by expressing the number of WNV-positive pools divided by the total number of pools tested as a percentage. This is an improvement over number of positive pools as an index of relative WNV transmission activity since it provides a rough estimate of the rate of WNV in the mosquitoes tested and can be used to compare WNV activity over time and place. However, the comparative value is limited unless the number of pools tested is relatively large and the number of mosquitoes per pool remains constant. Using percent of pools positive as an index of WNV activity is not recommended; as with the number of positive pools index, the same data can be used to produce the more informative Infection Rate and Vector Index measures of WNV activity.

The infection rate in a vector population is an estimate of the prevalence of WNV-infected mosquitoes in the population and is a good indicator of human risk. There are two commonly used methods for calculating and expressing the infection rate. The minimum infection rate (MIR) for a given mosquito species is calculated by dividing the number of WNV-positive pools by the total number of mosquitoes

tested (not the number of pools tested). The MIR is based on the assumption that infection rates are generally low and that only one mosquito is positive in a positive pool. The MIR can be expressed as a proportion or percent of the sample that is WNV positive, but is commonly expressed as the number infected/1000 tested because infection rates are usually low. The maximum likelihood estimate (MLE) of the infection rate does not require the assumption of one positive mosquito per positive pool, and provides a more accurate estimate when infection rates are high (Gu et al. 2008); thus, it is the preferred method of estimating infection rate particularly during outbreaks. The MLE and MIR are similar when infection rates are low. Estimates of infection rate provide a useful, quantitative basis for comparison, allowing evaluation of changes in infection rate over time and space. These indices also permit use of variable pool numbers and pool sizes while retaining comparability. Infection rate indices have been used successfully in systems associating infection rates with human risk (Bell et al. 2005). Larger sample sizes improve accuracy of the infection rate indicators. The MLE requires more complex calculations than the MIR; however, a Microsoft Excel® Add-In to compute infection rates from pooled data is available (http://www.cdc.gov/ncidod/dvbid/westnile/software.htm).

The Vector Index (VI) is an estimate of the abundance of infected mosquitoes in an area and incorporates information describing the vector species that are present in the area, relative abundance of those species, and the WNV infection rate in each species into a single index (Gujaral et al. 2007, Bolling et al. 2009, Jones et al. 2011). The VI is calculated by multiplying the average number of mosquitoes collected per trap night by the proportion infected with WNV, and is expressed as the average number of infected mosquitoes collected per trap night in the area during the sampling period. In areas where more than one WNV vector mosquito species is present, a VI is calculated for each of the important vector species and the individual VIs are summed to represent a combined estimate of the infected vector abundance. By summing the VI for the key vector species, the combined VI accommodates the fact that WNV transmission may involve one or more vectors in an area. Increases in VI reflect increases in risk of human disease (Bolling et al. 2009, Jones et al. 2011, Kwan et al. 2012, Colborn et al. 2013 in press) and have demonstrated significantly better predictive ability than estimates of vector abundance or infection rate alone, clearly demonstrating the value of combining information for vector abundance and WNV infection rates to generate a more meaningful risk index (Bolling et al. 2009). As with other surveillance indicators, the accuracy of the Vector Index is dependent upon the number of trap nights used to estimate abundance and the number of specimens tested for virus to estimate infection rate. Instructions for calculating the Vector Index in a system with multiple vector species present are in Appendix 2.

Use of Mosquito-Based Indicators

The mosquito-based surveillance indicators have two important roles in WNV surveillance and response programs. First, they can provide quantifiable thresholds for proactive vector control efforts. By identifying thresholds for vector abundance and infection rate that are below levels associated with disease outbreaks, integrated vector management programs can institute proactive measures to maintain mosquito populations at levels below which WNV amplification can occur. Second, if thresholds related to outbreak levels of transmission can be identified, surveillance can be used to determine when proactive measures have been insufficient to dampen virus amplification and more aggressive measures, such as wide-scale aerial application of mosquito adulticides and more aggressive public education messaging, are required to prevent or stop an outbreak.

Bird-Based WNV Surveillance

WNV amplifies in nature by replicating to high levels in a variety of bird species which then transmit the virus to mosquitoes during several days of sustained high-level viremia. In addition to infection from mosquito bites, some birds may get infected by consuming infected prey items such as insects, small mammals, or other birds, or in rare cases, from direct contact with other infected birds. Thus, monitoring infections in wild and/or captive birds can be effective for determining whether WNV may be active in a region, and in some cases, provide a quantitative index of risk for human infections. A hallmark of the North American strain of WNV is its propensity to kill a number of the birds it infects (326 affected species were reported to ArboNET through 2012), particularly among the corvids (species of the family Corvidae, including crows, ravens, magpies and jays) and a few other sensitive species (Komar 2003). More than a decade after the introduction of WNV across the North America continent, numerous bird species continue to suffer mortality from WNV infection, albeit at lower rates. Robust studies of the development of avian resistance to fatal WNV infections are lacking. However, one study of the American crow suggested that resistance is developing at a rate of approximately 1% per year (Reed et al. 2007). There are two basic strategies for avian mortality surveillance of WNV: virus testing of selected carcasses and spatial analysis of dead bird sightings.

While several bird species experience high mortality from WNV infection, most birds survive the infection and develop a lifelong immune response that can be detected by serology. Detection of antibodies in young birds, or seroconversions detected in older birds that are serially sampled is another approach to monitoring WNV transmission (Komar 2001).

Dead Bird Reporting

Dead birds need not be collected and tested in order to be useful in WNV surveillance programs. Simply collecting information about the temporal and spatial patterns of bird deaths in an area has provided information about WNV activity. In order to develop a dead bird reporting WNV surveillance program, public participation is essential and must be encouraged through an effective public education and outreach program. A database should be established to record and analyze dead bird sightings with the following suggested data: Caller identification and call-back number, date observed, location geocoded to the highest feasible resolution, species, and condition. Birds in good condition (unscavenged and without obvious decomposition or maggot infestation) may be sampled or retrieved for sampling and laboratory testing (see Avian morbidity/mortality testing).

Dead bird reporting systems, though they are passive surveillance systems, provide the widest surveillance net possible, extending to any area where a person is present to observe a dead bird. A strong carcass reporting system can guide selective sampling of carcasses for WNV testing. These systems have been used with some success to estimate risk of human infection (Eidson et al. 2001, Mostashiari et al. 2003, Carney et al. 2011)

Though useful, there are limitations to dead bird surveillance systems. Maintaining public interest and willingness to participate is essential to these programs, but is difficult to maintain. The surveillance is passive and qualitative, and can only be used to assess risk of infection to people in areas where sufficient data are collected to populate risk models such as DYCAST (Carney et al. 2011) and SaTScan (Mostashari et al. 2003). Over time, bird populations are becoming resistant to morbidity and mortality (Reed et al. 2009). A system for carcass reporting needs to be developed and communicated to multiple

agencies in a community and to the public (Eidson 2001). This system should include assistance to the public for identifying birds (specialized websites are available). Other causes of bird mortality may produce a false alarm for WNV activity. However, this alarm might alert the public health and wildlife disease communities to other pathogens or health threats. A subset of reported bird deaths should be investigated to confirm WNV activity through carcass testing.

Avian Morbidity/Mortality Testing

In programs where the objective of avian morbidity/mortality testing is simply early detection of WNV activity and not production of a quantitative index of human risk, testing of moribund or dead birds should be initiated when local adult mosquito activity begins in the spring and continue as long as local WNV activity remains undetected in the area. Once WNV is detected in dead birds in an area, or if prevention and control actions have been initiated, continued detection of WNV in carcasses in that area does not provide additional information about WNV activity and is not necessary or cost-effective. However, the number of WNV-infected dead birds can contribute to an effective human risk index (Kwan et al. 2012a).

Contact with WNV-infected carcasses presents a potential health hazard to handlers (Fonseca et al. 2005). Appropriate biosafety precautions should be taken when handling carcasses in the field and in the laboratory. More detailed guidelines for sampling avian carcasses are available in Appendix 3.

To maximize sensitivity of this surveillance system, a variety of bird species should be tested, but corvids should be emphasized if they are present (Nemeth et al. 2007a). In dead corvids and other birds, bloody pulp from immature feathers, and tissues collected at necropsy such as brain, heart, kidney, or skin harbor very high viral loads, and any of these specimen types is sufficient for sensitive detection of WNV (Panella et al. 2001, Komar et al. 2002, Docherty et al. 2004, Nemeth et al. 2009, Johnson et al. 2010). Oral swabs and breast feathers are easy specimens to collect in the field, avoid the need to transfer dead birds to the laboratory, do not require a cold chain, and are effective for detecting WNV in dead corvids (Komar et al. 2002, Nemeth et al. 2009). They are less sensitive for WNV detection in non-corvids; however, the reduced sensitivity of testing non-corvids using these tissue types can be offset by sampling more carcasses. The number of bird specimens tested will be dependent upon resources and whether WNV-infected birds have already been found in the area; triage of specimens by species or by geographic location may be appropriate in some jurisdictions.

Several studies have demonstrated the effectiveness of avian mortality testing for early detection of WNV activity in several studies (Eidson et al. 2001, Julian et al. 2002, Guptill et al. 2003, Nemeth et al. 2007b, Patnaik et al. 2007, Kwan et al. 2012a). Wildlife rehabilitation clinics can be a good source of specimens derived from carcasses (Nemeth et al. 2007b). Collecting samples from living birds that are showing signs of illness requires the assistance of a veterinarian or wildlife technician. Dead crows and raptors alarm the public and carcasses are easily spotted (Ward et al. 2008). However, in regions with few or no crows, carcasses may be less obvious. Eye aspirates have been shown to be a sensitive and fast sampling protocol for WNV detection in corvid carcasses brought to the laboratory for testing (Lim et al 2009).

Live Bird Serology

The use of living birds as sentinels for monitoring WNV transmission requires serially blood-sampling a statistically valid number of avian hosts. Captive chickens, frequently referred to as sentinel chickens, (though other species have been used) provide the most convenient source of blood for this purpose. Blood may be collected from a wing vein, the jugular vein, or on Nobuto® strips by pricking the chicken's comb with a lancet. There is no standard protocol for implementing a sentinel chicken program. It can be tailored to the specific circumstances of each surveillance jurisdiction, though sentinel chicken systems generally employ flocks of 6-10 birds at each site and bleed each bird weekly or every other week throughout the WNV transmission season. Sentinel chicken–based WNV surveillance systems have provided evidence of WNV transmission several weeks in advance of human cases (Healy et al. 2012).

While serially sampling free-ranging bird species is very labor intensive, it can provide information about seroconversion in amplifier hosts, similar to the data provided by sentinel chickens. Quantifying seroprevalence in free-ranging birds may provide additional information that benefits surveillance programs (Komar 2001). For example, a serosurvey of the local resident bird population (in particular, juvenile birds) following the arbovirus transmission season may help determine which local species may be important amplifiers of WNV in the surveillance area. This in turn could be used to map areas of greatest risk in relation to the populations of amplifier hosts. Furthermore, a serosurvey of adult birds just prior to arbovirus transmission season can detect pre-existing levels of antibody in the bird population. High levels would suggest less opportunity for WNV amplification because many adult bird species transfer maternal antibodies to their offspring, which can delay or inhibit WNV amplification among the population of juvenile birds that emerges each summer. In Los Angeles, California, serosurveys of local amplifier hosts during winter determined that subsequent outbreaks occurred only after seroprevalence dipped below 10% in these birds (Kwan et al. 2012b).

