CE update [blood banking | microbiology and virology | molecular diagnostics]
West Nile Virus: Laboratory Diagnosis and FDA Guidance

Henry O. Ogedegbe, PhD, BB(ASCP)SC, Halcyon St. Hill, EdD, MT(ASCP)
College of Health Professions, Florida Gulf Coast University, Fort Myers, FL

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After reading this article, the reader should understand the epidemiology and genetics of the West Niles virus in addition to understanding the current compliance requirements for blood banks and laboratory personnel. Blood banking exam 60301 questions and corresponding answer form are located after the “Your Lab Focus” section on p. 489.

Background

West Nile virus is a mosquito-borne flavivirus and human equine and avian neuropathogen. It is a small spherical RNA virus (about 50 nm), a member of the Flaviviridae family, and belongs to the Japanese encephalitis (JE) subgroup. It consists of a lipid envelope which encloses a single-stranded positive sense ribonucleic acid genome with approximately 11,000 nucleotides lacking a 3’prime poly A tract. The WNV like other lipid enveloped viruses is inactivated by heat and solvent detergent treatment. It is closely related to the St. Louis encephalitis, Kunjin virus, and Muray Valley encephalitis virus. It was first isolated in the West Nile Province of Uganda in 1937. Between 1950 and 1957, Israel recorded its first epidemics. In 1974, the largest epidemic was reported in South Africa. The virus is indigenous to Africa, Asia, Europe, and Australia, and has recently caused large epidemics in Romania, Russia, and Israel. Recent outbreaks of WNV encephalitis in humans have occurred in Algeria in 1994, Romania in 1996-1997, the Czech Republic in 1997, the Democratic Republic of the Congo in 1998, Russia in 1999, the United States in 1999-2001, and Israel in 2000. Epizootics of disease in horses occurred in Morocco in 1996, Italy in 1998, the United States in 1999-2001, and France in 2000. There were reports of epidemics in Western Europe in the Rhone delta of France in 1962. Three hundred ninety-three cases of West Nile meningoencephalitis (WNME) with subclinical human infection occurred in southeast Romania resulting in 17 deaths in 1996.5 A susceptible avian population, urban/suburban infrastructures, and related conditions that were favorable for the production of a large population of Culex pipien mosquitoes contributed to this epidemic. Results of serosurveys conducted as the epidemic waned implicated the novel introduction of WNV to Bucharest.5,6

Between 1963 and 1993, several strains of WNV were isolated from ticks, birds, and mosquitoes in the southeastern part of European Russia and western Siberia. Anti-WNV antibody was found in 0.4% to 8% of healthy adult donors in the same regions. However, the health authorities did not consider WNV infection as a potentially emerging infection. Consequently, the large outbreak of WNV in southern Russia which began in late July 1999 did not receive timely recognition.7 The recent geographical expansion of WNV into new areas which
were previously without WNV activity is highlighted by the detection of WNV in North America.8

North American Invasion of West Nile Virus

A WNV epidemic occurred in New York City and 2 surrounding New York counties in 1999. About the same time, an epizootic among American crows and other bird species was observed in 4 states. Until this epidemic, there had never been documentation of indigenous transmission of WNV in the western hemisphere. The epizootic expanded to 12 states including the District of Columbia in 2000, while the epidemic continued in New York City, 5 New Jersey counties, and 1 Connecticut county. Several large epidemics also occurred in other regions of the world where this disease was absent or rare less than 5 years ago. Isolates of many strains of the virus from recent outbreaks have demonstrated an extremely high degree of homology that suggests widespread circulation of potentially epidemic strains. There is no clear explanation for the high rates of severe neurological illness and death among humans, horses, and birds.9 Subsequent to WNV reaching North America in 1999, an outbreak of encephalitis occurred in humans in the New York area. At the same time, a number of cases of equine encephalitis, and deaths in American crows and a variety of North American natives and exotic bird species were reported. Since April 2000, several states have instituted surveillance for WNV and this has resulted in the detection of WNV in thousands of dead birds from several species in North America, as well as in several species of mammals. The surveillance system developed in North America through the testing of dead birds for the rapid detection of WNV presence has been a unique integration of public health and wildlife health agencies.10

