**TECHNICAL INFO: DETECTION OF HIGH AVIDITY IgG Abs TO WEST NILE VIRUS IN HORSES**

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| **Method / Test type** | **ELISA / Qualitative** |
| **Sample** | **(Equine) Serum or Plasma** |
| **Sample volume** | **1 ml** |
| **Min. volume accepted** | **0.2 ml** |
| **Sample collection** | **Serum or plasma shipped in pour off tubes** |
| **Shipping** | **Ship frozen or refrigerated, for next-day delivery. Frozen is recommended.** |
| **Stability** | **30 days at -20°C (Frozen); OR 14 days at 4°C (Refrigerated)** |
| **Rejection criteria** | **Sample that is not equine serum or plasma.**<br>**Clearly contaminated sample – bacterial, fungal, foreign objects.**<br>**Sample arriving outside of stability.** |
| **Reportable/Ref. range** | **Positive or Negative. Negative is normal.** |
| **Reporting time** | **3 days (from lab receipt)** |
| **Significance** | ***West Nile virus (WNV) is a mosquito-transmitted RNA (flavi)virus, with increasing prevalence in US due to expanding range of main vector, Culex pipiens. Approx. 10% of WNV infected horses develop clinical symptoms and in non-vaccinated horses 24-45% of clinical cases are fatal.*** **Initial symptoms are non-specific and can include fever, depression, loss of appetite, and colic. As infection progresses typical symptoms include ataxia and lameness due to neurological affects. Recovery ranges from few days to several weeks with long-term effects in 20% of horses with past clinical infection.**<br>**WNV diagnosis is by direct virus detection, or detection of specific antibodies, although due to short viremic phase, direct detection of WNV in live animals is often unsuccessful. From limited research on antibody titer development in WNV infected horses the detection of persistent IgG & IgM antibodies >12 months after infection complicates acute diagnosis.**<br>**For reliable differentiation between acute and old infections this testing relies on 2-phase testing.**<br>**1. Initial anti-WNV IgG (screen) must result positive to be considered for avidity testing.**<br>**2. Avidity detection calculate Relative Avidity Index (RAI): presence of low-avidity IgG antibodies evidence of an acute WNV infection, while high-avidity IgG antibodies indicate a late-stage / past WNV infection.**<br>**If the avidity test is negative despite the presence of clear symptoms of West Nile fever, a second sample should be tested for low-avidity antibodies some days later.**<br>**As antigenic similarity within Flaviviruses is high, antibody cross-reactivity is likely, and therefore positive results should be confirmed using different test system.** |

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| **Test specifics** | **Sandwich ELISA: 96-well plate coated with WNV antigen, if sample, or controls contain specific anti-WNV immunoglobulins (IgG) they bind to WNV antigen and amount (titer) of specific anti-WNV IgG bound is determined by secondary binding of IgG-specific conjugated antibody used to catalyze a colorimetric reaction (with TMB) to produce blue color at OD450nm.**<br><br>**A urea treatment is used to determine low-avidity antibody presence – substantial drop in OD with urea treatment due to lowered binding of low-avidity antibodies in presence of urea**<br><br>**Sample result is based on Relative avidity index (RAI) calculated as %:**<br><br>**(Extinction (OD) of urea-treated sample) * 100**<br>**(Extinction (OD) without urea-treatment)**<br><br>**<40% indicative of low avidity antibodies (acute infection)**<br><br>**40-60% Equivocal**<br><br>**>60% indicative of high avidity antibodies (late-stage / past infection)**<br><br>**NOTES:**<br><br>**Determination of RAI in samples WITHOUT urea treatment with OD< 0.140 is not considered accurate.**<br><br>**False high RAI values were obtained in samples WITHOUT urea treatment with OD>1.2, it is recommended to retry samples resulting OD>1.2 at higher sample dilution, UNLESS resulting as low avidity antibodies no further testing required.**<br><br>**For equivocal result recommended to collect another sample: >7 days later and retest old and new sample at same time.** |