There are numerous positive attributes of sentinel chicken and other live-bird serology surveillance systems. Sentinel chickens are captive, so a seroconversion event indicates local transmission and presence of infected mosquitoes in the area. Chickens are preferred blood-feeding hosts of *Cx. pipiens* and *Cx. quinquefasciatus*, which are important urban vectors of WNV. Chickens can be used to monitor seroconversions of multiple arboviruses of public health importance (i.e., WN, SLE, WEE and EEE viruses) simultaneously. However, there are also a number of important limitations related to these systems. Determination that a chicken has seroconverted occurs typically 3-4 weeks after the transmission event has occurred, and reporting of a positive chicken may not precede the first local case of human disease caused by WNV (Patnaik et al. 2007, Kwan et al. 2010, Unlu et al. 2009). However, a rapid screening assay can cut this time in half (Cheng & Su 2011) and improve the predictive capability of sentinel chicken systems (Healy 2012). Use of sentinel birds requires institutional animal use and care protocols, and other authorization permits. Linking patterns in sentinel chicken seroconversion with human risk requires multiple years of data collection.

Horses and Other Vertebrates

Horses are susceptible to encephalitis due to WNV infection; thus, equine cases of WNV-induced encephalitis may serve a sentinel function in the absence of other environmental surveillance programs. Equine health is an important economic issue, so severe disease in horses comes to the attention of the veterinary community. Use of horses as sentinels for active WNV surveillance is theoretically possible,

but practically infeasible. Widespread use of equine WNV vaccines decreases the incidence of equine WNV disease, and survivors of natural infections are protected from disease, reducing the usefulness of equines as sentinels. Veterinarians, veterinary service societies/agencies, and state agriculture departments are essential partners in any surveillance activities involving WNV infections in horses. Equine disease due to WNV is rare in tropical ecosystems. However, WNV frequently infects horses in the tropics. Detection of seroconversions in horses has been suggested as a sentinel system to detect risk of WNV transmission to people in Puerto Rico and other tropical locations (Phoutrides et al. 2011, Mattar et al. 2011).

Small numbers of other mammal species have been affected by WNV. Dead squirrels are tested for WNV along with dead birds in some jurisdictions (Reisen et al. 2013). Among domestic mammals, the most important has been the camelids, such as llamas and alpacas (Whitehead & Bedenice 2009). As with horses, these come to the attention of veterinarians and any veterinary case of disease due to WNV may be used for passive surveillance. Dogs and cats become infected with WNV (Austgen et al. 2004) and have been shown to seroconvert to WNV during human disease outbreaks (Kile et al. 2005, Resnick et al. 2008); however, their value as primary WNV surveillance tools has not been evaluated and is not recommended at this time. There is no evidence that dogs or cats develop sufficient viremia to become amplifier hosts (Austgen et al. 2004).

Evaluation of Environmental Surveillance Systems

The objective of environmental surveillance is to detect local WNV activity in advance of transmission to humans in order to guide the implementation of interventions and to prevent human infections and disease from occurring. The methods for mosquito and bird surveillance outlined above are widely used to monitor WNV transmission levels, but few studies have directly compared the performance of these early warning systems in their ability to measure or predict human risk.

Single factor surveillance systems, in which only one method of surveillance is used to monitor WNV activity and predict risk of subsequent human infection, have been shown to give valuable early information (Bolling et al. 2009, Carney et al. 2011, Ginsberg et al. 2010. Jones et al. 2011). Comparisons of single-factor surveillance systems have been done on a regional basis. In comparing mosquito and sentinel chicken systems in Louisiana, Unlu et al. (2009) demonstrated that positive mosquito pools occurred before detection of human cases, but seroconversions of sentinel chickens were detected after human cases occurred. They also compared mosquito trapping methods and found that while gravid traps collected more total mosquitoes and WNV-positive mosquitoes, sentinel chicken box traps detected WNV positive mosquitoes earlier. Another study showed that sentinel chicken seroconversions were the first indication of WNV activity (Blackmore et al. 2003). A comparison of mosquito, sentinel chicken and dead-bird systems found that all provided evidence of WNV transmission before human cases occurred, but that mosquito surveillance performed better than the other systems (Healy et al. 2012).

A few WNV surveillance programs have been developed that incorporate multiple WNV monitoring systems and other environmental parameters (e.g., temperature and rainfall patterns). These generally require a historical database of environmental and epidemiological surveillance data in order to develop and validate the complex quantitative associations that they employ. An example of a multi-factor system is the California Mosquito-Borne Virus Surveillance and Response Plan (CMVRA) (http://westnile.ca.gov/downloads.php?download_id=2321&filename=2012 CA Response Plan 5-8-

12.pdf) which uses mosquitoes, birds and environmental conditions to perform a mosquito-borne virus risk assessment. Individual components of the system are scored to provide a value that corresponds to a normal season, emergency planning or epidemic situation.

Kwan et al. (2012) compared the single-factor, mosquito-based Vector Index to the single-factor bird-based Dynamic Continuous-Area Space-Time (DYCAST) system, and the multiple-factor CMVRA system and found that the CMVRA system using avian, mosquito and environmental information provided better prediction of WNV high risk periods. However, the Vector Index and DYCAST systems also provided useful, predictive information.

ArboNET

ArboNET, the national arboviral surveillance system, was developed by CDC and state health departments in 2000 in response to the emergence of WNV in 1999 (CDC 2010). In 2003, the system was expanded to include other domestic and imported arboviruses of public health significance. ArboNET is an electronic surveillance system administered by CDC's Division of Vector-Borne Diseases (DVBD). Human arboviral disease data are reported from all states and three local jurisdictions. In addition to human disease cases, ArboNET maintains data on arboviral infections among human viremic blood donors, non-human mammals, sentinel animals, dead birds, and mosquitoes.

Data Collected

Variables collected for human disease cases include patient age, sex, race, and county and state of residence; date of illness onset; case status (i.e., confirmed, probable, suspected, or not a case); clinical syndrome (e.g., encephalitis, meningitis, or uncomplicated fever); whether illness resulted in hospitalization; and whether the illness was fatal. Cases reported as encephalitis (including meningoencephalitis), meningitis, or acute flaccid paralysis are collectively referred to as neuroinvasive disease; others are considered non-neuroinvasive disease. Acute flaccid paralysis can occur with or without encephalitis or meningitis. Information regarding potential non-mosquito-borne transmission (e.g., blood transfusion or organ transplant recipient, breast-fed infant, or laboratory worker) and recent donation of blood or solid organs should be reported if applicable. An optional set of variables related to clinical symptoms and diagnostic testing was added to ArboNET in 2012.

Blood donors identified as presumptively viremic by NAAT screening of the donation by a blood collection agency are also reported to ArboNET. For the purposes of national surveillance, a presumptively viremic donor is defined as a person with a blood donation that meets at least one of the following criteria: a) One reactive NAAT with a signal-to-cutoff (S/CO) ratio ≥ 17; or b) Two reactive NAATs. Reporting of donors who do not meet these criteria should wait until follow-up testing is completed. The date of blood donation is reported in addition to the variables routinely reported for disease cases.

WNV disease in non-human mammals (primarily horses) and WNV infections in trapped mosquitoes, dead birds, and sentinel animals are also reported to ArboNET. Variables collected for non-

human WNV infections include species, state and county, and date of specimen collection or symptom onset. The total number of mosquitoes or birds tested weekly can also be reported.

Detailed descriptions of all variables collected by ArboNET and instructions for reporting are included in the ArboNET User Guide, which can be requested from DVBD by phone (970-261-6400) or email (dvbid2@cdc.gov).

Data Transmission

Jurisdictions can transmit data to ArboNET using one or more of four methods supported by DVBD: 1) jurisdictions that have a commercially- or state-developed electronic surveillance system can upload records from their system using an Extensible Markup Language (XML) message; 2) jurisdictions can upload records from a Microsoft® Access database provided by CDC DVBD using an XML message; 3) jurisdictions may enter records manually using a CDC web site (https://wwwn.cdc.gov/arbonet/); or 4) jurisdictions can report cases through the CDC NEDSS Base System (NBS) and DVBD will download records directly from NBS to ArboNET. ArboNET data are maintained in a Microsoft® Structured Query Language (SQL) Server® database inside CDC's firewall. Users can access data via a password-protected website, but are limited to viewing data only from their own jurisdiction. The ArboNET website and database are maintained by CDC information technology staff and are backed up nightly.

Dissemination of ArboNET Data

CDC epidemiologists periodically review and analyze ArboNET surveillance data and disseminate results to stakeholders via direct communication, briefs in *Morbidity and Mortality Weekly Reports* and Epi-X, comprehensive annual summary reports, and DVBD's website. CDC also provides ArboNET data to the U.S. Geological Survey to produce maps of domestic arboviral activity which are then posted on its website (http://diseasemaps.usgs.gov/). Surveillance reports are typically updated weekly during the transmission season and monthly during the off-season. A final report is released in the spring of the following year. CDC provides limited-use ArboNET data sets to the general public by formal request. Data release guidelines have been updated to be consistent with those developed by CDC and the Council of State and Territorial Epidemiologists (CSTE).

Limitations of ArboNET Data

Human surveillance for WNV disease is largely passive, and relies on the receipt of information from physicians, laboratories, and other reporting sources by state health departments. Neuroinvasive disease cases are likely to be consistently reported because of the substantial morbidity associated with this clinical syndrome. Non-neuroinvasive disease cases are inconsistently reported because of a less severe spectrum of illness, geographic differences in disease awareness and healthcare seeking behavior, and variable capacity for laboratory testing. Surveillance data for non-neuroinvasive disease cases should be interpreted with caution and generally should not be used to make comparisons between geographic areas or over time. Accordingly, ratios of reported neuroinvasive disease cases to non-neuroinvasive disease cases should not be interpreted as a measure of WNV virulence in an area.

ArboNET does not routinely collect information regarding clinical signs and symptoms or diagnostic laboratory test results. Therefore, misclassification of the various syndromes caused by WNV cannot be detected. In addition, ArboNET does not routinely collect information regarding the specific laboratory methods used to confirm each case. The most common laboratory tests used to diagnose arboviral disease are IgM antibody-capture enzyme immunoassays (EIA. Although these assays are relatively specific, false-positive results and cross-reactions occur between WNV and other flaviviruses (e.g., St. Louis encephalitis or Dengue viruses). Positive IgM results should be confirmed by additional tests, especially plaque-reduction neutralization. However, such confirmatory testing often is not performed.

While the electronic mechanisms for data transmission allow for rapid case reporting, the inclusion of both clinical and laboratory criteria in the surveillance case definition creates delays between the occurrence of cases and their reporting. Provisional data are disseminated to allow for monitoring of regional and national epidemiology during the arboviral transmission season. However, these reports generally lag several weeks behind the occurrence of the cases comprising them, and the data may change substantially before they are finalized. For this reason, provisional data from the current transmission season should not be combined with or compared to provisional or final data from previous years.

