



Animal Health Diagnostic Center

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

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Equine Protozoal Myeloencephalitis (EPM) Testing

WHY TEST?

- EPM is a neurological disease of the Americas. It is most commonly caused by the parasite *Sarcocystis neurona* and more rarely by *Neospora hughesi*.
- The standard of practice for diagnosis is to perform a complete neurological exam, accompanied by laboratory tests that detect an immunological response to infection.
- Testing of cerebrospinal fluid (CSF), with a paired serum, is more predictive of active disease than serum alone.
- The reluctance to perform a spinal tap due to risk, cost or inexperience is understandable and although not the preferred approach, a positive serum IgG test in the presence of neurologic signs and history compatible with EPM, supports a diagnosis of EPM.
- Although response to treatment is often used as a diagnostic test, there are many false results that would have been predicted by prior serum and CSF testing.
- Testing of non-neurologic horses is generally not recommended.
- Repeat side-by-side testing with any quantitative test format can be useful in noting changes in the level of IgG with time, either due to natural response or in response to treatment.

AVAILABLE TESTS:

- All detect the IgG response to the causative parasite.
- Are distinguishable by the strain of parasite used as antigen source, assay format, validation samples, recommended diagnostic sample and result interpretation.
- Western blot (WB) is a complex, semi-quantitative method requiring much expertise. The presence of specific immunoreactive bands is the basis for the subjective interpretation of results. The designated bands vary by lab. This method is more sensitive to blood contamination in CSF samples, which could cause false positive results.
 - The original standard WB (Granstrom *et al.* 1993) was well validated with several hundred necropsied neurologic cases as the gold standard. Paired serum and CSF samples are recommended. The most current calculation of test performance was performed by Equine Diagnostic Solutions (EDS) using 66 necropsied neurologic horses with 30 diagnosed as EPM. Respectively, serum and CSF sensitivities were 90% and 83% and the specificities were 42% and 86%.
 - The modified WB (Rossano *et al.* 2000) includes a step using bovine sera with a high titer of *Sarcocystis cruzi* IgG under the premise of eliminating cross reactivities and increasing specificity. This species infects cattle but not horses. This test was validated using sera from 57 horses native to India and Germany, where EPM does not exist (true negatives) and 6 EPM necropsy positive cases (true positives). Using these two different standards, the sensitivity and specificity were reported as 100% and 98%, respectively. The specificity would be expected to be much lower if normal horses from EPM endemic regions were used as negative EPM cases.



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- The procedures and validation of WB performed at several academic labs (UC Davis, Auburn and Oregon State) have not been publically presented or published.
- Enzyme linked immunosorbent assay (ELISA) is a simple, quantitative method which generates titers. Depending on the antigen used, the actual titer values and their significance vary.
 - The immunodominant surface antigen designated 1 (SAG1) ELISA (Ellison *et al.* 2003) was validated (serum and CSF) using 6 experimentally infected horses. Based on this limited set, the sensitivities were reported on marketing material as 94% (serum) and 100% (CSF) and specificities as 86% (serum) and 94% (CSF). A serum titer of 1:32 is said to be clinically significant. The same strain of *S. neurona* was used for both the SAG1 ELISA test development and experimental infection. It is now well documented that a number of *S. neurona* isolates lack the SAG1 gene and will test as false negative.
 - The surface antigens 2, 3 and 4 (Howe *et al.* 2011) are the basis of the newest EPM test. They were validated with paired sera and CSFs from 66 necropsy (neurologic) cases and ~200 well characterized field cases (presented at 2010 ACVIM, manuscript in preparation). While individual serum and CSF titers can be determined, the ratio of serum:CSF titers is very predictive of an EPM diagnosis. As intrathecal IgG production increases, the titer ratio decreases and ratios of <100 strongly correlate with EPM. A <100 ratio has a sensitivity = 83% and a specificity = 97%. Although a wide range of serum titers was observed for necropsy positive EPM cases, there was a trend for higher serum titers ($\geq 1:4000$) to correlate better with EPM. A recent paper about EPM like disease in sea otters (Wendte *et al.* 2010) raised the question about potential cross reactivities due to *S. falcatula*, but research has shown that horses do not raise an IgG response when experimentally infected by this species (Cutler *et al.* 1999).
- Immunofluorescent antibody (IFA) is a complex method with subjective interpretation. Parasites are immobilized on glass slides and the IgG titers of a sample are determined by visual assessment of indirect detection of surface immunofluorescence (Duarte *et al.*, 2004). It was validated using paired sera and CSFs from a set of 110 general necropsy cases including 8 EPM positive cases with reported sensitivities of 83% (serum) and 100% (CSF) and specificities of 97% (serum) and 99% (CSF). A subsequent paper (Duarte *et al.*, 2006) included samples from 182 horses - many with multiple collections - with either natural infections, experimental infections or enrolled in a vaccine study to examine persistence of antibody. The analysis of this entire set (102 paired sera & CSFs, 326 serum only and 253 CSF only) was used to generate theoretical probabilities of clinical EPM based on mathematical modeling/simulation. Using serum and CSF titers said to have <50% probability of EPM as negative (not likely EPM), the calculated sensitivities and specificities are far lower than as originally claimed, with sensitivities of 67% (serum) and 64% (CSF) and specificities of 80% (serum) and 89% (CSF). This assay also has a known cross reactivity with *S. fayeri*, a *Sarcocystis* that can infect horses but does not cause EPM.



