



## **AAEP Guidelines for Serology in Horses with Adverse Events from Vaccination**

### **Introduction**

These guidelines are intended to be a reference for veterinarians who wish to utilize serological testing to determine levels of circulating antibodies (titers) to specific pathogens. It should be recognized that levels of circulating antibodies are only one component of a complex immune system and that for most vaccine antigens the correlation between serological response to vaccination and protection in the horse has not been well established. As such, obtaining and assessing levels of circulating antibodies to specific pathogens has limitations. The only definitive way to assess whether an animal is resistant to infection is through experimental or natural challenge. These guidelines are neither regulations nor directives and should not be interpreted as such. It is the responsibility of attending veterinarians, through an appropriate veterinarian-client-patient relationship, to utilize all available relevant information to determine optimal health care programs for their patients and provide serology and vaccination recommendations to their clients. It is incumbent upon each individual practitioner to interpret the results of serological data and reach a decision on the significance of those results based upon the circumstances of each unique situation and his or her professional experience.

Recently there has been an increased interest by both horse owners and veterinarians to obtain information regarding serological titers in the horse and determine how that information may or may not correlate with protection from disease. Serological testing is generally done to *help* assess the immunological status of a patient and in certain situations *help* assess vaccine responses and the need to administer booster vaccinations. Serological data may also be used to enhance

risk/benefit analysis decisions in horses with a history of systemic vaccine-associated adverse events. When the risk and consequences of a systemic vaccine-associated adverse event exceeds the risk and consequences from the disease, it is rational to attempt to predict immunity by serologic testing to either eliminate vaccination for the disease in question or extend the revaccination interval for as long as possible. Serological testing may also be indicated to help assess immunological response to vaccination in horses that may be “poor or non-responders.” Examples would include horses with pituitary pars intermedia dysfunction, horses with combined variable immunodeficiency, or in older horses where immunosenescence may play a role in response to vaccination.

### **Immunology Considerations**

When assessing a possible role for serological testing in a clinical setting, it is important to recognize the basics of equine immune function and how different types of vaccines stimulate the immune system. Both innate and acquired immune responses are involved in recovery from an infection with microbial pathogens, and typically involve a combination of humoral and cellular mechanisms. These immune responses can occur systemically, providing evidence of pathogen-specific antibodies in the bloodstream, or locally – such as a mucosal surface – in which case the antibodies may or may not be present in the bloodstream and instead remain on the mucosal surface or in mucosal secretions. Many factors contribute to the type of immune response, including the specific pathogen, route of infection, the precise pathogenesis and the ability of the pathogen to evade or down-regulate immune responses. For some pathogens, levels of circulating antibody correlate well with protection, while for other pathogens protective indicators may involve systemic or local cellular responses or local humoral responses that cannot be readily quantified.

## **Vaccine Types & Immune Responses**

Vaccination remains an important approach to helping prevent infectious disease. There are various types of vaccines available to stimulate an intended immune response without causing disease. In general, the effectiveness of a vaccine is determined by how closely it can mimic the response to natural infection.

Inactivated parenterally administered vaccines are generally good inducers of systemic antibody responses, but less effective at inducing cell-mediated and local immune responses. Replicating modified-live virus (MLV) or vectored vaccines more closely mimic how a natural viral or bacterial infection is processed. Attenuated parenterally administered live vaccines are generally effective at inducing both cellular and humoral responses, whereas intranasally administered vaccines may induce local mucosal responses and cellular responses without inducing significant increases in the level of circulating antibodies and effector T cells. As such, route of administration as it relates to route of infection frequently plays an important role in protection induced by vaccines.

## **Core Serology Guidelines**

### **Eastern/Western Equine Encephalomyelitis (EEE/WEE)**

Serologic correlates with protection against EEE and WEE are not well established in the horse. However, infection with EEE and WEE virus is acquired by way of vascular injection (mosquito bite), and proper and appropriate administration of inactivated vaccines (which induce primarily humoral responses) are for the most part highly efficacious. Therefore, circulating virus neutralizing antibodies are likely to play a prominent role in protection from disease.

