Animal Health Diagnostic Center
College of Veterinary Medicine, Cornell University
In Partnership with the NYS Dept of Ag & Markets

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West Nile Virus Diagnostic Testing

West Nile virus (WNV) is a member of the *Flaviviridae* family in the genus *Flavivirus*. Other members of the genus include St. Louis encephalitis virus, dengue virus, Zika virus and yellow fever virus. The hallmark of this group of viruses is their transmission by various species of mosquitoes. Up until 1999, WNV was considered an "Old World" virus, but in that year its introduction into the US in the New York City area triggered an epizootic that spread the virus to all 48 contiguous states. Now the virus is enzootic in much of North and South America. The virus is maintained in a mosquito – bird – mosquito cycle with spill over into other animals such as reptiles and mammals being incidental to life cycle of the virus. The virus persists in passerines but infection does occur in other bird species such as raptors and corvids. Over 300 species of birds have been documented as having been infected with WNV, but this number is likely a gross under estimate. WNV seems to be able to infect anything that a mosquito will bite. A recent publication indicates that WNV is continuing to exert a negative impact on the populations of certain bird species such as purple finches, wrentits, and white-crowned sparrows.

The most significant impact of WNV in veterinary medicine excluding wildlife is the incidental infection of equines which can lead to viral encephalitis. Even though a viremia develops early in the infection of the horse, the amount of virus in blood is too low to infect mosquitoes. WNV can infect other animals such as dogs and cats, but clinical signs do not usually develop.

Testing strategies

The type of tests and timing of the sampling for diagnosing WNV infections depends on the species of the test subject:

Avian

1. Agent detection

A. RT-PCR testing

a. Whole blood – EDTA preferred (**DO NOT** use heparin for anti-coagulant as heparin is inhibitory in PCR assays.

b. Fresh tissue samples: Liver, spleen, kidney, bone marrow, brain, heart.

c Formalin-fixed tissue: same sample set as fresh tissue (a surcharge will be applied for the additional costs related to extraction of formalin-fixed tissues.)

d. Oral or cloacal swabs preferably in small volume of transport medium or saline (oral swabs may not be appropriate for all bird species such as raptors).

B. Virus isolation

a. Virus isolation can be done on all samples suitable for PCR EXCEPT formalin-fixed tissues.

2. **Antibody detection** preferred sample is serum. Can be done with plasma, but starting dilution of assay will be higher to dilute out anti-coagulant. As with most serological assays, paired samples are needed for an accurate assessment of a recent infection (or vaccination).

A. Plaque-reduction neutralization assay (PRNT)

Traditional test used for flavivirus antibody detection where cross-reactivity with other viruses may occur, e.g. St Louis encephalitis virus.

B. Microplate serum (virus) neutralization assay can be done by special request.

Mammals

1. Agent detection

A. RT-PCR testing

a. Whole blood or serum **IS NOT** a standard sample for agent detection as viremia levels in mammals are low and usually undetectable at the time clinical signs are noted.

b. Fresh tissues: brain, spinal cord, CSF.

c Formalin-fixed tissue: same sample set as fresh tissue (a surcharge will be applied for the additional costs related to extraction of formalin-fixed tissues.)

B. Virus isolation

a. Virus isolation can be done on all samples suitable for PCR EXCEPT formalin fixed tissues.

2. **Antibody detection** preferred sample is serum. Can be done with plasma, but starting dilution of neutralization assays will be higher in order to dilute out anti-coagulant. As with most serological assays, paired samples are needed for an accurate assessment of a recent infection (or vaccination).

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A. Microplate serum (virus) neutralization assay (SN). Microplate test gives titer values 2-4 fold higher than the plaque reduction assay and is more economical. - Plaque reduction neutralization assay on mammalian samples will be done only by special request.

B. Antibody capture ELISA tests (**EQUINE and CANINE ONLY**)

a. IgM ELISA test. (serum, CSF, and plasma by special request). Test is used to detect an early immune response due to infection. Test interpretation must take into account vaccination status of the animal.

b. IgG ELISA test. (serum, CSF, and plasma by special request). Test is used to define the infection or vaccination status of the horse. Can be done in combination with the IgM ELISA to obtain a more comprehensive immune status of the horse. Paired serum samples may be necessary to define the infection status of a vaccinated horse.

Reptiles and Amphibians

A. Use testing strategies listed for Avian.