



Anti-West Nile Virus ELISA Horse (IgG) Avidity determination



- High specificity due to the use of a recombinant antigen
- Alternative principle for the detection of fresh infections (no influence from persisting IgM antibodies)
- Efficient automation solutions available

Technical data

Antigen	Recombinant glycoprotein E antigen of WNV
Avidity determination	Calculation of a relative avidity index (RAI) from the extinction of a sample which is analysed twice, with and without urea treatment
Result interpretation	EUROIMMUN recommends interpreting results as follows: RAI < 40%: Indication of low-avidity IgG antibodies RAI 40-60%: Borderline range RAI > 60%: Indication of high-avidity IgG antibodies
Sample dilution	Equine serum or plasma, 1 : 101 in sample buffer
Reagents	Ready for use, with the exception of the wash buffer (10x); colour-coded solutions
Test procedure	30 min (37 °C) / 10 min (room temperature) / 30 min (37 °C) / 15 min (room temperature), fully automatable
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Test kit format	96 break-off wells; kit includes all necessary reagents
Order no.	EI 2662-9601-1 GE

Clinical significance

West Nile virus (WNV) is an enveloped single-stranded RNA virus of the *Flaviviridae* family. WNV is present not only in tropical areas, but also in moderate climate regions. An increasing number of infections in humans and animals and studies on the detection of WNV in birds and mosquitoes have confirmed that WNV is increasingly spreading to areas of moderate climate in Europe and to Mediterranean regions. WNV is transmitted by a variety of mosquitoes, with the genera *Culex* and *Aedes* being the main arthropod hosts. Birds represent the vertebrate reservoir. Acting as incidental hosts, mammals can also become infected when bitten by an infected mosquito. However, the disease generally only manifests in horses and humans.

After an incubation period of 3 to 15 days, WNV infection in horses mostly leads to a short viraemic phase with low virus titers. Only around 10% of infected horses show clinical symptoms. The first symptoms are rather unspecific and include fever, depression, loss of appetite and colic. When the infection proceeds, neurological disorders often follow, leading to ataxia and lameness (paresis), which are considered as typical clinical symptoms. In rare cases, problems of the facial nerves, photosensitivity and blindness, sub-sultus as well as general sensitivity and personality changes are observed. 20% of horses with past clinical infection show long-term sequelae such as weight loss, lethargy, ataxia and cerebral nerve problems. In non-vaccinated horses, the infection is fatal in 24% to 45% of clinical cases. Intensive medical care is the only possibility to positively influence the illness. A vaccine with formalin-inactivated WNV is available for horses.

Differential diagnosis includes further arbovirus-caused encephalitides (e.g. tick-borne encephalitis, equine encephalomyelitis and Japanese encephalitis), equine herpesvirus 1, Borna disease and rabies. Since the degree of antigenic similarity within the Flavivirus genus is high, antibody cross reactions can occur.

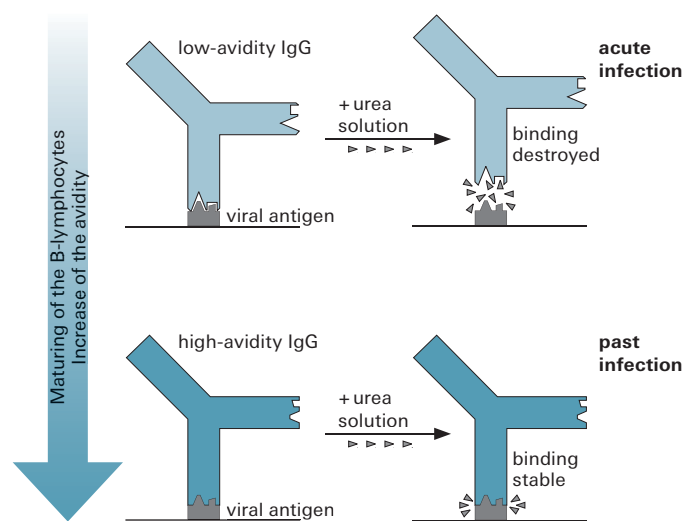


Diagnostic application

Diagnosis of WNV can be established by means of direct virus detection or detection of specific antibodies. Isolation of the virus from serum and CSF or virus detection via reverse transcriptase polymerase chain reaction (RT-PCR) is often unsuccessful because the viraemic phase is short and the virus titer is generally low. The detection of specific anti-WNV antibodies by means of serological methods is of particular importance. Specific IgM antibodies can be detected in equine serum after seven to ten days. The development of antibody titers in WNV-infected horses has only been investigated to a limited extent. Generally, IgM antibodies can be detected one to two months after infection. In human WNV infections, however, IgM antibodies could be detected six months to more than one year later. Persisting IgM antibodies can therefore complicate the diagnosis of acute WNV infections. Additionally, the determination of IgM antibodies is often unreliable and problematic since alongside the persistence, further disturbing factors such as weak, inexistent, or delayed IgM formation or unspecific IgM formation due to B cell stimulation may occur. By investigating low-avidity antibodies of class IgG, an additional parameter that significantly enhances serological analyses has been established. Fresh WNV infections can be serologically detected with the EUROIMMUN Anti-WNV ELISA Horse (IgG) Avidity determination (EI 2662-9601-1 GE) by means of low-avidity IgG antibodies.

Principle of the test: Determination of low-avidity IgG antibodies

The first reaction of the immune system to an infection is the formation of low-avidity antibodies. As the infection proceeds, antigen-adapted IgG is increasingly formed, and the avidity grows. If high-avidity IgG is detected in the serum, it can be assumed that the infection is in a late stage. In order to identify low-avidity antibodies in a patient sample, two samples are investigated in parallel in the ELISA. After sample incubation, one of the samples is treated with urea. Here, low-avidity antibodies detach from the antigens. The second sample remains untreated. Subsequently, the samples are incubated with peroxidase-labelled anti-horse IgG. The presence of low-avidity antibodies in a patient's serum has been proved if the ELISA extinction is significantly reduced by urea treatment. For an objective interpretation the relative avidity index (RAI) can be calculated from the measured values with and without urea incubation and expressed as a percentage.



Sensitivity and specificity

For the determination of sensitivity and specificity, pre-characterised horse samples were investigated with the Anti-WNV ELISA Horse (IgG) Avidity determination. In fresh WNV infections, low-avidity IgG antibodies were found in 100% of cases, and in past infections, high-avidity IgG antibodies were found in 100% of cases. Borderline results were not included in the evaluation.

Additionally, data from four horses experimentally infected with West Nile virus were investigated using the Anti-West Nile Virus ELISA Horse (IgG) Avidity determination. Low-avidity IgG antibodies could be already detected in all horses 14 days after infection. Since IgG antibodies are generally only detectable approximately 14 days after infection, an avidity determination seven days after infection is not evaluable.

n = 32		ELISA from other manufacturer	
		IgM positive (acute infection)	IgM negative (past infection)
EUROIMMUN Anti-WNV ELISA Horse (IgG) Avidity determination	low-avidity IgG	16	0
	borderline range	1	0
	high-avidity IgG	0	15

Horse	Days past infection	EUROIMMUN Anti-West Nile Virus ELISA	
		IgG	IgG Avidity determination
No. 1	before infection	-	n.e.
	7	-	n.e.
	14	-/+	low avidity
	21	+	low avidity

As an example, data of one experimentally infected horse are presented. Negative (-), borderline (-/+), positive (+), n.e. (not evaluable, since too few IgG-antibodies are present).