A positive plaque reduction neutralization test (PRNT) against EEE or WEE provides evidence of previous infection or vaccination. Although titer levels do not reliably predict protection against infection and disease, the degree of susceptibility can be

inferred. Horses with titers <1:10 are likely susceptible to disease while horses with titers >1:100 are most likely protected. However, during interpretation of these results and making decisions regarding vaccinations, one needs to consider the high morbidity and mortality rates associated with EEE and the documented need for frequent revaccination in highly endemic areas such as the southeastern US. Although horses residing in the western US are at low risk for EEE, they remain at risk for WEE.

As with all diseases, interpretation and utilization of serologic data to guide vaccination decisions for EEE and WEE should be done with consideration being given to all other factors including but not limited to risk of disease, consequences of disease, risk of systemic vaccine associated adverse events, prior vaccination history, and the health status of the animal.

PRNT and immunoglobulin M antibody capture (MAC) ELISA tests are available through the National Veterinary Services Laboratories in Ames, Iowa. MAC-ELISA is exclusively utilized for diagnosis of EEE as it can differentiate between vaccinal and viral-induced titers. A positive MAC-ELISA titer provides evidence of recent infection, whereas a negative result does not predict susceptibility to viral challenge.

## **Rabies**

Rabies is a fatal disease that is known to be reliably prevented in horses by routine annual administration of an approved rabies vaccine. While most jurisdictions do not require rabies vaccination of horses, mandated management of horses (and other domestic animals) that are exposed to rabies, but are not currently vaccinated, may include a 6-month strict quarantine period or euthanasia. Before considering the use of serology as an alternative to routine vaccination, veterinarians should make their client aware that: failing to vaccinate may increase the risk of a fatal outcome following a rabies exposure; and that there may be state and local rabies statutes

and regulations that dictate the management of an unvaccinated, or not currently vaccinated, horse.

Although a definitive “protective” titer against rabies virus infection has not been established, research in several species indicates that the primary correlate to provide evidence of an immune response to rabies vaccine is the presence of rabies virus neutralizing antibody (RVNA). The rapid fluorescence focus inhibition test (RFFIT) is the current gold standard serological assay recommended by both the Advisory Committee on Immunization Practices (ACIP) and the World Health Organization (WHO) to measure RVNA. The RFFIT results are expressed as either a RVNA endpoint titer or as International Units of antibody per milliliter (IU/ml) of serum, which is a value for RVNA potency. For humans, the WHO guidelines consider a RVNA level of  $\geq 0.5$  IU/ml to be indicative of an adequate response to vaccination while the ACIP considers a RVNA titer of 1:5 indicative of an adequate response to vaccination. Titer levels or IU/ml values of  $\geq 0.5$  IU/ml are indicative of a robust response to immunization. This RVNA level is also the level recognized by regulatory authorities as evidence of an adequate response to vaccination for importation of dogs and cats into rabies free areas.

Specific levels of RVNA and the role of cellular immunity needed to confer protection in the horse are unknown. An overview of rabies challenge studies in dogs and cats suggest that RVNA levels predict survival on more of a qualitative than quantitative basis. The same likely holds true for horses. Per Title 9 of the Code of Federal Regulations (specifically 9 CFR 113.209) an alternate to challenging all vaccinates as part of licensure requirements is to challenge at least 10 vaccinates with the lowest titers. For at least one of the equine rabies vaccines, this method was selected, and all 10 vaccinates with the lowest titers selected for challenge survived challenge 14 months after the administration of a single dose. Low titers do not necessarily mean that the horse is susceptible to disease whereas high titers likely correlate with, but are not a guarantee of, protection. Low titers do provide justification for booster

vaccination. All things considered, it seems reasonable to extrapolate information from studies in humans, studies in other species and challenge studies for licensing of equine rabies vaccines to support the use of the RVNA level of  $\geq 0.5$  IU/ml as a guideline to provide evidence of an immune response to the vaccine in the horse.

Rabies virus is a potent antigen which generally stimulates a robust immune response in immunocompetent individuals. Horses generally respond very well to inactivated rabies vaccines with challenge studies for most vaccines supporting at least a 1-year duration of immunity following a single dose. Horses should receive a booster vaccination 1 year after the priming dose. Published data (Harvey et al. 2016) demonstrated that additional boosters (after the primary and 1-year booster) reliably resulted in rapid, robust, and long-lasting increase in RVNA antibody levels that persisted above the 0.5 IU/ml level for at least 36 months.

