West Nile Virus

Diagnosis of West Nile Fever Using IgM Antibody Detection
West Nile fever clinical signs raise the suspicion of WNV infection, but because cases of WNV infection may have no apparent signs, diagnosis requires both clinical assessment and laboratory tests. West Nile fever can be diagnosed by detecting the virus using virology or polymerase chain reaction methods. But the most useful method is one that detects IgM antibodies to WNV. IgM antibodies can be detected for up to three months after infection. Therefore, the presence of IgM antibodies reveals the presence of recent WNV infection, making this detection method useful for epidemiological studies, as well as for disease diagnosis. The OIE Terrestrial Manual calls the IgM-capture ELISA “particularly useful in detecting recent natural exposures and infections by West Nile virus.”

IgM Detection and West Nile Virus Infection

<table>
<thead>
<tr>
<th>Viremia</th>
<th>IgM Ab</th>
<th>IgG Ab</th>
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<tbody>
<tr>
<td>Infection</td>
<td>Onset of clinical signs (only 20%–30% of horses show clinical signs)</td>
<td>3 months &gt; 1 year</td>
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</table>

Accurate Timing of Infection Allows Appropriate Response
Because IgM are detectable from seven days to three months after WNV exposure, a positive result from an IgM-capture ELISA means the horse was exposed to West Nile Virus within the last three months. Such a recent infection indicates that mosquitoes are spreading WNV in the area. Armed with this knowledge, you can take appropriate action to protect animals and eliminate mosquitoes.

Protective Measures
The key to protecting against West Nile virus is to control the mosquito population and reduce exposure to mosquitoes. Insect screens and repellents are useful. Other recommendations include isolating horses from mosquitoes, using fans to keep mosquitoes away and switching off lights at night.

Sensitivity and Specificity Studies
The IDEXX IgM WNV Ab Test demonstrated 99% sensitivity and 100% specificity in tested populations. Although there is no reference serum for use with West Nile ELISAs, the IDEXX IgM WNV Ab Test was tested using a positive serum from the French National Reference Laboratory. The IDEXX test detected the positive serum at a dilution of 1:104,800.

IDEXX IgM WNV Ab Test Product Information
2-plate kit: Ref P00730-2
5-plate kit: Ref P00730-5
• IgM for West Nile virus is detectable from 7 days through 3 months post infection
• The presence of WNV IgM indicates the animal was recently infected with WNV

For more information about West Nile Virus, please contact your IDEXX representative or visit idexx.com/wnv.

Reference
West Nile Virus

About 70% of horses do not present clinical signs, while 20% present mild signs. Neuro-invasive forms of infection are observed in about 1%–2% of infected horses; these forms include meningitis, encephalitis, panencephalitis, meningoencephalitis, and myalgia. Between 20% and 40% of horses left with neuro-invasive infections die. There is no specific treatment for West Nile fever.

Origin of West Nile Fever in Uganda

West Nile fever is a disease caused by West Nile virus (WNV), a single-stranded RNA virus of the Flaviviridae family. Other viruses in this family cause Japanese encephalitis, yellow fever, and Saint Louis encephalitis. The name of the virus comes from the West Nile district of Uganda where the disease was first recognized in 1937. It was then spread to the Western Hemisphere where it first appeared in the New York area and then entered Canada, Mexico, and Latin America. There are two virus strains. Lineage 1 is found mostly in Europe, North Africa, Central Africa, Israel, India, Central America, North America, Argentina and Columbia. Lineage 3 is endemic in Central Africa, Southern Africa and Madagascar.

Clinical Signs

West Nile fever primarily affects birds, humans, and horses. The disease is generally asymptomatic, but in about 20% of cases, flu-like symptoms such as fever, headache, anorexia and myalgia are reported. The disease can also cause encephalitis and aseptic meningitis. Humans and horses are considered “dead-end” hosts because WNV does not replicate enough within them to reach a transmissible level. In horses, the incubation period is 3–15 days. Infected horses usually show afebrile and can also show weakness, muscle fasciculation and cranial nerve deficits.

West Nile Virus Transmission

The IDEXX IgM WNV Ab Test is a two-step capture ELISA that accurately detects IgM antibodies against West Nile virus (WNV) in horse serum.

West Nile Fever Transmission

Because IgM antibodies can be detected for only three months after infection, positive test results indicate both recent WNV exposure and the presence of WNV circulating in the area.

Immune Status Ratio (ISR): Provides a Clear Interpretation of Results

Results of the ELISA are expressed as an Immune Status Ratio (ISR), which is the ratio between the optical density of the WNRA well and the optical density of the NCA well for each sample. Results are interpreted as follows:
- ISR > 3.00 = Positive (the sample does contain IgM antibodies against WNV)
- ISR < 2.00 = Negative (the sample does not contain IgM antibodies against WNV)
- ISR between 2.00 and 3.00 = Doubtful (this sample requires a confirmatory test).

Example of IDEXX IgM WNV Ab Test Result Interpretation

Two-Step Capture ELISA Method

The following figure illustrates the two-step capture IgM ELISA method employed by the IDEXX IgM WNV Ab Test.

1. Each sample is placed in two wells of the microplate. If the sample contains IgM antibodies, they become fixed to the anti-iM-specific antigen in the microplate wells.
2. The microplate is washed, then West Nile Recombinant Antigen (WNRA) is added to one well and Normal Cell Antigen (NCA) is added to the other well for each sample.
3. If the sample contains WNV-specific IgM antibodies, the WNRA becomes fixed to the IgM in the wells.
4. The microplate is washed, then alkaline phosphatase conjugate against West Nile is added to all wells. The conjugate binds to any WNRA fixed to the IgM.
5. The microplate is washed, then TMB enzyme substrate is added to the wells. If the conjugate was bound to the WNRA in step 3, the TMB enzyme forms a blue compound that becomes more intense after stop solution is added.
6. The microplate is read at 450 nm.

The following figure illustrates the two-step IgM capture method employed by the IDEXX IgM WNV Ab Test.

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2. The microplate is washed, then WNRA becomes fixed to the IgM in the wells.
3. If the sample contains WNV-specific IgM antibodies, the WNRA becomes fixed to the IgM in the wells.
4. The microplate is washed, then TMB enzyme substrate is added to the wells. If the conjugate was bound to the WNRA in step 3, the TMB enzyme forms a blue compound that becomes more intense after stop solution is added.
5. The microplate is read at 450 nm. The intensity of the color is proportional to the amount of antibody in the sample.

Confirmation of West Nile Virus Transmission

Results of the ELISA are expressed as an Immune Status Ratio (ISR), which is the ratio between the optical density of the WNRA well and the optical density of the NCA well for each sample. Results are interpreted as follows:
- ISR > 3.00 = Positive (the sample does contain IgM antibodies against WNV)
- ISR < 2.00 = Negative (the sample does not contain IgM antibodies against WNV)
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IDEXX IgM WNV Ab Test detects recent exposure to West Nile Virus

Validation criteria (All four criteria must be met. All readings are at 450 nm)