The collection and reporting of non-human WNV surveillance data are highly variable among states (and even between regions within states) and changes from year to year. Because of this variability, non-human surveillance data should not be used to compare arboviral activity between geographic areas or over time.

For more information about ArboNET, please contact the Division of Vector-Borne Diseases by phone: 970-261-6400 or email: dvbid2@cdc.gov.

Laboratory Diagnosis and Testing

Public health programs responsible for monitoring WNV activity and implementing effective interventions rely on accurate information about human disease and environmental indicators of risk. To be successful, these programs must be supported by diagnostic laboratories capable of performing the required range of tests. Numerous serological and virus detection testing protocols have been developed to diagnose human infections and cases, and to enable environmental surveillance programs to monitor the presence of WNV in vector mosquitoes and non-human vertebrate hosts. The characteristics and uses of these tests are outlined in the sections below.

Biocontainment - Laboratory Safety Issues

Laboratory-associated infections with WNV have been reported in the literature. The Subcommittee on Arbovirus Laboratory Safety in 1980 reported 15 human infections from laboratory accidents. One of these infections was attributed to aerosol exposure. In addition, two parenteral inoculations have been reported during work with animals.

WNV may be present in blood, serum, tissues and CSF of infected humans, birds, mammals and reptiles. The virus has been found in the oral fluids and feces of birds. Parenteral inoculation with contaminated materials poses the greatest hazard; contact exposure of broken skin is a possible risk. Sharps precautions should be strictly adhered to when handling potentially infectious materials. Workers performing necropsies on infected animals may be at high risk of infection.

Manipulation of infectious stocks of WNV should be conducted in BSL-3 laboratory space. However, diagnostic specimens from any source that have not been tested may be handled in BSL-2 conditions. If the specimen is suspected of harboring infectious WNV, it is recommended that it be manipulated in BSL-3 conditions, such as within a Class II Type A biological safety cabinet. Necropsies of birds or other animals expected to harbor high-titered WNV infections should be practiced under BSL-3 conditions. Containment specifications are available in the Centers for Disease Control and Prevention/National Institutes of Health publication Biosafety in Microbiological and Biomedical Laboratories (BMBL). This document can be found online at: http://www.cdc.gov/biosafety/publications/bmbl5/.

Shipping of Agents

Shipping and transport of WNV and clinical specimens should follow current International Air Transport Association (IATA) and Department of Commerce recommendations. Because of the threat to the domestic animal population, a U.S. Department of Agriculture (USDA) shipping permit is required for transport of known WNV isolates. For more information, visit the IATA dangerous goods Web site at: http://www.iata.org/publications/dgr/Pages/index.aspx, and the USDA Animal and Plant Health Inspection Service (APHIS), National Center for Import /Export's Web site at: http://www.aphis.usda.gov/animal_health/vet_biologics/vb_notices.shtml.

Human Diagnosis

In most patients, infection with WNV and many of the other arboviruses that cause encephalitis is clinically inapparent or causes a nonspecific viral syndrome. Numerous pathogens cause encephalitis, aseptic meningitis and febrile disease with clinical symptoms and presentations similar to those caused by WNV and should be considered in the differential diagnosis. Definitive diagnosis of WNV can only be made by laboratory testing using specific reagents. Selection of diagnostic test procedures should take into consideration the range of pathogens in the differential diagnosis, the criteria for classifying a WNV case as confirmed or probable, as well as the capability of the primary and confirming diagnostic laboratories. The case definition for neuroinvasive and non-neuroinvasive disease caused by WNV and the other arboviral pathogens present in the United States specifies presence of clinically-compatible symptoms accompanied by laboratory evidence of recent infection (Appendix 1).

Appropriate selection of diagnostic procedures and accurate interpretation of findings requires information describing the patient and the diagnostic specimen. For human specimens, the following data must accompany sera, CSF or tissue specimens for results to be properly interpreted and reported: 1) symptom onset date (when known); 2) date of sample collection; 3) unusual immunological status of patient (e.g., immunosuppression); 4) state and county of residence; 5) travel history (especially in flavivirus-endemic areas); 6) history of prior vaccination (e.g., yellow fever, Japanese encephalitis, or Tick-borne encephalitis viruses); and 7) brief clinical summary including clinical diagnosis (e.g., encephalitis, aseptic meningitis). Minimally, onset and sample collection dates are required to perform and interpret initial screening tests. The remaining information is required to evaluate any specimens positive on initial screening. If possible, a convalescent serum sample taken at least 14 days following the acute sample should be obtained to enable confirmation by serological testing.

Serology

The front-line screening assay for laboratory diagnosis of human WNV infection is the IgM assay. Currently, the FDA has cleared four commercially-available test kits from different manufacturers, for detection of WNV IgM antibodies. These four kits are used in many commercial and public health laboratories in the United States. In addition the CDC-defined IgM and IgG ELISA can be used; protocols and reagents are available from the CDC DVBD Diagnostic Laboratory (Martin et al. 2000; Johnson et al. 2000). There is also a microsphere-based immunoassay for the detection of IgM antibodies that can differentiate WNV from SLE (Johnson et al. 2005).

Because the IgM and IgG ELISA tests can cross-react between flaviviruses (e.g., SLE, dengue, yellow fever, WN), they should be viewed as screening tests only. For a case to be considered confirmed, serum samples that are antibody-positive on initial screening should be evaluated by a more specific test; currently the plaque reduction neutralization test (PRNT) is the recommended test for differentiating between flavivirus infections. Though WNV is the most common cause of arboviral encephalitis in the United States, there are several other arboviral encephalitides present in the country and in other regions of the world. Specimens submitted for WNV testing should also be tested by ELISA and PRNT against other arboviruses known to be active or be present in the area or in the region where the patient traveled.

Virus Detection Assays

Numerous procedures have been developed for detecting viable WNV, WNV antigen or WNV RNA in human diagnostic samples, many of which have been adapted to detecting WNV in other vertebrates and in mosquito samples. These procedures vary in their sensitivity, specificity, and time required to conduct the test (Table 1). Among the tests listed in the Table 1, the VectorTest, Antigen Capture ELISA, and Rapid Analyte Measurement Platform were developed specifically for testing mosquitoes for WNV antigen, were subsequently adapted to testing bird and other vertebrate samples, and are not used for human diagnostic testing. Additional details about these tests are contained in the following sections on mosquito and bird diagnostic tests. The remaining tests have been used in various human diagnostic assays.

Table 1. Characteristic sensitivity and time required for West Nile infectious virus, viral RNA or viral antigen detection assays.

Test	Detects	Detection Level (pfu/ml)	Assay Time
VectorTest®	Viral antigen	100,000	15 min
Antigen Capture ELISA	Viral antigen	10,000	24 hours
Rapid Analyte Measurement Platform (RAMP) ®	Viral antigen	1,500	90 min
Virus isolation in suckling mouse	Infectious virus	100	4-10 days
Virus isolation in Vero cell culture	Infectious virus	100	3 days
Standard RT-PCR	Viral RNA	5	8 hours
Nucleic Acid Sequence Based Amplification (NASBA)	Viral RNA	0.1	4 hours
TaqMan® (Real Time RT-PCR)	Viral RNA	0.1	4 hours
Transcription Mediated Amplification	Viral RNA	0.02	4 hours

Among the most sensitive procedures for detecting WNV in samples are those using RT-PCR to detect WNV RNA in human CSF, serum and other tissues. Fluorogenic 5' nuclease techniques (real-time PCR) and nucleic acid sequence-based amplification (NASBA) methods have been developed and validated for specific human diagnostic applications (Briese et al. 2000; Shi et al. 2001; Lanciotti et al. 2000; Lanciotti et al. 2001) and for detecting WNV RNA in blood donations (Busch et al. 2005).

WNV presence can be demonstrated by isolation of viable virus from samples taken from clinically ill humans. Appropriate samples include CSF (serum samples may be useful very early in infection) and brain tissue (taken at biopsy or postmortem). Virus isolation should be performed in known susceptible mammalian (e.g., Vero) or mosquito cell lines (e.g., C6/36). Mosquito origin cells may not show obvious cytopathic effect and must be screened by immunofluorescence or RT-PCR. Appropriate samples for virus isolation from clinically ill humans include CSF (serum samples may be useful very early in infection) and brain tissue (taken at biopsy or postmortem). Confirmation of virus isolate identity can be accomplished by indirect immunofluorescence assay (IFA) using virus-specific monoclonal antibodies or nucleic acid detection. The IFA using well-defined murine monoclonal antibodies (MAbs) is an efficient, economical, and rapid method to identify flaviviruses. MAbs are available that can differentiate WNV and SLE virus from each other and from other flaviviruses. Incorporating MAbs specific for other arboviruses known to circulate in various regions will increase the rapid diagnostic capacities of state

and local laboratories. Nucleic acid detection methods including RT-PCR, TaqMan and nucleic acid sequence based amplification (NASBA) methods may be used to confirm virus isolates as WNV (Briese et al. 2000; Shi et al. 2001; Lanciotti et al. 2000).

While these tests can be quite sensitive, virus isolation and RT-PCR to detect WNV RNA in sera or CSF of clinically ill patients have limited utility in diagnosing human WNV neuroinvasive disease due to the low level viremia present in most cases at the time of clinical presentation. However, one study demonstrated that combining detection of IgM with detection of WNV RNA in plasma significantly increased the number of WNV non-neuroinvasive (i.e., fever) cases detected (Tilley et al 2006). Virus isolation or RT-PCR on serum may be helpful in confirming human WNV infection in immunocompromised patients when antibody development is delayed or absent.

Immunohistochemistry (IHC) using virus-specific MAbs on brain tissue has been very useful in identifying both human and avian cases of WNV infection. In suspected fatal cases, IHC should be performed on formalin fixed autopsy, biopsy, and necropsy material, ideally collected from multiple anatomic regions of the brain, including the brainstem, midbrain, and cortex (Bhatnagar et al. 2007).

To maintain Clinical Laboratory Improvements Amendments (CLIA) certification, CLIA recommendations for performing and interpreting human diagnostic tests should be followed. Laboratories doing WNV serology or RNA-detection testing are invited to participate in the annual proficiency testing that is available from CDC's Division of Vector-Borne Diseases in Fort Collins, Colorado. To obtain additional information about the proficiency testing program and about training in arbovirus diagnostic procedures, contact the Division of Vector-Borne Diseases by phone: 970-261-6400 or email: dvbid2@cdc.gov.

Non-human laboratory diagnosis

The following sections discuss techniques that may be applied to mosquitoes and non-human vertebrate specimens that are collected for the purpose of diagnosing WNV infections. Many of the virus detection procedures are identical those described in the Human Diagnosis section, but several procedures have been developed specifically for these sample types.

Mosquitoes

Identification and Pooling

Mosquitoes should be identified to species or lowest taxonomic unit. Specimens are placed into pools of 50 specimens or less based on species, sex, location, trap- type, and date of collection. Larger pool sizes can be used in some assays with loss of sensitivity (Sutherland and Nasci 2007). If resources are limited, testing of mosquitoes for surveillance purposes can be limited to the primary vector species.