Horses that have had a life-threatening adverse reaction to rabies vaccine, may benefit from consideration of extending the revaccination interval beyond the recommended one year, providing the horse had previously received at least two (and preferably three) doses of rabies vaccine and that the horse has circulating RVNA levels  $\geq 0.5$  IU/ml. Titers on these individuals should be checked regularly and the interval between tests should not exceed 1 year. Additional factors that should be considered when deciding whether or not to use serology to monitor RVNA levels as opposed to routine vaccination, include: local rabies epidemiology, potential for exposure opportunities, and the public health consequences of development of disease (e.g. how much contact with the public does the animal have).

## **Tetanus**

Circulating levels of tetanus toxin binding (To-Bi) antibody are thought to correlate well with protection in the horse, with levels  $>0.01$  IU/ml considered to be protective. This level is referenced numerous times in the literature and is most likely a combination of information extrapolated from a limited number of challenge

studies in horses and recommendations for humans, which in turn are based on studies in guinea pigs. Tetanus toxoid is a very potent antigen that stimulates a strong antibody response following a 2 or 3 dose primary series in immunocompetent horses. Following proper immunization with at least two-doses of tetanus toxoid, antibodies generally persist at levels  $>0.01$  IU/ml for at least 12 months. Depending upon the immunological status of the horse, the product used, and the number of doses administered, To-Bi antibody levels may remain above the 0.01 IU/ml level for several years. However, one should use caution when extrapolating data from different studies using different vaccines. Measuring and monitoring To-Bi antibody levels may be justified for horses known to have systemic vaccine associated adverse events to tetanus toxoid or when it is thought to be beneficial to the health of the horse to decrease the number of antigens being administered. In these situations, the revaccination interval may be extended for as long as the To-Bi antibody levels remain above 0.01 IU/ml. It is worth noting that based upon the low amount of toxin per unit of body weight needed to induce lethal disease, horses are one of the more susceptible species to tetanus. In addition, clostridial spores are often present in environments where horses reside, so risk of exposure is always a consideration. Utilization of serological data to guide booster vaccination decisions should be done on a case by case basis utilizing evidence-based medicine and benefit/risk analysis to guide decisions.

A toxin-binding enzyme-linked immunosorbent (ELISA) assay is currently available at the Animal Health Diagnostic Center, Cornell University.

### **West Nile Virus (WNV)**

Similar to EEE and WEE, serologic correlates for protection against WNV are not well established in the horse. The plaque reduction neutralization test (PRNT) is available for detection of WNV-specific neutralizing antibodies in serum. There is currently no test available that can differentiate whether a positive PRNT titer to WNV is the result of vaccination or previous infection, although a positive MAC-ELISA result

provides evidence of recent infection. Based on research in hamsters, a minimal PRNT of 1:5 has been established. Horses with titers <1:5 are likely susceptible to disease, whereas horses with titers  $\geq$ 1:5 are most likely protected. Use of PRNT titers to assess disease susceptibility follows the same general principles as described above for EEE and WEE.

Though WNV is endemic in almost all areas of North America, the mortality rate is lower than for EEE infection. In addition, because many WNV infections are subclinical, serological evidence of exposure is widespread, even in non-vaccinated horses. Though appropriate administration of inactivated vaccines is for the most part highly efficacious, there is evidence that vaccinated horses may become infected and experience a “boost” in immunity, which may explain why some previously vaccinated horses show an increase in circulating antibody levels despite not having been revaccinated.

As with all diseases, interpretation and utilization of serologic data to guide vaccination decisions for WNV should be done with consideration being given to all other factors, including but not limited to risk of disease, consequences of disease, risk of systemic vaccine associated adverse events, prior vaccination history, and the health status of the animal.

The PRNT and immunoglobulin M antibody capture (MAC) ELISA tests are available through the National Veterinary Services Laboratories (NVSL) in Ames, Iowa. MAC-ELISA is exclusively utilized for diagnosis of WNV as it can differentiate between vaccinal and viral-induced PRNT titers. As with EEE, a positive MAC-ELISA titer provides evidence of recent infection, whereas a negative result does not predict susceptibility to viral challenge.

### **Risk-Based Serology Guidelines (limited to Equine Influenza, EHV, and Strangles)**

Circulating antibody titers associated with protection have not been reported for most risk-based diseases; therefore, there is little rationale for serologic testing of



horses to assess protection or the need for revaccination. However, since many questions arise regarding serologic correlates to protection against equine influenza, equine herpesvirus and strangles, and since these are the most commonly administered risk-based vaccines, comments regarding risk-based diseases have been included in these guidelines.