The emergence of one or more new strains of WNV is believed to be responsible for the recent upsurge in clinical WNV infection in wild and domestic animals. It was found that the virus isolated in New York in 1999 is identical to that isolated in Israel. As the invasion of WNV in the western hemisphere continues, new disease characteristics and patterns have been observed and many more are evolving. More research involving animals are required to determine bird migration as a mechanism of virus dispersal, vector and vertebrate host relationships, virus persistence mechanisms, laboratory diagnosis, viral pathogenesis, risk factor studies, vaccine development, and WNV impact on wildlife. Another area of research interest focuses on the primary reservoir host species and suitable sentinel species for active surveillance.10

From 1999 thru 2002, WNV extended its range throughout much of the eastern parts of the United States, and its range within the western hemisphere continues to expand. Enzootic activity has been documented in 44 states and the District of Columbia since its first identification in New York City in 1999. More than 1,745 human cases of WNV resulting in 84 deaths were verified by the CDC in 2002.11

Investigation and Reports of WNV in the United States

The CDC, the FDA, the Health Resources and Services Administration (HRSA), blood collection agencies, and state and local health departments continue to investigate WNV infections in blood, blood components, and transfusion recipients. The CDC received reports from 14 states during the period of August 28 through October 16, 2002, of 25 patients with WNME and 4 with other WNV-associated illnesses. In the month prior to the illness onset, the patients reported having received blood component transfusions. All 29 patients resided in areas that were known to have high levels of WNV activity.12 The CDC also received reports of another group of 47 persons who might have contracted possible transfusion-related WNV infection during the period of August 28 to October 26, 2002. Further investigations indicated that 14 of the individuals might not have had WNV infection, and the WNV infection was not acquired through transfusion. The other 33 persons who had confirmed WNV infection had received blood components in the month prior to onset of illness.13,14

The CDC, the FDA, the American Red Cross (ARC), and state health departments in Georgia and Florida investigated the transmission of WNV from a single organ donor to 4 organ recipients. The organ donor had been transfused with numerous blood and blood components to treat injuries he had received which eventually proved fatal. Four organs recovered from the donor were subsequently transplanted into 4 people. The CDC performed PCR on the donor serum collected during procurement of the organs, which revealed the presence of WNV. The case definition for WNV encephalitis was met by 3 of the 4 organ recipients.15

The recipient of one of the donor kidneys developed a febrile illness 13 days after transplant. The illness progressed to encephalitis, which required transient mechanical ventilation. The cerebrospinal fluid (CSF) gave a positive result for WNV immunoglobulin M (IgM) antibody. A second kidney recipient developed a febrile illness 17 days after transplant, which progressed to fatal encephalitis. Brain tissues obtained at autopsy were strongly positive for WNV by quantitative PCR and also were positive by flavivirus specific immunohistochemical staining. Ataxia was observed in a third patient who received a heart transplant 8 days following transplant. The patient later became unresponsive, required mechanical ventilation, and his CSF and serum was strongly positive for WNV IgM antibody. A fourth patient was given a liver transplant and he developed fever, cough, and malaise 7 days following transplantation. The patient had no clinical evidence of encephalitis, his symptoms resolved, and he was subsequently discharged from the hospital.15

Four other patients with WNV infection who were diagnosed after receiving units of blood in the weeks before WNV diagnosis have also come to the attention of the CDC. It is likely that these individuals were infected through mosquito exposure since they lived in areas known to have high levels of WNV activity. Investigations
are ongoing in order to rule out blood transfusion-associated transmission through follow-up and WNV testing on donors of blood given to these patients. In each instance, precautionary measures taken have included withdrawal of any remaining blood products obtained from the donors from inventory.16