Homogenizing and Centrifugation

After adding the appropriate media, mosquito pools can be macerated or ground by a variety of techniques including mortar and pestel, vortexing sealed tubes containing one or more copper clad BBs,

or by use of tissue homogenizing apparatus that are commercially available (Savage et al. 2007). After grinding, samples are centrifuged and an aliquot is removed for WNV testing. Because mosquito pools may contain WNV and other pathogenic viruses which may be aerosolized during processing, laboratory staff should take appropriate safety precautions including use of a Class II Type A biological safety cabinet and wearing appropriate personal protective equipment (PPE) and biosafety practices.

Virus Detection

Virus isolation in Vero cell culture remains the standard for confirmation of WNV positive pools (Beaty et al. 1989, Savage et al. 1999, Lanciotti et al. 2000). Virus isolation provides the benefit of detecting other viruses that may be contained in the mosquitoes, a feature that is lost using test procedures that target virus-specific nucleotide sequence or proteins. However, Vero cell culture is expensive and requires specialized laboratory facilities; thus, nucleic acid assays have largely replaced virus isolation as detection and confirmatory assay methods of choice. Virus isolation requires that mosquito pools be ground in a media that protects the virus from degradation such as BA-1 (Lanciotti et al. 2000), and preservation of an aliquot at -70^{o}C to retain virus viability for future testing.

Nucleic acid detection assays are the most sensitive assays for virus detection and confirmation of WNV in mosquito pools (Lanciotti et al. 2000, Nasci et al. 2002). Real-Time RT-PCR assays with different primer sets may be used for both detection and confirmation of virus in mosquito pools. Standard RT-PCR primers are also available (Kuno et al 1998). Nucleic acids may be extracted from an aliquot of the mosquito pool homogenate by hand using traditional methods or with kits, or with automated robots in high-through-put laboratories (Savage et al 2007).

West Nile virus antigen detection assays are available in ELISA format (Tsai et al. 1987, Hunt et al. 2002) and in two commercial kits that employ lateral flow wicking assays, developed specifically for testing mosquitoes (, Komar et al. 2002, Panella et al. 2001, Burkhalter et al. 2006). The antigen capture ELISA of Hunt et al. 2002 and the RAMP (Rapid Analyte Measurement Platform, Response Biomedical Corp, Burnaby, British Columbia, Canada) WNV test are approximately equal in sensitivity and detect WNV in mosquito pools at concentrations as low as $10^{3.1}$ PFU/ml (Burkhalter et al. 2006). The VecTest (Medical Analysis Systems, Inc., Camarillo, CA) is less sensitive and detects WNV in mosquito pools at concentrations of $10^{5.17}$ PFU/ml. The VecTest (evaluated by Burkhalter et al. 2006) is no longer available, but is similar to a lateral flow wicking assay marketed as VecTOR Test (VecTOR Test Systems, Inc., Thousand Oaks, CA). Although the antigen detection assays are less sensitive than nucleic acid detection assays, they have been evaluated in operational surveillance programs (Mackay et al. 2004. Lampman et al. 2006. Williges et al. 2009, Kesavaraju et al. 2012) and can provide valuable WNV infection rate data when employed consistently in a mosquito surveillance program.

Non-Human Vertebrates

Serology

Diagnostic kits for serologic diagnosis of WNV infection in clinically ill domestic animals are not commercially available. IgM-capture ELISA has been developed for use in horses, and can be readily adapted to other animal species where anti-IgM antibody reagents are commercially available. Alternatively, seroconversion for IgG, neutralizing antibodies, and haemagglutinin inhibiting (HAI) assays

in acute and convalescent serum samples collected 2-3 weeks apart can be used as screening assays. The latter two approaches do not require species-specific reagents and thus have broad applicability. The ELISA format may be used when employed as inhibition or competition ELISAs, which avoids the use of species-specific reagents. A popular blocking ELISA has been applied to a variety of vertebrate species with very high specificity and sensitivity, reducing the necessity of a second confirmatory test (Blitvich et al 2003a, 2003b). Similarly, the microsphere immunoassay, when used comparatively with WNV antigen-coated beads and SLEV antigen-coated beads, performs with high specificity and sensitivity (Johnson et al. 2005). Typically, a confirmatory 90% plaque-reduction neutralization test ($PRNT_{90}$) with end-point titration is used to confirm serology in non-human vertebrates. Plaque-reduction thresholds below 80% are not recommended. Because of the cross-reactive potential of anti-flavivirus antibodies, the PRNT must be comparative, performed simultaneously with St. Louis encephalitis virus (SLEV).

PRNT requires the use of a BSL3 laboratory and Vero cell culture. The PRNT has been adapted to BSL-2 laboratory conditions using a recombinant chimeric virus featuring the WNV envelope glycoprotein gene in a yellow fever virus backbone (Chimeravax®, originally developed as a live-attenuated vaccine candidate). For PRNTs, the Chimeravax provided equivalent results for bird sera, and 10-100 fold lower titers for equine sera (Komar et al. 2009).

The same serologic techniques applied to clinically ill animals may also be used for healthy subjects for vertebrate serosurveys or for healthy sentinel animals serially sampled as sentinels. Serologic techniques for WNV diagnosis should not be applied to carcasses, as in many cases of fatal WNV infection, the host will die before a detectable immune response develops. Furthermore, some morbid or moribund animals that have WNV antibodies due to past infection may be currently infected with a pathogen other than WNV. Fatal cases should have readily detectable WNV in their tissues.

As with human diagnostic samples, serologic results from non-human vertebrates must be interpreted with caution and with an understanding of the cross-reactive tendencies of WNV and other flaviviruses. For primary WNV infections, a low rate of cross-reactivity is expected (<5%) and misdiagnoses are avoided by the requirement that the reciprocal anti-WNV titer be a minimum of 4-fold greater than the corresponding anti-SLEV titer. In rare cases, a secondary flavivirus infection due to WNV in a host with a history of SLEV infection may boost the older anti-SLEV titer to greater levels than the anti-WNV titer, resulting in a misdiagnosis of SLEV infection, a phenomenon known as “original antigenic sin”. Some serum samples will have endpoint titers for WNV and SLEV that are the same or just 2-fold different. While it is possible that this serologic result is due to past infections with both of these viruses, it is impossible to rule out cross-reaction from one or the other, or even from a third indeterminate flavivirus. Such a result should be presented as “Undifferentiated flavivirus infection.”

Virus Detection

Methods for WNV detection, isolation and identification are the same as described for human and mosquito diagnostics. Specimens typically used are tissues and/or fluids from acutely ill and/or dead animals. Virus detection in apparently healthy animals is very low-yield and inefficient, and therefore not cost-effective, and should not be considered for routine surveillance programs. In bird, mammal and reptile carcasses, tissue tropisms have varied among individuals within a species, and across species. Some animals, like humans, have few tissues with detectable virus particles or viral RNA at necropsy, such as horses. Others, such as certain bird species, may have fulminant infections with high viral loads in almost every tissue.

Prevention and Control

Integrated Vector Management

Mosquito abatement programs successfully employ integrated pest management (IPM) principles to reduce mosquito abundance, providing important community services to protect quality of life and public health (Rose 2001). Prevention and control of WNV and other zoonotic arboviral diseases is accomplished most effectively through a comprehensive, integrated vector management (IVM) program applying the principles of IPM. IVM is based on an understanding of the underlying biology of the arbovirus transmission system, and utilizes regular monitoring of vector mosquito populations and WNV activity levels to determine if, when, and where interventions are needed to keep mosquito numbers below levels which produce risk of human disease, and to respond appropriately to reduce risk when it exceeds acceptable levels.

Operationally, IVM is anchored by a monitoring program providing data that describe:

- Conditions and habitats that produce vector mosquitoes.
- Abundance of those mosquitoes over the course of a season.
- WNV transmission activity levels expressed as WNV infection rate in mosquito vectors.
- Parameters that influence local mosquito populations and WNV transmission.

These data inform decisions about implementing mosquito control activities appropriate to the situation, such as:

- Source reduction through habitat modification.
- Larval mosquito control using the appropriate methods for the habitat.
- Adult mosquito control using pesticides applied from trucks or aircraft when established thresholds have been exceeded.
- Community education efforts related to WNV risk levels and intervention activities.

Monitoring also provides quality control for the program, allowing evaluation of:

- Effectiveness of larval control efforts.
- Effectiveness of adult control efforts.
- Causes of control failures (e.g., undetected larval sources, pesticide resistance, equipment failure).

Surveillance Programs

Effective IVM for WNV prevention relies on a sustained, consistent surveillance program that targets vector species. The objectives are to identify and map larval production sites by season, monitor adult mosquito abundance, monitor vector infection rates, document the need for control based on established thresholds, and monitor control efficacy. Surveillance can be subdivided into three categories based on the objective of the surveillance effort. However, the surveillance elements are complementary, and in combination provide the information required for IVM decisions.

Larval Mosquito Surveillance

Larval surveillance involves identifying and sampling a wide range of aquatic habitats to identify the sources of vector mosquitoes, maintaining a database of these locations, and a record of larval control measures applied to each. This requires trained inspectors to identify larval production sites, collect larval specimens on a regular basis from known larval habitats, and to perform systematic surveillance for new sources. This information is used to determine where and when source reduction or larval control efforts should be implemented.

Adult Mosquito Surveillance

Adult mosquito surveillance is used to quantify relative abundance of adult vector mosquitoes, and to describe their spatial distribution. This process also provides specimens for evaluating the incidence of WNV infection in vector mosquitoes (see Surveillance chapter for more information). Adult mosquito surveillance programs require standardized and consistent surveillance efforts in order to provide data appropriate for monitoring trends in vector activity, for setting action thresholds, and evaluating control efforts. Various methods are available for monitoring adult mosquitoes. Most frequently used in WNV surveillance are the CO_2-baited CDC miniature-style light traps for monitoring host-seeking *Culex tarsalis* (and potential bridge vector species) and gravid traps to monitor *Cx. quinquefasciatus, Cx. pipiens* and *Cx. restuans* populations. Adult mosquito surveillance should consist of a series of collecting sites at which mosquitoes are sampled using both gravid and light traps on a regular schedule. Fixed trap sites allow monitoring of trends in mosquito abundance and virus activity over time and are essential for obtaining information to evaluate WNV risk and to guide control efforts. Additional trap sites can be utilized on an ad hoc basis to provide additional information about the extent of virus transmission activity and effectiveness of control efforts.

WNV Transmission Activity

Monitoring WNV transmission activity in the environment before human cases occur is an essential component of an IVM program to reduce WNV risk. Without this information, it is impossible to set thresholds for vector mosquito population management and to take appropriate action before an outbreak is in progress (Table 2). WNV transmission activity can be monitored by tracking the WNV infection rate in vector mosquito populations, WNV-related avian mortality, seroconversion to WNV in sentinel chickens, seroprevalence/seroconversion in wild birds, and WNV veterinary (primarily horse) cases. The methods for monitoring and calculating indices of WNV activity are described in detail in the Environmental Surveillance section of the Surveillance chapter.