## **Equine Influenza**

Immunity generated by inactivated influenza vaccines administered intramuscularly is primarily reliant upon stimulation of circulating antibodies to the hemagglutinin (HA) glycoprotein. Consequently, high titers of circulating antibody correlate well with protection, depending on the test used to measure antibody. In contrast, immunity following administration of the cold-adapted intranasal vaccine or natural infection generates a broad range of adaptive humoral and cellular immune responses in both the systemic and mucosal compartments, the result being less reliance upon circulating antibodies to induce protection. Therefore, measurement of circulating antibodies is typically of limited value in predicting susceptibility to infection in horses vaccinated with the intranasal vaccine or those that have recovered from natural infection. In these horses, low antibody titers do not necessarily mean that the horse is susceptible to disease. Following natural infection, solid immunity persists even when circulating antibody levels have dropped to low or undetectable levels.

Levels of circulating antibody to equine influenza HA are primarily measured by the hemagglutination inhibition (HI) or single radial hemolysis (SRH) tests. SRH is more reproducible and has a higher sensitivity than the HI test. SRH results seem to be a good predictor of protection following vaccination with inactivated vaccines, with levels  $>140 \text{ mm}^2$  indicative of protection. However, correlation between HI titer and protection has yet to be established unequivocally. Unfortunately, the SRH test is not commercially available in the USA and although the HI test is available through NVSL, HI levels are not reliable for correlating titers to protection from disease.

Utilization of serology to help determine if one may extend vaccination intervals for equine influenza does not seem reasonable or advisable considering that serologic responses are only indicative of protection following administration of inactivated vaccines, that partial protection induced by inactivated vaccines is of limited duration (generally 6 – 7 months), and that the SRH test, which best correlates with protection, is not commercially available in the USA. For horses with known or expected systemic vaccine associated adverse events to intramuscular, inactivated influenza vaccines, one may want to consider administration of the cold-adapted intranasal influenza vaccine.

### **Equine Herpesvirus – 1 and Equine Herpesvirus - 4 (EHV-1&4)**

Systemic humoral immune responses alone are not sufficient to protect horses against infection with equine herpesvirus (EHV). An effective immune response requires a combination of mucosal, local (lymphoid), and systemic cellular and humoral responses. Although attenuation of clinical disease and reduction of viral shedding have been well documented in horses with high levels of circulating antibody, no clear relationship exists between protection from EHV infection and concentration of circulating antibody induced by either vaccination or infection. Infection and induction of clinical disease occur regularly in horses with high levels of circulating virus neutralizing antibody. Therefore, utilization of serology to assess protection or predict the need for vaccination cannot be recommended.

### **Strangles (*Streptococcus equi subspecies equi*)**

Evaluation of serum antibody levels in horses that have recovered from strangles and been challenged, as well as studies evaluating serum antibody levels in vaccinated horses, suggest that serological values for *S. equi* cannot be correlated to protection.

Veterinarians may utilize serology in evaluation of *Streptococcus equi subspecies equi* cases. The ELISA tests available to measure antibodies to the cell wall M-protein (Se-M) of *S. equi* are available from IDEXX and Equine Diagnostic Solutions in the US

and the Animal Health Trust in England. The ACVIM Revised Consensus Statement on *Streptococcus equi* Infections in Horses provides detailed guidelines on interpretation of serology for *S. equi* and can be accessed here

<https://onlinelibrary.wiley.com/doi/full/10.1111/jvim.15043>.

Vaccination is not recommended in horses with SeM titers  $\geq 1:3,200$  because of increased risk of systemic adverse reactions, including purpura hemorrhagica.

### **Summary**

Interpretation and utilization of serologic data to guide vaccination decisions should be done with caution and with consideration of all other factors associated with occurrence of disease. These include, but are not limited to risk of disease, consequences of disease, risk of systemic vaccine-associated adverse events, prior vaccination history, and data to support titer correlates to protection against the disease. It should be emphasized and recognized that circulating antibodies are only one component of a very complex immune system. Levels of circulating tetanus toxin binding antibody and rabies virus neutralizing antibody appear to correlate with protection in the horse. For most other vaccine antigens, the correlation between vaccine-induced serologic response and protection has either not been established or is not plausible based on the biology of the disease in question.