A case of WNV transmission through blood transfusion was noted in a 47-year-old man who had received a liver transplant and 24 units of blood products. He was discharged from the hospital but was readmitted 10 days later with a complaint of fever. Subsequently, he developed encephalopathy and a lumbar puncture revealed elevated protein, a lymphocytic pleocytosis, and WNV IgM antibody. The patient eventually recovered and was discharged. Kinetic quantitative PCR assay were performed on the available retention segments from 20 donors. One retention segment was positive and the remaining 19 were negative.17 A 40-year-old woman who had delivered a healthy infant and was transfused with 2 units of red blood cells (RBC) for anemia was discharged but later developed intermittent nausea, malaise, and fever. She was readmitted to the hospital 13 days after discharge with a fever of 39.3°C. A lumbar puncture revealed mildly elevated protein, lymphocytic pleocytosis, and WNV IgM antibody. Records of the blood center showed that one of the transfused RBC units was derived from the same donation that was found to be PCR positive as the blood component received by the liver transplant recipient. The patient had started breast-feeding on the day of delivery and a sample of breast milk obtained 16 days later tested positive for WNV and for WNV-specific IgM antibody. The patient recovered and was discharged. The infant continued to be breast-fed and remains healthy.17

Genetics of West Nile Virus and Viral Antigen

The complete nucleotide sequences for 8 WNV strains include: Egypt 1951, Romania 1996-MQ, Italy 1998-equine, New York 1999-equine, MD 2000-crow265, NJ 2000MQ5488, NY 2000-crow3282, and NY 2000-crow3356.18 Construction of phylogenetic trees from the aligned nucleotide sequences of these 8 strains have revealed the presence of 2 genetic lineages of WNV. Lineage 1 isolates have been from the northeastern United States, Europe, Israel, Africa, India, Russia, and Australia while lineage 2 isolates have been from sub-Saharan Africa and Madagascar.18

Mishra and colleagues19 studied the distribution of WNV antigen in different tissues of mosquitoes in 3 species including *Culex tritaeniorhynchus*, *C. vishnui*, and *C. pseudovishnui* and found that overall percent positivity was higher in the mosquitoes that were intrathoracically inoculated when compared to the mosquitoes that were orally infected. This suggested that a midgut barrier existed. The salivary glands were found to be negative in a small number of mosquitoes even though fluorescence was seen in the respective head squashes. This suggested the presence of a salivary gland barrier in the mosquitoes. There were no differences in the percent salivary gland and salivary gland area positivity between the 3 species. A finding of epidemiological importance that suggested the possibility of trans-ovarial transmission of virus even in the first gonotrophic cycle was the presence of virus antigen in the ovaries of the 3 species on the third post-infection day.19

Disease Prevalence

Statistically, a person’s risk of contracting WNV is low. In most areas where the virus is established, only 1% of the area’s mosquitoes carry the virus. Less than 1% of people bitten by these infected mosquitoes develop serious complications from the virus, while the remainder exhibit flu-like symptoms or no symptoms at all. Individuals at highest risk are the elderly and people with weakened immune systems.11 There were reports of 142 cases of neuroinvasive WNV disease of the central nervous system (CNS), which included 18 fatalities and 7 cases of uncomplicated WNV fever in the United States from 1999 through 2001. Clinical infection can range in severity from uncomplicated WNV fever to fatal meningoencephalitis; however, most human infections are subclinical.8,10,20,21

Severe neurological illness may be seen in 1 in 150 WNV infections. Advanced age appears to be the most important risk factor for neurological disease as well as the most severe clinical outcome once disease develops. One hundred forty-nine persons with WN- related illness were identified in 10 states through surveillance programs with the most commonly reported illness being encephalitis. Often, a clinical clue to the presence of WNV infection is muscle weakness and flaccid paralysis. The incidence of WNV infection peaks in late summer, although there have been reported cases of onset from July through December.21

Natural Reservoir of West Nile Virus

West Nile virus is a ubiquitous arbovirus, which occurs over a broad geographical range in a diverse variety of vertebrate host and vector species. Clinical disease resulting from natural WNV infection in wild or domestic animals is not as commonly reported as reports of infection. Records of morbidity and mortality in wild birds were confined to a few infrequently reported cases and infections. Between 1996 and 2001, there was an upsurge of illness due to WNV in animals and humans, which included reports of WNV encephalitis in horses in Italy in 1998 and in France in 2000. Between 1997 and 1999, the first report of disease and deaths caused by WNV infection in domestic birds and hundreds of young geese was noted in Israel.10