Mosquito Control Activities

Guided by the surveillance elements of the program, integrated efforts to control mosquitoes are implemented to maintain vector populations below thresholds that would facilitate WNV amplification and increase human risk. Failing that, efforts to reduce the abundance of WNV-infected biting adult mosquitoes must be quickly implemented to prevent risk levels from increasing to the point of a human disease outbreak. Properly implemented, a program monitoring mosquito abundance and WNV activities in the vector mosquito population will provide a warning of when risk levels are increasing. Because of delays in onset of disease following infection, and delays related to seeking medical care,

diagnosis, and reporting of human disease, WNV surveillance based on human case reports lags behind increases in risk and is not sufficiently sensitive to allow timely implementation of outbreak control measures.

Larval Mosquito Control

The objective of the larval mosquito control component of an IVM program is to manage mosquito populations before they emerge as adults. This can be an efficient method of managing mosquito populations if the mosquito breeding sites are accessible. However larval control may not attain the levels of mosquito population reduction needed to maintain WNV risk at low levels, and must be accompanied by measures to control the adult mosquito populations as well. In outbreak situations, larval control complements adult mosquito control measures by preventing new vector mosquitoes from being produced. However, larval control alone is not able to stop WNV outbreaks once virus amplification has reached levels causing human infections.

Numerous methods are available for controlling larval mosquitoes. Source reduction is the elimination or removal of habitats that produce mosquitoes. This can range from draining roadside ditches to properly disposing of discarded tires and other trash containers. Only through a thorough surveillance program will mosquito sources be identified and appropriately removed. In order to effectively control vector mosquito populations through source reduction, all sites capable of producing vector mosquitoes must be identified and routinely inspected for the presence of mosquito larvae or pupae. This is difficult to accomplish with the WNV vector species *Cx. quinquefasciatus* and *Cx. pipiens* that readily utilize cryptic sites such as storm drainage systems, grey water storage cisterns and storm water runoff impoundments. Vacant housing with unmaintained swimming pools, ponds and similar water features are difficult to identify and contribute a significant number of adult mosquitoes to local populations.

To manage mosquitoes produced in habitats that are not conducive to source reduction, pesticides registered by EPA for larval mosquito control are applied when larvae are detected. Several larval mosquito control pesticides are available and a discussion of their attributes and limitations is beyond the scope of this overview. More detailed information about pesticides used for larval mosquito control is available from the U.S. EPA (http://www2.epa.gov/mosquitocontrol/controlling-mosquitoes-larval-stage). No single larvicide product will work effectively in every habitat where WNV vectors are found. An adequate field staff with proper training is required to properly identify larval production sites and implement the appropriate management tools for that site.

Adult Mosquito Control

Source reduction and larvicide treatments may be inadequate to maintain vector populations at levels sufficiently low to limit virus amplification. The objective of the adult mosquito control component of an IVM program is to complement the larval management program by reducing the abundance of adult mosquitoes in an area, thereby reducing the number of eggs laid in breeding sites. Adult mosquito control is also intended to reduce the abundance of biting, infected adult mosquitoes in order to prevent them from transmitting WNV to humans and to break the mosquito-bird transmission cycle. In situations where vector abundance is increasing above acceptable levels, targeted adulticide applications using pesticides registered by EPA for this purpose can assist in maintaining vector abundance below threshold levels. More detailed information about pesticides used for adult mosquito

control is available from the U.S. EPA (http://www2.epa.gov/mosquitocontrol/controlling-adult-mosquitoes). Pesticides for adult mosquito control can be applied from hand-held application devices or from trucks or aircraft. Hand-held or truck-based applications are useful to manage relatively small areas, but are limited in their capacity to treat large areas quickly during an outbreak. In addition, gaps in coverage may occur during truck-based applications due to limitations of the road infrastructure. Aerial application of mosquito control adulticides is required when large areas must be treated quickly, and can be particularly valuable because controlling WNV vectors such as *Cx. quinquefasciatus* or *Cx. pipiens* often requires multiple, closely spaced treatments (Andis et al. 1987). Both truck and aerially-applied pesticides for adult mosquito control are applied using ultra-low-volume (ULV) technology in which a very small volume of pesticide is applied per acre in an aerosol of minute droplets designed to contain sufficient pesticide to kill mosquitoes that are contacted by the droplets. Information describing ULV spray technology and the factors affecting effectiveness of ground and aerially-applied ULV pesticides is reviewed in Mount et al. 1996, Mount 1998, and Bonds 2012.

Risk and Safety of Vector Control Pesticides and Practices

Insecticides to control larval and adult mosquitoes are registered specifically for that use by the U.S. Environmental Protection Agency (EPA). Instructions provided on the product labels prescribe the required application and use parameters, and must be carefully followed. Properly applied, these products do not negatively affect human health or the environment. Research has demonstrated that ULV application of mosquito control adulticides did not produce detectable exposure or increases in asthma events in persons living in treated areas (Karpati et al. 2004, Currier et al. 2005, Duprey et al. 2008). The risks from WNV demonstrably exceed the risks from mosquito control practices (Davis and Peterson 2008, Macedo et al. 2010, Peterson et al. 2006)

Legal Action to Achieve Access or Control

Individually-owned private properties may be major sources of mosquito production. Examples include accumulations of discarded tires or other trash, neglected swimming pools and similar water features that become stagnant and produce mosquitoes. Local public health statutes or public nuisance regulations may be employed to gain access for surveillance and control, or to require the property owner to mitigate the problem. Executing such legal actions may be a prolonged process during which adult mosquitoes are continuously produced. Proactive communication with residents and public education programs may alleviate the need to use legal actions. However, legal efforts may be required to eliminate persistent mosquito production sites.

Quality Control

Pesticide products and application procedures (for both larval and adult control) must periodically be evaluated to ensure an effective rate of application is being used and that the desired degree of control is obtained. Application procedures should be evaluated regularly (minimally once each season) to assure equipment is functioning properly to deliver the correct dosages and droplet parameters and to determine appropriate label rates to use locally. Finally, mosquito populations should routinely be evaluated to ensure insecticide resistance is not emerging.

Records

Surveillance data describing vector sources, abundance and infection rates, records of control efforts (e.g., source reduction, larvicide applications, adulticide applications), and quality control data must be maintained and used to evaluate IVM needs and performance. Long-term data are essential to track trends and to evaluate levels of risk.

Insecticide Resistance Management

In order to delay or prevent the development of insecticide resistance in vector populations, integrated vector management programs should include a resistance management component (Florida Coordinating Council on Mosquito Control 1998). Ideally, this should include annual monitoring of the status of resistance in the target populations to:

- Provide baseline data for program planning and pesticide selection before the start of control operations.
- Detect resistance at an early stage so that timely management can be implemented.
- Continuously monitor the effect of control strategies on insecticide resistance.

Monitoring resistance in the vector population is essential, and is useful in determining the potential causes for control failures, should they occur. CDC has developed an assay to determine if a particular pesticide formulation (combination of the active ingredient in the insecticide and inactive ingredients) is able to kill mosquito vectors. The technique, referred to as the CDC bottle bioassay, is simple, rapid, and economical compared with alternatives. The results can help guide the choice of insecticide used for spraying. A practical laboratory manual that describes how to perform and interpret the CDC bottle bioassay is available online (http://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf). For additional information about obtaining and performing the bottle bioassay, contact CDC at bottleassay@cdc.gov.

The integrated vector management program should include options for managing resistance that are appropriate for the local conditions. The techniques regularly used include the following:

- Management by moderation - preventing onset of resistance by:
    - Using dosages no lower than the lowest label rate to avoid genetic selection.
    - Using less frequent applications.
    - Using chemicals of short environmental persistence.
    - Avoiding slow-release formulations that increase selection for resistance.
    - Avoiding the use of the same class of insecticide to control both adults and immature stages.
    - Applying locally**.** Currently, most districts treat only hot spots. Area-wide treatments are used only during public health alerts or outbreaks.
    - Leaving certain generations, population segments**,** or areas untreated.
    - Establishing high thresholds for pest mosquito mitigation using insecticides except during public health alerts or outbreaks.

- Management by continued suppression. This strategy is used in regions of high value or persistent high risk (e.g., heavily populated regions or locations with recurring WNV outbreaks) where mosquitoes must be kept at very low densities. This does not mean saturation of the

environment by pesticides, but rather the saturation of the defense mechanisms of the insect by insecticide dosages that can overcome resistance. This is achieved by the application of dosages within label rates but sufficiently high to be lethal to heterozygous individuals that are partially resistant. If the heterozygous individuals are killed, resistance will be slow to emerge. This method should not be used if any significant portion of the population in question is fully resistant. Another approach more commonly used is the addition of synergists that inhibit existing detoxification enzymes and thus eliminate the competitive advantage of these individuals. Commonly, the synergist of choice in mosquito control is piperonyl butoxide (PBO).

- Management by multiple attack consists of achieving effective control through the action of several different and independent pressures such that selection for any one of them would be below that required for the development of resistance in the mosquito population. This strategy involves the use of insecticides with different modes of action in mixtures or in rotations. There are economic limitations associated with this approach (e.g., costs of switching chemicals or having storage space for them), and critical variables in addition to the pesticide mode of action that must be taken into consideration (i.e., mode of resistance inheritance, frequency of mutations, population dynamics of the target species, availability of refuges, and migration). General recommendations are to evaluate resistance patterns at least annually and the need for rotating insecticides at annual or longer intervals.

Continuing Education

Continuing education for operational vector control workers is required to instill or refresh knowledge related to practical mosquito control. Training focusses on safety, applied technology, and requirements for the regulated certification program mandated by most states. Training should also include information on the identification of mosquito species, their behavior, ecology, and appropriate methods of control.

Vector Management in Public Health Emergencies

Intensive early season adult mosquito control efforts can decrease WNV transmission activity (Lothrop *et al*. 2008) and result in reduced human risk. However, depending on local conditions, proactive vector management may not maintain mosquito populations at levels sufficiently low to avoid development of outbreaks. As evidence of sustained or intensified virus transmission in a region increases, emergency vector control efforts to reduce the abundance of infected, biting adult mosquitoes must be implemented. This is particularly important in areas where vector surveillance indicates that infection rates in *Culex* mosquitoes are continually increasing, or being sustained at high levels, that potential accessory vectors (e.g., mammal-feeding mosquito species) are infected with WNV, or multiple human cases or viremic blood donors have been reported. Delaying adulticide applications until numerous human cases occur negates the value and purpose of the surveillance system. Timely application of adulticides interrupts WNV transmission and prevents human cases (Carney *et al*. 2005).

Guidelines for a Phased Response to WNV Surveillance Data

The objective of a phased response to WNV surveillance data is to implement public health interventions appropriate to the level of WNV risk in a community (Table 2). A surveillance program adequate to monitor WNV activity levels associated with human risk must be in place in order to provide detection of epizootic transmission in advance of human disease outbreaks. The surveillance programs and environmental surveillance indicators described above demonstrate that enzootic/epizootic WNV transmission can be detected several weeks before the onset of human disease, allowing for implementation of effective interventions (Bolling *et al*. 2009, Jones *et al.* 2011, Mostashari *et al*. 2003, Unlu *et al.* 2009).