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TEST COMPARISON STUDIES (published or presented):

- Duarte *et al.* (2003): 48 sera from UC Davis necropsy cases of horses with and without neurologic disease (EPM confirmed = 9) were tested by standard WB (SWB), modified WB (MWB) and IFA with the resulting calculated test parameters. The sensitivities were identical for all three at 89%. Specificities were reported as 100% (IFA), 87% (SWB) and 69% (MWB).
- Saville (2007): 20 sera from an experimental infection study, field submissions with EPM (1 confirmed by necropsy) or Wobbler diagnoses and native European horses were tested by 4 commercial *S. neurona* IgG tests. The standard WB results (performed by the lab now known as EDS) correlated well with sample histories, especially when CSF was also tested (as recommended). The Michigan State modified WB generated many false positives, including non-infected controls and native European horses. The SAG1 ELISA (Pathogenes) generated many false negatives, including the EPM necropsy positive case. The UC Davis IFA results also correlated well with sample histories except for giving a false positive for a horse experimentally infected with *S. fayeri*.
- Johnson *et al.* (2010): Hospital cases were distinguished by necropsy as EPM (T=9) or not EPM (T=17) or by clinical characterization as EPM suspect (T=10) or not EPM suspect (T=29). Testing of subsets of this group by SAG1 ELISA (Antech Diagnostics) and IFA (UC Davis) revealed calculated test performance parameters that were generally lower than as originally published for each assay. IFA sensitivities were 94% (serum) and 92% (CSF) and specificities were 85% (serum) and 90% (CSF). SAG1 ELISA serum sensitivity was 13% and specificity was 97% (CSF was not tested).
- Reed *et al.* (2010): 20 well characterized cases (necropsied = 12) diagnosed as either EPM or Wobbler were tested by 4 *S. neurona* IgG tests using the sample(s) recommended by each lab. For an EPM diagnosis, false negatives were 15% (EDS CSF SWB and EDS/Howe SAG 2, 4/3 serum:CSF titer ratios), 46% (UCD serum IFA) and 69% (Pathogenes serum SAG1 ELISA). False positives were 0% (EDS/Howe SAG 2, 4/3 serum:CSF titer ratios), 14% (EDS CSF SWB), 29% (Pathogenes serum SAG1 ELISA) and 43% (UCD serum IFA).

RESULT INTERPRETATION:

- Serum
 - A positive serum IgG result means that the horse has been exposed to the causative parasite. However, in the presence of accompanying clinical signs that are characteristic of EPM, a positive serum is supportive of an EPM diagnosis.
 - Different levels of IgG may reflect the stage of infection, not necessarily correlation to a given likelihood of clinical EPM, although there seems to be some relationship between magnitude and disease. Further, individual horses may not respond to the same level.
 - The seroprevalence (% of a horse population that is seropositive) varies by geography and a positive serum result may be more meaningful in areas of low seroprevalence (positive



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predictive value). It has been estimated that ~50% of all horses in the US have been exposed to *S. neurona*.

- Few (<1%) of exposed horses develop clinical EPM.
- A negative result is usually a good “rule-out” for EPM although with all tests, the sensitivity may be <90%. An exception to this is the acute onset of disease prior to a detectable level of an IgG response. Retesting in 10-14 days may be helpful.
- CSF
 - A positive CSF IgG result strongly supports an EPM diagnosis with accompanying clinical signs characteristic of EPM.
 - Contamination of CSF by blood may result in a false positive; some *S. neurona* IgG tests are less impacted than others. The CSF Index test can be helpful in evaluating intrathecal (in the CNS) production of IgG.
 - A negative result is usually a good “rule-out” for EPM except in cases with acute onset. Retesting in 10-14 days may be helpful if clinical signs persist, as some EPM horses test negative in CSF early in infection.
- Polymerase chain reaction (PCR) testing may be useful in acute onset cases. While technically very specific and sensitive, its clinical sensitivity (ability to detect parasite DNA in a given CSF collection) is poor i.e. few samples test positive. A negative PCR does not rule out EPM.