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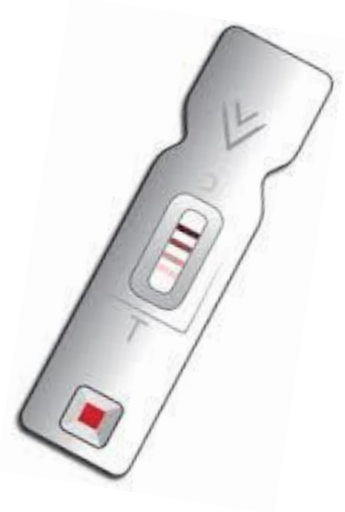
EquiChek™

Inflammation Detection Made Simple



Testing for Inflammation in Horses

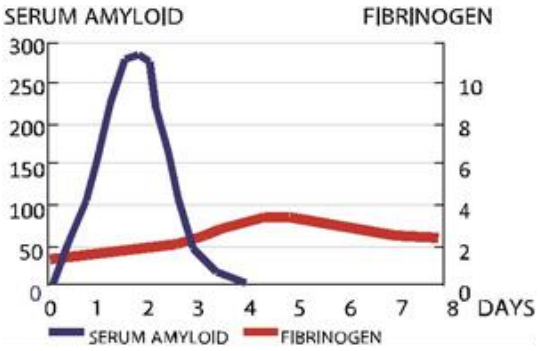
Serum Amyloid A in Horses

The use of Serum Amyloid A (SAA) to aid diagnosis of equine conditions is well documented[1,2,3]. However, the need for investment in laboratory equipment and the time taken to gain results have restricted the use of SAA for the ambulatory practitioner.

Fibrinogen vs Serum Amyloid

Many vets still use fibrinogen as a way of assessing a horse's inflammatory condition. This does produce a number but what does this number really mean? Can it be relied upon to truly assess inflammatory conditions in horses?

Fibrinogen has long been the traditional marker of inflammation in horses and has been used either alone or in conjunction with white blood cells. However, in recent years Serum Amyloid A (SAA) has started to gain traction as an alternative indicator of inflammation and with good reason. A quick look at the figure below demonstrating inflammation followed by recovery explains why.



Serum Amyloid A

Serum Amyloid A (SAA) is a major acute phase protein of inflammation in horses. Very low levels are seen in normal healthy conditions but it increases to levels 100-1000 fold above normal within hours of a problem. This rise in SAA during an inflammatory response is much clearer than the change in fibrinogen. SAA increases are high and rapid. Many clinical and subclinical conditions will be quickly recognized. In contrast, fibrinogen is a minor acute phase protein in horses, with reference levels in most labs ranging between 100-4004 or 200-400 mg/dl[4,5] and only rising 0.5-2 fold above normal. The increase in SAA is substantially higher and hence more reliable. A recent publication[3] concluded that the relatively wide reference interval for fibrinogen concentrations in healthy horses and a lengthy response period after an inflammatory stimulus has rendered fibrinogen a fairly insensitive indicator of inflammation. Hence, a horse with an active inflammatory condition can have an increase in fibrinogen but still be within the normal range given the small increases that occur.

A recent study[1] of 212 horses by an equine lab in Miami demonstrated a clinically significant and very distinct increase in SAA for horses with active inflammatory conditions. Importantly, SAA alone indicated subclinical conditions several horses. These horses all developed clinical symptoms in following days yet neither fibrinogen nor white blood cells were raised. The overall conclusion from the report was that Serum Amyloid A is the most reliable and the most accurate indicator of an active inflammatory condition.

The Road to Recovery

Monitoring recovery requires knowing when to administer and when to cease administration of therapeutics. With inflammation, it is essential to know when the horse has recovered to avoid unnecessary use of therapeutics. With fibrinogen, the levels remain elevated for as long as two weeks after the horse has returned to normal. A very significant advantage of SAA is the rapid fall in levels to normality following successful intervention[7,8], giving a clear signal of recovery.

EquiChek™ SAA Test

The test is simple to use with results in less than 10 minutes. A semi-quantitative visual readout indicates whether the horse has normal, moderate or clinically significant inflammation.

Uses of the EquiChek™ SAA Test Include:

- Confirming the presence of an active inflammatory condition in the field or at the stable
- Detection of sub – clinical inflammation where there is a suspicion something is wrong
- Real time monitoring of recovery of disease activity
- Real time monitoring following therapeutic intervention
- Pre-breeding check for inflammation status (e.g. detecting endometritis; high SAA strongly correlated with high incidence of early embryonic loss)
- Detection of sub-clinical inflammation due to trauma as result of over training
- Monitoring health status before an event to assess the horses ability/potential to perform at peak level.
- Assessing whether an event horse is suffering from inflammation.
- Assessing horse health prior to or after transportation
- An indicator to prompt for more detailed diagnostic testing

EquiChek™ uses a novel competitive assay format to detect SAA in whole blood. The test delivers a semi-quantitative visual readout to help distinguish between normal and mild to clinically significant inflammation. The test is based on competition between SAA present in a sample and SAA printed onto the test strip with antibody coated gold nanoparticles. EquiChek is not affected by the high dose hook effect, unlike other tests.

Test Procedure

Step 1: Remove test cassette from foil pouch.

Step 2: Remove cap from blood tube and insert sample applicator. Just touch sample applicator to surface of the blood. DO NOT IMMERSE SAMPLE APPLICATOR IN BL0OD TUBE. DO NOT SQUEEZE the sample applicator; blood will be drawn up automatically up to the red line on the sample applicator.

Step 3: Apply sample to the sample port on the test strip. Touch sample applicator to the sample port and gently squeeze to expel blood into the test window.

References

1. Assessment of Serum Amyloid A testing and its clinical application in a specialized equine practice. In press JAVMA 2013.
2. Dynamics in serum of the inflammatory markers Serum Amyloid A, haptoglobin, fibrinogen and alpha 2 globulins during induced non-infectious arthritis in the horse. Equine Vet J, 34: 699-704, 2002.
3. Blood proteins and inflammation in the horse. Vet Clin of North America; Equine Practice 24;285-297, 2008. 4. Why perform the CBC, and how can the information be used to manage cases. AAEP Proceedings, 45: 285-290, 1999.
4. Measurement of equine fibrinogen using the Abaxis VetScan VSpro analyzer. Special Equine section,14-22, 2012.
5. Serum Amyloid A as an aid to management of infectious disease in the foal: comparison with total leukocyte count, neutrophil count and fibrinogen. Equine Vet J. 34:693-698 , 2002.
6. Use of serum amyloid A and other acute phase reactants to monitor the inflammatory response after castration in horses: a field study. Equine Vet J, 37:552-556, 2005.
7. Acute Phase Response to Surgery of Varying Intensity in Horses: A Preliminary Study; Veterinary Surgery 38:762–769, 2009.

Step 4 : Add 3 drops of liquid from the dropper bottle into the sample port.

Step 5: The result can be seen in the Test Window (Figure 1). It is recommended that the test is read at between 5 - 10 minutes after starting. However, a normal result can be seen within 2-3 minutes (4 lines will be visible). Do not read after 10 minutes as lines may fade.

Interpretation of Results

A normal healthy horse will have 4 clear lines in the test.

A horse with a strong inflammatory problem such as infection will have just 1 line visible.

Where inflammatory status is borderline, 3 lines may be visible and where there is mild to moderate inflammation, 2-3 lines will be visible. Horses with evidence of low grade inflammation should be retested within 24 hours to establish inflammatory status. As with all diagnostic tests, a definitive clinical diagnosis should not be based on a single result, but should only be made after all clinical and lab findings have been considered.

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