The natural reservoir hosts for WNV are birds, and while the virus is maintained in nature in a mosquito-bird-mosquito transmission cycle primarily involving *Culex* mosquitoes, humans can be incidental hosts. Incidental mosquito-borne infection may also occur in other animals including horses, cats, squirrels, and domestic animals. The Camargue region of southeastern France includes the cities of Arles and Avignon and a large protected wildlife area mainly composed of marshes whose ecosystem fa-
West Nile virus infection can cause severe, potentially fatal neurological illnesses such as encephalitis and meningitis. Acute infection also has been associated with acute flaccid paralysis (AFP). This is attributed to a peripheral demyelinating process, such as Guillain Barre Syndrome (GBS) or to an anterior myelitis. The exact etiology of AFP has not been assessed thoroughly with electrophysiologic, laboratory, and neuroimaging data. However, WNV-associated AFP might be due to a pathologic process involving anterior horn cells and motor axons which is similar to that seen in acute poliomyelitis. Clinicians should evaluate patients with AFP for evidence of WNV infection and conduct tests to differentiate GBS from other causes of AFP. A brief viremia, which causes fever, headache, myalgias, and enlarged lymph nodes, follows an incubation period of 4 to 21 days. In about half of the WNV cases, invasion of the CNS is marked by stupor, disorientation, coma, tremors, convulsion, paralysis, high fever, muscle weakness, and at times may result in death. The illness may occasionally be complicated by meningoencephalitis, which results in a mortality rate of between 5% and 20%. Pleocytosis is usually demonstrated on examination of CSF and shows an increase in lymphocytes and high concentrations of protein, neopterin, and β2 microglobulin. Evidence of a pathologic process is indicated by the presence of meningeal congestion and inflammation, brain edema, and widespread encephalitis.

Prevention depends on elimination of mosquito breeding sites, judicious use of pesticides, and avoidance of mosquito bites through the use of mosquito repellents and public education. Prompt reporting of cases along with swift responses from local and state health departments and the CDC can prevent larger outbreaks of the virus.

McCarthy and colleagues did a study to estimate the seroprevalence of WNV infection and assess the risk perception and practices with regards to potential exposures to mosquitoes of persons living in an area that had intense epizootics in 1999 and 2000. A serosurvey of persons aged 12 years or greater was conducted in southwestern Connecticut using household-based stratified cluster sampling. Participants were asked to complete a questionnaire regarding concern for and personal measures taken with respect to WNV and provide a blood sample for WNV testing. Seven hundred and thirty persons from 645 households were recruited for the study and none of the participants tested positive for WNV. About 44% of the participants used mosquito repellent, 56% practiced 2 or more personal precautions to avoid mosquitoes, and 61% of households did 2 or more mosquito-source reduction activities. In addition, McCarthy and colleagues found that using mosquito repellent was associated with persons less than 50 years in age, using English as the primary language in the home, being worried about WNV, having concerns about pesticides, and finding mosquitoes frequently in the home. Also females and persons who were very worried about WNV were more likely to practice 2 or more personal precautions. The study showed that an intense epizootic could occur in an area without having a high risk for infection to humans. McCarthy and colleagues further suggested that a better understanding of why certain people do not take personal protective measures, especially among those aged 50 years and over and those whose primary language is not English, might be needed if educational campaigns are to prevent future WNV outbreaks.

Educational campaigns in the spoken language of participants may prove to be an effective means to assist in understanding the importance of and using personal protective measures.

Laboratory Diagnosis

There is currently no FDA approved tests for WNV diagnosis or donor screening. The ongoing American epidemic of human infections with WNV has strongly suggested that WNV can be transmitted through blood transfusions, even though the level of risk is under investigation. Nearly 80% of infected persons are asymptomatic even in the presence of transient viremia. Consequently, screening of blood and plasma donors for WNV may be necessary to protect blood safety if there is a persistence of the epidemic. The FDA is encouraging manufacturers to develop tests for WNV that can be implemented on a large scale. Until these tests are available, diagnostic test for WNV based on detecting IgM antibodies to WNV can be obtained from state or local health departments. However, this test is not appropriate for donor blood screening. The FDA will recommend routine licensed donor screening tests to detect WNV infection when such tests become available. The FDA also plans to allow widespread use of appropriate tests under an Investigational New Drug (IND) application once the tests are available.