All communities should prepare for WNV activity. For reasons that are not well understood, some regions are at risk of higher levels of WNV transmission and epidemics than others (CDC 2010), but there is evidence of WNV presence and the risk of human disease and outbreaks in most counties in the contiguous 48 states. The ability to develop a useful phased response depends upon the existence of some form of WNV monitoring in the community to provide the information needed to gauge risk levels. Measures of the intensity of WNV epizootic transmission in a region, preferably from environmental surveillance indicators, should be considered when determining the level of the public health response. As noted previously, human case reports lag weeks behind human infection events and are poor indicators of current risk levels. Effective public health actions depend on interpreting the best available surveillance data and initiating prompt and aggressive intervention when necessary.

Table 2. Recommendations for a phased response to WNV surveillance data.

Risk category	Probability of human outbreak	Definition	Recommended activities and responses
0	None	• No adult mosquito biting activity (vector species).	• Develop and review WNV response plan. • Review mosquito control program. • Maintain source reduction projects. • Secure surveillance and control resources necessary to enable emergency response. • Review and update community outreach and public education programs.
1	Low	• Biting adult mosquitoes active (vector species). -or- • Epizootic activity expected based on onset of transmission in prior years. -or- • Limited or sporadic epizootic activity in birds or mosquitoes.	• Response as in category 0, plus: • Conduct Integrated Vector Management program to monitor and reduce vector mosquito abundance. • Conduct environmental surveillance to monitor virus activity (mosquitoes, sentinel chickens, avian mortality, etc.). • Initiate community outreach and public education programs focused on personal protection and residential source reduction.

2	High	• Sustained transmission activity in mosquitoes or birds. -or- • Horse cases reported. -or- • Human case or viremic blood donor reported.	• Response as in category 1 plus: • Intensify and expand adult mosquito control in areas using ground and/or aerial applications where surveillance indicates human risk. • Intensify visible activities in community to increase attention to WNV transmission risk and personal protection measures. • Work with collaborators to address high risk populations. • Intensify and expand surveillance for human cases.
3	Outbreak in progress	• Conditions favor continued transmission to humans (i.e., persistent high infection rate in mosquitoes, continued avian mortality, seasonal mosquito population decreases not anticipated for weeks) -or- • Multiple confirmed human cases or viremic blood donors.	• Response as in category 2 plus: • Intensify emergency adult mosquito control program repeating applications as necessary to achieve adequate control. • Monitor effectiveness of vector control efforts • Emphasize urgency of personal protection, including use of repellents, through community leaders and media.

Individual and Community –Based Prevention Education

Without an effective human vaccine, the only way to prevent human infection and disease is to prevent infected mosquitoes from biting people. This can be accomplished by the efforts of community-based integrated vector management programs, as described above, and by effective personal protection behaviors and practices, such as mosquito-avoidance, use of personal repellents, and removal of residential mosquito sources.

Prevention messages and programs for promoting individual and community measures to decrease risk of WNV infection should be employed. Public education and risk communication activities should reflect the degree of WNV risk in a community, as noted in Table 2. Messages should acknowledge the seriousness of the disease without promoting undue fear or panic in the target population. Fear-driven messages may heighten the powerlessness people express in dealing with vector-borne diseases like WNV. Messages should be clear and consistent with the recommendations of coordinating agencies. Use plain language, and adapt materials for lower literacy and non-English speaking audiences.

Individual-Level Actions and Behaviors to Reduce WNV Risk

MESSAGING ABOUT PERSONAL AND HOUSEHOLD PREVENTION

The best way to prevent West Nile virus disease is to avoid mosquito bites.

- Use insect repellents when you go outdoors.
- Wear long sleeves and pants during dawn and dusk.
- Repair or install screens on windows and doors.
- Use air conditioning, if you have it.
- Remove mosquito sources from around your home.

Repellents

CDC recommends the use of products containing active ingredients which have been registered by the U.S. Environmental Protection Agency (EPA) for use as repellents applied to skin and clothing. EPA registration of repellent active ingredients indicates the materials have been reviewed and approved for efficacy and human safety when applied according to the instructions on the label.

Repellents for use on skin and clothing: CDC evaluation of information contained in peer-reviewed scientific literature and data available from EPA has identified several EPA-registered products that provide repellent activity sufficient to help people avoid the bites of disease carrying mosquitoes. Products containing these active ingredients typically provide reasonably long-lasting protection**:**

- DEET (Chemical Name: N,N-diethyl-m-toluamide or N,N-diethly-3-methyl-benzamide)
- Picaridin (KBR 3023, Chemical Name: 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester)
- Oil of Lemon Eucalyptus* or PMD (Chemical Name: para-Menthane-3,8-diol) the synthesized version of oil of lemon eucalyptus
- IR3535 (Chemical Name: 3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester)

  * Note: This recommendation refers to EPA-registered repellent products containing the active ingredient oil of lemon eucalyptus (or PMD). “Pure” oil of lemon eucalyptus (e.g. essential oil) has not received similar, validated testing for safety and efficacy, is not registered with EPA as an insect repellent, and is not covered by this CDC recommendation.

EPA characterizes the active ingredients DEET and Picaridin as “conventional repellents” and Oil of Lemon Eucalyptus, PMD, and IR3535 as “biopesticide repellents”, which are derived from natural materials. For more information on repellent active ingredients see (http://www2.epa.gov/mosquitocontrol) .

Published data indicate that repellent efficacy and duration of protection vary considerably among products and among mosquito species, and are markedly affected by ambient temperature, amount of perspiration, exposure to water, abrasive removal, and other factors. In general, higher concentrations

of active ingredient provide longer duration of protection, regardless of the active ingredient, although concentrations above ~50% do not offer a marked increase in protection time. Products with <10% active ingredient may offer only limited protection, often from 1-2 hours. Products that offer sustained release or controlled release (micro-encapsulated) formulations, even with lower active ingredient concentrations, may provide longer protection times.

Repellents for use on clothing: Certain products containing permethrin are recommended for use on clothing, shoes, bed nets, and camping gear, and are registered with EPA for this use. Permethrin is highly effective as an insecticide and as a repellent. Permethrin-treated clothing repels mosquitoes, and retains this effect after repeated laundering. The permethrin repellents should be reapplied following the label instructions. Some commercial clothing products are available pretreated with permethrin. Additional information about CDC's repellent recommendations is available at (http://www.cdc.gov/ncidod/dvbid/westnile/RepellentUpdates.htm).

Mosquito bites can be avoided simply by not going outdoors when mosquitoes are biting, and recommendations to avoid outdoor activity when and where high WNV activity levels have been detected are a component of prevention programs. Recommendations to avoid being outdoors from dusk to dawn may conflict with neighborhood social patterns, community events, or the practices of persons without air-conditioning. It is important to communicate that the primary WNV vectors are active from dusk until dawn. Emphasize that repellent use is protective, and should be used when outdoors during the prime mosquito-biting hours.

Reduce Mosquito Production at the Home

Encourage residents to regularly empty standing water from items outside homes such as rain gutters, flowerpots, old tires, empty containers, buckets, and wading pools. Water in birdbaths should be changed at least once per week. CDC provides resources that list these and other steps for reducing mosquito populations (www.cdc.gov/ncidod/dvbid/westnile/qa/habitats.htm).

Community-Level Actions to Reduce WNV Risk

MESSAGING ABOUT COMMUNITY PROTECTION

Using insecticides to control adult mosquitoes, whether applied from trucks at ground level or by aircraft, is a common, safe and effective practice in the United States.

- In many parts of the country, spraying insecticides is part of an annual effort to reduce the number of adult mosquitoes, which in turn reduces the risk of WNV and other diseases spread by mosquitoes.
- Insecticides used in aerial spraying are no different from those sprayed by trucks. Aerial spraying can cover larger areas faster, which helps to reduce the number of infected mosquitoes and slow the spread of illness.
- These products are specifically designed to reduce the number of adult mosquitoes. Insecticides used in mosquito control are reviewed for safety and effectiveness and registered by EPA.

Community Protection Measures

At the community level, reporting dead birds and nuisance mosquito problems, advocating for organized mosquito abatement, and participating in community mobilization projects to address sources of mosquitoes such as trash, standing water or neglected swimming pools are activities that can help protect individuals and at-risk groups.

Communicating About Mosquito Control

Area-wide control of mosquitoes that transmit WNV is most effectively done at a community level. Public understanding and acceptance of emergency adult mosquito control operations using insecticides is critical to its success, especially where these measures are unfamiliar. Questions about the products being used, their safety, and their effects on the environment are common. Improved communication about surveillance and how decisions to use mosquito adulticides are made may help residents weigh the risks and benefits of control. When possible, provide detailed information regarding the schedule for adulticiding through newspapers, radio, government-access television, the internet, recorded phone messages, or other means your agency uses to successfully communicate with its constituencies.

Community Engagement and Education

Knowing the norms and cultural practices of communities is invaluable when informing the public, and for gaining support and assistance for routine vector-management practices and to enhance personal protection during outbreaks. It is essential to know how targeted audiences communicate with each other and with government officials, and to identify the key audiences and how to reach them. Identifying what risk communication principles work best can better prepare the community to effectively provide information during an outbreak. Translating complex scientific jargon into understandable concepts promotes community understanding and acceptance. The following provides a description of selected best practices for targeting high-risk groups, offers suggestions for cultivating partnerships with media and communities, and provides select outreach measures for mobilizing communities.

Prevention Strategies for High-Risk Groups

Audience members have different disease-related concerns and motivations for action. Proper message targeting (including use of plain language) permits better use of limited communication and prevention resources. The following are some population segments that require specific targeting:

Persons over age 50

While persons of any age can be infected with WNV, surveillance data indicate that persons over age 50 are at higher risk for severe disease and death due to WNV infection. Collaborate with organizations that have an established relationship with mature adults, such as the AARP, senior centers, and programs for adult learners. Include images of older adults in your promotional material. Identify activities in your area where older adults may be exposed to mosquito bites (e.g., jogging, walking, golf, and gardening).

Persons with Outdoor Exposure

Data suggest that persons engaged in extensive outdoor work or recreational activities are at greater risk of being bitten by mosquitoes which may be infected with WNV. Develop opportunities to inform people engaged in outdoor activities about WNV. Encourage use of repellent and protective clothing, particularly if outdoor activities occur during dusk to dawn hours. Local spokespersons (e.g., union officials, job-site supervisors, golf pros, sports organizations, lawn care professionals, public works officials, gardening experts) may be useful collaborators. Place messages in locations where people engage in outdoor activities (e.g., parks, golf courses, hiking trails).

Homeless Persons

Extensive outdoor exposure and limited financial resources in this group present special challenges. Application of repellents to exposed skin and clothing may be most appropriate prevention measures for this population. Work with social service groups in your area to educate and provide repellents to this population segment.

Residences Lacking Window Screens.

The absence of intact window/door screens is a likely risk factor for exposure to mosquito bites. Focus attention on the need to repair screens and provide access to resources to do so. Partner with community organizations that can assist older persons or others with financial or physical barriers to screen installation or repair.