**Tables, References, Authors and Disclaimer on following pages.**

**Table 1:** Summary of serologic testing guidelines and cautionary statements for AAEP **core equine diseases**, including laboratories offering routine antibody testing.\* Please note: Expected duration of protection based on titers should not preclude horses from receiving core vaccines in accordance with manufacturer labels and AAEP guidelines.

Pathogen or toxin	Titer associated with protection	Expected duration of protection following revaccination of primed horses	Cautionary comments and limitations	Laboratories offering antibody testing
EEE and WEE	>1:100 (PRNT)	1 year	EEE/WEE documented in vaccinated horses	National Veterinary Services Laboratories, Ames, Iowa
Tetanus toxin	> 0.01 IU/mL	2 to 8 years	Tetanus documented in vaccinated horses	Animal Health Diagnostic Center, Cornell University
Rabies virus	≥0.5 IU/mL	2 to 3 years	Adequate titer level only available for humans	Kansas State Veterinary Diagnostic Laboratory Atlanta Health Associates, Cumming, Georgia
West Nile Virus	1:5 (PRNT)	1 year	Protective titer only available for hamsters	National Veterinary Services Laboratories, Ames, Iowa

\*Wilson WD. 2017. Assessment of Vaccination Status and Susceptibility to Infection. In: Pusterla N, Higgins J, editors. Interpretation of Equine Laboratory Diagnostics. 1<sup>st</sup> ed. Hokoken (NJ): Wiley-Blackwell. p. 376-400. (Table adapted with permission from Drs. Nicola Pusterla and David Wilson.)

**Table 2:** Summary of serologic testing guidelines and cautionary statements for AAEP **risk-based equine diseases (EIV, EHV & Strangles)**, including laboratories offering routine antibody testing (where applicable) \*

<b>Pathogen or toxin</b>	<b>Titer associated with protection</b>	<b>Expected duration of protection following revaccination of primed horses</b>	<b>Cautionary comments and limitations</b>	<b>Laboratories offering antibody testing</b>
EIV	SRH levels >140mm <sup>2</sup>	<1 year	Antibody titer not a measure of protection for modified-live intranasal vaccine	National Veterinary Services Laboratories, Ames, Iowa
EHV-1, EHV-4	Not reported	Not reported	Antibody titers don't predict susceptibility or resistance to infection	N/A
<i>Streptococcus equi</i> ss. <i>equi</i> (Strangles)	Not reported	Not reported	Titer-specific interpretations regarding risk associated with vaccination	National Veterinary Services Laboratories, Ames, Iowa
<i>Neorickettsia risticii</i> (Potomac Horse Fever)	Not reported	Not reported	Antibody levels do not provide useful information regarding susceptibility to infection or the need for vaccination	National Veterinary Services Laboratories, Ames, Iowa
EVA	Not reported	Not reported	Documentation of seronegative status prior to export or prior to vaccination	National Veterinary Services Laboratories, Ames, Iowa
<i>Clostridium botulinum</i> type B toxoid (Botulism)	Not reported	Not reported	Vaccine as an aid in prevention of the shaker foal syndrome	N/A
Equine rotavirus	Not reported	Not reported	Antibody levels do not provide useful information regarding	N/A

			susceptibility to infection or the need for vaccination	
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\*Wilson WD. 2017. Assessment of Vaccination Status and Susceptibility to Infection. In: Pusterla N, Higgins J, editors. Interpretation of Equine Laboratory Diagnostics. 1<sup>st</sup> ed. Hoboken (NJ): Wiley. p. 376-400.

*(Table adapted with permission from Drs. Nicola Pusterla and David Wilson.)*

### Primary Reference

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### Supporting References

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### **Guideline Disclaimer**

*AAEP guidelines are created to simply serve as guidelines for the practitioner and the equine industry. As such, they do not have the force of law. All guidelines issued by the AAEP should be regarded as one of several tools a practitioner may take into consideration in the context of his or her practice. All practitioners are encouraged first and foremost to understand and comply with the laws, regulations and standard of care of their appropriate jurisdiction. Whereas guidelines are intended to promote a standard for veterinary practice, lack of adherence to any specific AAEP guideline does not constitute grounds for disciplinary action. The AAEP can exercise disciplinary action only in connection with its own members and its action is limited to denial of membership in the AAEP. The AAEP shall have no liability whatsoever for any guideline.*

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