Although cross-reactions are possible in patients who are recently vaccinated against or who are recently infected with related flaviviruses, IgM antibody testing of serum specimens and CSF is the most efficient method of diagnosis. Plaque reduction neutralization assays can be performed to distinguish among the flaviviruses. Both standard reverse transcription-polymerase chain reaction (RT-PCR) and Taqman RT-PCR may be used to establish the diagnosis of WNV infection from CSF. Indirect fluorescent anti-
body assay may also be used to establish the diagnosis from serum or CSF samples. The assay detects the presence of IgM and/or IgG antibody to WNV in serum and/or CSF. The samples are tested with positive and negative controls on IFA slides on which are fixed whole cells infected with WNV. The level of fluorescence of each sample is determined, and if positive, the sample is titrated to its endpoint.23

Koraka and colleagues29 evaluated a newly developed, commercially-available immunofluorescence assay (IFA) for the detection of IgM and IgG antibodies against dengue virus, yellow fever virus, JE, and WNV. Immunofluorescence assay was compared with standard diagnostic enzyme immunosassays (EIAs) specific for the detection of IgM and IgG antibodies against these viruses. Forty-seven serum samples were tested from patients with a defined flavivirus infection, and serum samples from individuals with antibodies against tick-borne encephalitis virus and hepatitis C virus as well as healthy individuals were used as controls. The results of the study indicated that IFA had a significantly better discrimination for flavivirus specific IgM antibodies than did the standard IgM specific EIAs with the overall cross-reactivity varying between 4% and 10% by IFA and 30% to 44% by EIA for the respective viruses. In contrast, the detection of flavivirus specific IgG antibodies showed high cross-reactions in both IFA and EIAs with overall cross-reactivity of 16% to 71% and 62% to 84%, respectively. The study underscores the complexity of flavivirus diagnosis, implying that the use of one assay or search for one virus only with the current methods are not reliable due to cross reactivity. The flavivirus IFA may be useful for the identification of flavivirus infections during the acute stage of disease and especially in samples obtained from travelers who have been accidentally exposed to these viruses. Testing can be arranged through local or state health departments,8,10,20,21,29

**FDA Guidance for Blood Donors Suspected of WNV Infection**

The provision of a safe and sufficient blood supply is critical to patient care. Volunteers are carefully screened for health problems prior to donation, which helps to maintain adequate and safe blood supply in the United States.2 The FDA requires that all donated blood are tested for various transmissible viruses and bacteria including human immunodeficiency virus (HIV-1 and -2), human T-cell lymphotrophic viruses (HTLV-I and II), hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV), and syphilis. Testing for cytomegalovirus (CMV) is generally done after collection when CMV seronegative products are required. Testing is not routinely done for human parvovirus B19, hepatitis A virus (HAV), hepatitis G virus (HGV), or hepatitis E virus (HEV).2 Similarly, screening is also not done at the present time for WNV. However, many safety layers are used to protect the blood supply from WNV transmission, including careful screening of blood donors that includes disqualifying and deferring those who may be experiencing symptoms of WNV such as fever and chills. On October 25, 2002, the FDA issued a final guidance for industry, recommendations for the assessment of donor suitability and blood and blood product safety in cases of known or suspected West Nile virus infection.1 This final guidance applies to whole blood and blood components that are intended for transfusion purposes. It also includes recovered plasma, source leukocytes, and source plasma intended for use in further manufacturing into injectable or non-injectable products.1,28

Presented below are numbered statements, which reflect the FDA final guidance recommendations, followed by a brief discussion as applicable.

1) WNV diagnosis and illness:
Defer potential donor diagnosed with WNV infection 14 days after the condition is resolved and at a minimum of 28 days from the onset of symptoms or diagnosis, whichever date is later.1

2) Suspected WNV cases: Donors with an otherwise unexplained post-donation febrile illness suggesting WNV infection and who are in a community or setting of active WNV transmission should be deferred for 28 days after the onset of illness, or 14 days after resolution of the condition, whichever date is later.1,28

When a potential donor lacks current or recent symptoms, an IgM antibody test result should not indicate deferral. Blood collection centers are required to encourage donors to report post-donation illness that could be construed as WNV infection that occurs within 2 weeks of blood donation in a community where WNV transmission is active.