Partnerships with Media and the Community

Cultivate relationships with the media (radio, TV, newspaper, web-based news outlets). Obtain media training for at least one member of your staff, and designate that individual as the organization's spokesperson. Develop clear press releases and an efficient system to answer press inquiries. Many communities have heard similar prevention messages repeated for several years. Securing the public's attention when risk levels increase can be a challenge. Evaluate and update WNV prevention messages annually, and test new messages with different population segments to evaluate effectiveness. Develop partnerships with agencies/organizations that have relationships with populations at higher risk (such as persons over 50) or are recognized as community leaders (e.g., churches, service groups). Working through sources trusted by the target audience can heighten the credibility of and attention to

messages. Partnerships with businesses that sell materials to fix or install window screens or that sell insect repellent may be useful in some settings (e.g., local hardware stores, grocery stores).

Community Mobilization and Outreach

Community mobilization can improve education and help achieve behavior change goals. Promote the concept that health departments and mosquito control programs require community assistance to reduce WNV risk. A community task force that includes civic, business, public health, and environmental concerns can be valuable in achieving buy-in from various segments of the community, and in developing a common message. Community mobilization activities can include clean-up days to get rid of mosquito breeding sites. Community outreach involves presenting messages in person, in addition to media and educational materials, and involving citizens in prevention activities. Hearing the message of personal prevention from community leaders can validate the importance of the disease. Health promotion events and activities reinforce the importance of prevention in a community setting. Select tools and resources are listed here that may enhance your outreach efforts:

Social Media

The increasing popularity of social media has been accompanied with a rise in health information-seeking using these new interactive outlets. Outreach can be conducted using Twitter, Facebook, YouTube, blogs, and other websites that may reach constituents less connected to more traditional media sources. Social media posts can be used as an attention-grabber, providing links that direct users to webpages or other resources with more complete information.

Online Resources

The Internet has become a primary source of health information for many Americans. Unfortunately, it is impossible to police the vast amount of content available online for quality and accuracy. Encourage constituents to seek advice from credible sources. Make sure local official public health agency websites are clear, accurate and up to date. Useful information is available from a number of resources:

Federal public health resources: The CDC WNV web pages are updated frequently to reflect new findings and recommendations (www.cdc.gov/westnile). Materials on the CDC web site are in the public domain, and serve as a resource for state and local health departments and other organizations. CDC staff can provide technical assistance in the development of audience research and strategies for public education and community outreach. Contact CDC/Division of Vector-Borne Diseases' health communications staff in Fort Collins, CO at 970-221-6400.

The Environmental Health Services Branch in CDC's National Center for Environmental Health, Division of Emergency and Environmental Health Services, sponsors free online training on mosquito control methods following disasters and to prevent vector-borne disease on the web site of the National Environmental Health Association. The training can be accessed at www.nehacert.org/moodle and clicking on Free CDC and EPA Training Courses. The relevant courses are listed as:

- CDC0702 (Biology and Control of Insects and Rodents)
- CDC1001 (Biology and Control of Vectors and Public Health Pests: The Importance of

The U.S. Environmental Protection Agency (EPA) is the government's regulatory agency for insecticide and insect repellent use, safety, and effectiveness. Information about mosquito control insecticides and repellents is available at (http://www2.epa.gov/mosquitocontrol). These include guidance for using repellents safely (http://epa.gov/pesticides/insect/safe.htm) and a search tool to assist in finding a repellent that is right for you (http://cfpub.epa.gov/oppref/insect/#searchform) which allows the user to examine the protection time afforded by registered repellents containing various concentrations of the active ingredients.

There are a number of organizations that have developed useful tools and information that can be adapted for local needs. Examples include: the American Mosquito Control Association (www.mosquito.org) and the National Pesticide Information Center (NPIC) (www.npic.orst.edu).

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Appendix 1: Arboviral Diseases, Neuroinvasive and Non-Neuroinvasive Case Definitions.

CSTE Position Statement Numbers: 10-ID-18, 10-ID-20, 10-ID-21, 10-ID-22, 10-ID-23, 10-ID-24

- California Serogroup Viruses, (i.e., California encephalitis, Jamestown Canyon, Keystone, La Crosse, Snowshoe hare, and Trivittatus viruses)
- Eastern Equine Encephalitis Virus
- Powassan Virus
- St. Louis Encephalitis Virus
- West Nile Virus
- Western Equine Encephalitis Virus

Background

Arthropod-borne viruses (arboviruses) are transmitted to humans primarily through the bites of infected mosquitoes, ticks, sand flies, or midges. Other modes of transmission for some arboviruses include blood transfusion, organ transplantation, perinatal transmission, consumption of unpasteurized dairy products, breast feeding, and laboratory exposures. More than 130 arboviruses are known to cause human disease. Most arboviruses of public health importance belong to one of three virus genera: ***Flavivirus***, ***Alphavirus***, and ***Bunyavirus***.

Clinical description

Most arboviral infections are asymptomatic. Clinical disease ranges from mild febrile illness to severe encephalitis. For the purposes of surveillance and reporting, based on their clinical presentation, arboviral disease cases are often categorized into two primary groups: neuroinvasive disease and non-neuroinvasive disease.

Neuroinvasive disease: Many arboviruses cause neuroinvasive disease such as aseptic meningitis, encephalitis, or acute flaccid paralysis (AFP). These illnesses are usually characterized by the acute onset of fever with stiff neck, altered mental status, seizures, limb weakness, cerebrospinal fluid (CSF) pleocytosis, or abnormal neuroimaging. AFP may result from anterior ("polio") myelitis, peripheral neuritis, or post-infectious peripheral demyelinating neuropathy (i.e., Guillain-Barré syndrome). Less common neurological manifestations, such as cranial nerve palsies, also occur.

Non-neuroinvasive disease: Most arboviruses are capable of causing an acute systemic febrile illness (e.g., West Nile fever) that may include headache, myalgias, arthralgias, rash, or gastrointestinal symptoms. Rarely, myocarditis, pancreatitis, hepatitis, or ocular manifestations such as chorioretinitis and iridocyclitis can occur.

Clinical criteria for diagnosis
A clinically compatible case of arboviral disease is defined as follows:

Neuroinvasive disease

- Fever (≥100.4°F or 38°C) as reported by the patient or a health-care provider, **AND**
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, **AND**
- Absence of a more likely clinical explanation.

Non-neuroinvasive disease

- Fever (≥100.4°F or 38°C) as reported by the patient or a health-care provider, **AND**
- Absence of neuroinvasive disease, **AND**
- Absence of a more likely clinical explanation.

Laboratory criteria for diagnosis

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid, **OR**
- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera, **OR**
- Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, **OR**
- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred, **OR**
- Virus-specific IgM antibodies in CSF or serum.

Case classification

Confirmed:

Neuroinvasive disease*:* A case that meets the above clinical criteria for neuroinvasive disease and one or more the following laboratory criteria for a confirmed case:

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid, **OR**
- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera, **OR**
- Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, **OR**
- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.

Non-neuroinvasive disease: A case that meets the above clinical criteria for non-neuroinvasive disease and one or more of the following laboratory criteria for a confirmed case:

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid, **OR**
- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera, **OR**
- Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, **OR**

- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.

Probable:

Neuroinvasive disease*:* A case that meets the above clinical criteria for neuroinvasive disease and the following laboratory criteria:

- Virus-specific IgM antibodies in CSF or serum but with no other testing.

Non-neuroinvasive disease*:* A case that meets the above clinical criteria for non-neuroinvasive disease and the laboratory criteria for a probable case:

- Virus-specific IgM antibodies in CSF or serum but with no other testing.

Comment:

Interpreting arboviral laboratory results

- **Serologic cross-reactivity.** In some instances, arboviruses from the same genus produce cross-reactive antibodies. In geographic areas where two or more closely-related arboviruses occur, serologic testing for more than one virus may be needed and results compared to determine the specific causative virus. For example, such testing might be needed to distinguish antibodies resulting from infections within genera, e.g., flaviviruses such as West Nile, St. Louis encephalitis, Powassan, Dengue, or Japanese encephalitis viruses.
- **Rise and fall of IgM antibodies.** For most arboviral infections, IgM antibodies are generally first detectable at 3 to 8 days after onset of illness and persist for 30 to 90 days, but longer persistence has been documented (e.g, up to 500 days for West Nile virus). Serum collected within 8 days of illness onset may not have detectable IgM and testing should be repeated on a convalescent-phase sample to rule out arboviral infection in those with a compatible clinical syndrome.
- **Persistence of IgM antibodies.** Arboviral IgM antibodies may be detected in some patients months or years after their acute infection. Therefore, the presence of these virus-specific IgM antibodies may signify a past infection and be unrelated to the current acute illness. Finding virus-specific IgM antibodies in CSF or a fourfold or greater change in virus-specific antibody titers between acute- and convalescent-phase serum specimens provides additional laboratory evidence that the arbovirus was the likely cause of the patient's recent illness. Clinical and epidemiologic history also should be carefully considered.
- **Persistence of IgG and neutralizing antibodies.** Arboviral IgG and neutralizing antibodies can persist for many years following a symptomatic or asymptomatic infection. Therefore, the presence of these antibodies alone is only evidence of previous infection and clinically compatible cases with the presence of IgG, but not IgM, should be evaluated for other etiologic agents.
- **Arboviral serologic assays.** Assays for the detection of IgM and IgG antibodies commonly include enzyme-linked immunosorbent assay (ELISA), microsphere immunoassay (MIA), or immunofluorescence assay (IFA). These assays provide a presumptive diagnosis and should have

confirmatory testing performed. Confirmatory testing involves the detection of arboviral-specific neutralizing antibodies utilizing assays such as plaque reduction neutralization test (PRNT).

- **Other information to consider.** Vaccination history, detailed travel history, date of onset of symptoms, and knowledge of potentially cross-reactive arboviruses known to circulate in the geographic area should be considered when interpreting results.

Imported arboviral diseases

Human disease cases due to dengue or yellow fever viruses are nationally notifiable to CDC using specific case definitions. However, many other exotic arboviruses (e.g., chikungunya, Japanese encephalitis, tick-borne encephalitis, Venezuelan equine encephalitis, and Rift Valley fever viruses) are important public health risks for the United States as competent vectors exist that could allow for sustained transmission upon establishment of imported arboviral pathogens. Health-care providers and public health officials should maintain a high index of clinical suspicion for cases of potentially exotic or unusual arboviral etiology, particularly in international travelers. If a suspected case occurs, it should be reported to the appropriate local/state health agencies and CDC.

Appendix 2: Calculation and Application of a Vector Index (VI) Reflecting the Number of WN Virus Infected Mosquitoes in a Population.

BACKGROUND. The establishment of West Nile (WN) virus across North America has been accompanied by expanded efforts to monitor WN virus transmission activity in many communities. Surveillance programs use various indicators to demonstrate virus activity. These include detecting evidence of virus in dead birds, dead horses, and mosquitoes; and detection of antibody against WN virus in sentinel birds, wild birds, or horses (Reisen & Brault 2007). While all of these surveillance practices can demonstrate the presence of WN virus in an area, few provide reliable, quantitative indices that may be useful in predictive surveillance programs. Only indices derived from a known and quantifiable surveillance effort conducted over time in an area will provide information that adequately reflects trends in virus transmission activity that may be related to human risk. Of the practices listed above, surveillance efforts are controlled and quantifiable only in mosquito and sentinel-chicken based programs. In these programs, the number of sentinel chicken flocks/ number of chickens, and the number of mosquito traps set per week is known and allows calculation of meaningful infection rates that reflect virus transmission activity.

PREMISE BEHIND DEVELOPING THE VECTOR INDEX.

Mosquito-based arbovirus surveillance provides three pieces of information: The variety of species comprising of the mosquito community; density of each species population (in terms of the number collected in each trap unit of a given trap type); and if the specimens are tested for the presence of arboviruses, the incidence of the agent in the mosquito population. Taken individually, each parameter describes one aspect of the vector community that may affect human risk, but the individual elements don't give a comprehensive estimate of the number of potentially infectious vectors seeking hosts at a given time in the surveillance area.

Parameter	Information Provided	Value in Surveillance Program
Mosquito Community Composition	Diversity of species in the area	Documents the presence of competent vector species in the area
Mosquito Population Density	Relative abundance of mosquito species in terms of trapping effort	Quantifies the number of individuals of each mosquito species at a given point in time, particularly important for key vector species.
Infection Rate of Virus in Mosquito Population	Proportion of the mosquito population carrying evidence of the disease agent	Quantifies incidence of infected and potentially infectious mosquitoes in the key vector population. Demonstrates if important bridge vectors are involved

VECTOR INDEX

To express the arbovirus transmission risk posed by a vector population adequately, information from all three parameters (vector species presence, vector species density, vector species infection rate) must be considered. The Vector Index (VI) combines all three of the parameters quantified through standard mosquito surveillance procedures in a single value (Gujaral et al. 2007, Bolling et al. 2009, Jones et al. 2011, Kwan et al. 2012, Colborn et al. 2013 in press). The VI is simply the estimated average number of infected mosquitoes collected per trap night summed for the key vector species in the area. Summing the VI for the key vector species incorporates the contribution of more than one species and recognizes the fact that West Nile (WN) virus transmission may involve one or more primary vectors and several accessory or bridge vectors in an area.

Deriving the Vector Index from routine mosquito surveillance data

The Vector Index is expressed as:

$$\text{Vector Index} = \sum_{i=species} \overline{N}_i \hat{P}_i$$

Where: $\overline{N} = \text{Average Density}$ (number per trap night for a given species)

$\hat{P} = \text{Estimated Infection Rate}$ (proportion of the mosquito population WNV positive)

Calculating the vector index in an area where two primary WNV vector species occur:

Step 1: Calculate mosquito density

Trap Site	*Cx. tarsalis*	*Cx. pipiens*
1	68	21
2	42	63
3	139	49
4	120	31
5	42	12
6	31	57
Total	442	233
Average per Trap Night	74	39
Standard Deviation	41	21

Step 2: Calculate the WNV infection rate for each species (as a proportion)

Pools Tested for Virus			
Pool Number	Species	Number in pool	Positives
1	*Cx. tarsalis*	50	0
2	*Cx. tarsalis*	50	0
3	*Cx. tarsalis*	50	1
4	*Cx. tarsalis*	50	0
5	*Cx. tarsalis*	50	0
6	*Cx. tarsalis*	50	0
7	*Cx. pipiens*	50	1
8	*Cx. pipiens*	50	0
9	*Cx. pipiens*	50	0
10	*Cx. pipiens*	50	0
11	*Cx. pipiens*	50	0
Cx. tarsalis			
Infection Rate	Lower limit	Upper limit	Confidence interval
0.0033	0.0002	0.0169	0.95
Cx. pipiens			
Infection Rate	Lower limit	Upper limit	Confidence interval
0.0040	0.0002	0.0206	0.95

Step 3. Calculate individual species Vector Index (VI) values, multiplying the average number per trap night by the proportion infected. Calculate combined VI value by summing the individual species VIs.

Vector Index Calculation	*Cx. tarsalis*	*Cx. pipiens*
Avg / trap night	74	39
Proportion infected	0.0033	0.004
VI (individual species)	0.24	0.16
VI (Combined)	**0.40**	

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Appendix 3: Interim Guidance for States Conducting Avian Mortality Surveillance for West Nile Virus (WNV) and/or Highly Pathogenic H5N1 Avian Influenza Virus.

This guidance, which is directed to state health departments, supplements current *Guidelines for WNV Surveillance, Prevention, and Control* (www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf) and *An Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds: U.S. Interagency Strategic Plan* (www.doi.gov/issues/birdflu_strategicplan.pdf).

Surveillance of dead birds for WNV has proven useful for the early detection of WNV in the United States. In recent months, it has also proven useful for the early detection of highly pathogenic H5N1 avian influenza A (HPAI H5N1, hereafter referred to as H5N1 virus) in Europe. Given the potential for H5N1 to infect wild birds in North America in the future, the following interim guidance is offered to support the efforts of states conducting avian mortality surveillance.

General Considerations for States Conducting Avian Mortality Surveillance

- If different agencies within a state are separately responsible for conducting surveillance for WNV or H5N1 among wild birds, the sharing of resources, including dead birds submitted for testing, may increase the efficiency of both systems.
- Any dead bird might be infected with any one of a number of zoonotic diseases currently present in the United States (US), such as WNV. However, in countries where H5N1 has been found in captive and wild birds, it frequently has resulted in multiple deaths within and across species, and if H5N1 enters the US, it is likely to result in the death of wild birds. If wild birds in the US are exposed to the virus, both single and groups of dead birds should be considered potentially infected.
- Avian mortality due to the introduction of H5N1 could occur at any time of the year, whereas WNV is more often detected when mosquitoes are active.
- To date, no human infections of WNV have been confirmed due to contact with live or dead wild birds in outdoor settings.
- Most human H5N1 cases overseas have been associated with close contact with infected poultry or their environment; however, a very small number of cases appear to be related to the handling of infected wild birds or their feathers or feces without the use of proper personal protective equipment (PPE). There is no evidence of H5N1 transmission to humans from exposure to H5N1 virus-contaminated water during swimming; however this may be theoretically possible. (http://www.who.int/csr/disease/avian_influenza/guidelines/pharmamanagement/en/www.who)
- Although handling infected birds is unlikely to lead to infection, persons who develop an influenza-like illness after handling sick or dead birds should seek medical attention. Their health care provider should report the incident to public health agencies if clinical symptoms or laboratory test results indicate possible H5N1 or WNV infection.

Infection Control and Health and Safety Precautions

These guidelines are intended for any person handling dead birds. The risk of infection with WNV from such contact is small. The risk of infection with H5N1 from handling dead birds is difficult to quantify and is likely to vary with each situation. Risk is related to the nature of the work environment, the number of birds to be collected, and the potential for aerosolization of bird feces, body fluids, or other tissues. The most important factor that will influence the degree of infection risk from handling wild birds is whether H5N1 has been reported in the area. Local public health officials can be consulted to help in selecting the most appropriate PPE for the situation.

General Precautions for Collection of Single Dead Birds (These precautions are applicable to employees as well as the general public)

When collecting dead birds, the risk of infection from WNV, H5N1, or any other pathogen may be eliminated by avoiding contamination of mucous membranes, eyes, and skin by material from the birds. This can be accomplished by eliminating any direct contact with dead birds via use of the following safety precautions:

- When picking up any dead bird, wear disposable impermeable gloves and place it directly into a plastic bag. Gloves should be changed if torn or otherwise damaged. If gloves are not available, use an inverted double–plastic bag technique for picking up carcasses or use a shovel to scoop the carcass into a plastic bag.
- In situations in which the bird carcass is in a wet environment or in other situations in which splashing or aerosolization of viral particles is likely to occur during disposal, safety goggles or glasses and a surgical mask may be worn to protect mucous membranes against splashed droplets or particles.
- Bird carcasses should be double bagged and placed in a trash receptacle that is secured from access by children and animals. If the carcass will be submitted for testing, hold it a cool location until it pickup or delivery to authorities. Carcasses should not be held in close contact with food (e.g., not in a household refrigerator or picnic cooler).
- After handling any dead bird, avoid touching the face with gloved or unwashed hands.
- Any PPE that was used (e.g. gloves, safety glasses, mask) should be discarded or disinfected* when done, and hands should then be washed with soap and water (or use an alcohol-based hand gel when soap and water are not available). http://www.cdc.gov/cleanhands/
- If possible, before disposing of the bird, members of the public may wish to consult with their local animal control, health, wildlife or agricultural agency or other such entity to inquire whether dead bird reports are being tallied and if the dead bird in question might be a candidate for WNV or H5N1 testing.

Additional Precautions for Personnel Tasked with Collecting Dead Birds in Higher-Risk Settings (e.g., when collecting large numbers or in confined indoor spaces, particularly once H5N1 has been confirmed in an area)

- Minimize any work activities that generate airborne particles. For example, during the cleanup phase of the bird removal, avoid washing surfaces with pressurized water or cleaner (i.e., pressure washing), which could theoretically aerosolize H5N1 viral particles that could then be inhaled. If aerosolization is unavoidable, the use of a filtering face-piece respirator (e.g., N95)

would be prudent, particularly while handling large quantities of dead birds repeatedly as part of regular work requirements.

- If using safety glasses, a mask, or a respirator, do not remove until after gloves have been removed and hands have been washed with soap and water (or use an alcohol-based hand gel when soap and water are not available). After PPE has been removed, hands should immediately be cleaned again. http://www.cdc.gov/cleanhands/ Personal protective equipment worn (e.g., gloves, mask, or clothing) should be disinfected* or discarded.
- Discuss appropriate biosafety practices and PPE use with your employer.

*Recommendations for PPE Disinfection

For machine-washable, reusable PPE: Disinfect PPE in a washing machine with detergent in a normal wash cycle. Adding bleach will increase the speed of viral inactivation as will hot water but detergent alone in cold water will be effective. Follow manufacturer recommendations for drying the PPE. Non machine-washable, reusable PPE should be cleaned following the manufacturer's recommendations for cleaning.

Laboratory Biosafety Recommendations

Laboratory handling of routine diagnostic specimens of avian carcasses requires a minimum of BSL-2 laboratory safety precautions. However, if either WNV or H5N1 infection of the specimens is suspected on the basis of previous surveillance findings, at a minimum BSL-3 precautions are advisable. Consult your institutional biosafety officer for specific recommendations. Biosafety levels are described at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm.

Additional Information Sources

Interim Guidance for Protection of Persons Involved in U.S. Avian Influenza Outbreak Disease Control and Eradication Activities http://www.cdc.gov/flu/avian/professional/protect-guid.htm

Interim Guidelines for the Protection of Persons Handling Wild Birds with Reference to Highly Pathogenic Avian Influenza H5N1 can be found at
http://www.nwhc.usgs.gov/publications/wildlife_health_bulletins/WHB_05_03.jsp

Avian Influenza: Protecting Workers at Risk http://www.osha.gov/dts/shib/shib121304.html