3) Retrieval, quarantine of blood and blood products, and notification of prior transfusion recipients: The FDA recommends retrieval and quarantine of previously collected in-date units of blood and blood components intended for transfusion and unpoled source plasma, recovered plasma, and source leukocytes intended for further manufacturing into injectable products and notification of prior transfusion recipients in the following situations.1,28

(a) When a blood establishment learns that a donor has been diagnosed with WNV, it should consider tracing records and notifying transfusion services so they can alert treating physicians of prior recipients of blood and blood components collected from the donor. Relevant units are those dating from 14 days before through 28 days after the onset of illness in the donor. (b) In cases where it is determined that a specific donor is the likely source of WNV transmission, the FDA recommends that the blood establishments consider tracing records and notifying transfusion services so that they can alert treating physicians of prior recipients of blood and blood components collected from the donor. In this case, the relevant units would be those dating from 28 days before to 28 days after the date of the donation implicated in WNV transmission.1,28 Notification
of transfusion services would not be indicated if a donor is potentially associated with WNV transmission, but the investigation has not established him or her as a likely source of WNV transmission. Although the guidance is intended to address WNV, the FDA states that these recommendations also may be useful in reducing the risk of SLE, which is also transmissible to humans by mosquito bite, and like WNV, is a member of the flaviviridae family. While there is currently no known transmission of SLE through blood transfusion, such a possibility cannot be completely excluded.

(4) Labeling products: Blood and blood products used for research or intended for manufacturing non-injectable products should be labeled appropriately and consistent with the following recommended labels as applicable: (a) “Bio-hazard”; (b) “Collected from a donor determined to be at risk for West Nile virus,” or “Collected from a donor positive for evidence of infection with West Nile virus”; and (c) “For laboratory research only” or “Intended only for further manufacturing into non-injectable products.”

(5) Reporting of biologic deviation and fatality: In accordance with 21 CFR 606.171, the Regulations on Reporting of Product Deviations by Licensed Manufacturers, Unlicensed Blood Establishments, and Transfusion Services are applicable. Under these regulations post donation WNV cases with product retrieval, quarantine, and/or recipient notification must be followed up with submission of a biological product deviation report. In addition, under FDA [21 CFR 606.170(b)], when fatality occurs in a transfusion, blood establishments are required to report it to the FDA. Reports of WNV should be reported to the CDC.

**Treatment of West Nile Virus Infection**

There is no specific therapy for WNV infection. In situations in which the disease is severe, intensive supportive therapy may be indicated, including hospitalization, intravenous (IV) fluids and nutrition, ventilator support if needed, and prevention of secondary infections. There are currently no vaccines available for WNV infection. Immunosuppressed patients have a much higher case-fatality rate, compared to non-immunosuppressed patients. Hamdan and colleagues described a 42-year-old male lung-transplant recipient who had a serologically confirmed WNV encephalitis and deteriorating level of consciousness. The patient was treated with 0.4 g/kg IV immunoglobulin preparation from donors that contained a high titer of WNV antibodies. The patient showed rapid improvement within 24 hours and complete recovery within 48 hours. According to Hamdan, this is the second case of an immunosuppressed patient responding to the same preparation of intravenous immunoglobulin.

Kanesa-Hasan and colleagues investigated whether immunization with licensed inactivated JE vaccine or experimental live attenuated dengue vaccines could result in induction of cross-neutralizing antibodies against WNV. Protective neutralizing antibody titers to WNV were not detected in any volunteer despite successful immunization to related flaviviruses. Vaccination against JE or dengue is unlikely to prevent WNV infections. There are currently no vaccines needed, and prevention of secondary infections is urgent. The patient was treated with 0.4 g/kg IV immunoglobulin preparation from donors that contained a high titer of WNV antibodies. The patient showed rapid improvement within 24 hours and complete recovery within 48 hours. According to Hamdan, this is the second case of an immunosuppressed patient responding to the same preparation of intravenous immunoglobulin.

**Conclusion**

West Nile virus infection is an emerging public health problem. Efforts should be intensified to increase surveillance activities and public education. Although most cases of WNV infection present with sub clinical disease, it is still important to minimize exposure to disease causing mosquitoes and to protect recipients of blood, blood products, and donated organs. It is important to follow the FDA guidance for industry recommendations regarding donor assessment and suitability of products discussed herein.


