Measurement of Equine Fibrinogen Using the Abaxis VetScan VSpro Analyzer

Test Utilization, Results Interpretation and Comparison to Other Methodologies

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Fibrinogen, also known as plasma coagulation factor I, is synthesized by hepatocytes and plays a key role in hemostasis. Decreases in its concentration can be seen with DIC, hepatic insufficiency, and primary hypofibrinogenemia. Fibrinogen is also an acute phase protein that increases in response to inflammation and is a useful tool to detect infection or inflammation in cattle and horses.

Uses of Fibrinogen

Fibrinogen is a sensitive indicator of inflammation. The fibrinogen level can increase up to 10 times in concentration within 24 to 72 hours of an inflammatory stimulus. Its half-life is approximately three days and concentrations return to baseline within one to two weeks after resolution of the inciting cause, making it an excellent test for monitoring response to therapy by comparing results to either baseline or the initial values of the ill patient. Fibrinogen can also be an excellent early indicator of inflammation as concentrations often increase before WBC changes are noted on a CBC. Fibrinogen concentrations are also often increased in states of chronic inflammation when WBC counts are elevated from baseline, but remain within population reference intervals, thus providing valuable information not immediately evident in a CBC analysis alone.

The primary clinical uses of fibrinogen concentration evaluation are to:

- Aid in diagnosis of inflammation
- Observe earlier warnings of inflammation than with a CBC alone
- Provide indication of chronic inflammatory processes when the CBC may be “normal”
- Assess severity of the inflammatory process
- Monitor inflammatory disease processes and response to therapy
- Confirm resolution of inflammatory disease processes

Unlike other biochemical markers of inflammation in horses (serum iron, haptoglobin, serum amyloid A, C-reactive protein and α1-acid glycoprotein), fibrinogen has been validated, standardized, and, most importantly, is available as a point-of-care test in clinical practice.

Validation, accuracy and precision of the VetScan VSpro Fibrinogen Test cartridge

When a test is validated, it is compared against a predicate device, or a device that has previously been determined to provide accurate and precise values for the particular test in a particular species in question. The VSpro Fibrinogen test has been validated against two such analyzers. The results were excellent with the VSpro, showing r values of 0.94 vs. the ACL 1000 and a value of 0.926 against the STA Compact both with p<0.001. Both of these analyzers had been previously validated for fibrinogen in the horse. Correlation coefficients, or “r” values, of 1 indicate perfect correlation and those above 0.90 are generally considered excellent.

The results comparing the VSpro Analyzer with other fibrinogen methods are shown graphically on the following page.
The precision of the test was also evaluated by calculation of the coefficient of variability. This value ranged from 7% to 15%, and is considered to be very good.

**Advantages of the VSpro Fibrinogen Test cartridge over other methods**

Understanding that the VSpro provides accurate and precise results in comparison to two of the most popular reference laboratory analyzers provides the veterinarian with confidence in the results. Currently, the most common method to obtain point-of-care results in equine practice is the heat precipitation method. Heat precipitation is based on the property of fibrinogen to precipitate at 56-58°C. Heat precipitation is considered a semi-quantitative method while the VSpro are considered quantitative, should provide a higher level of comfort in the accuracy and precision of the results from the VSpro against this method. In addition, the VSpro still allows for point-of-care results without the additional cost and time necessary to send the sample to a commercial laboratory.

Confidence in the results is maintained since accuracy and precision of the VSpro are similar to instruments used in commercial laboratories and the fact that results can be obtained patient-side at a lower cost allows the veterinarian to provide a higher level of medical care and customer service versus waiting for results from a commercial lab.

What if the values I obtain from the VSpro are different from what I am used to seeing?

There are several methods used to evaluate the fibrinogen that are based on its physical or biochemical properties, or its activity. The current gold standard method is the total clottable fibrinogen assay however this assay requires a great deal of time and expertise, and is impractical as an in-house methodology.

The most common routine methods used in human medicine to measure fibrinogen concentration are clotting rate assays. These assays measure the functional property of soluble fibrinogen to be converted into insoluble fibrin by activators of coagulation, usually thrombin. These methods are becoming more widely accepted in the veterinary market as automated in-house analyzers are validated and made available, and the VSpro uses such a method. Clotting rate assays are generally easier and faster to perform than total clottable fibrinogen assays, and have greater sensitivity and precision than heat precipitation methods. The increased sensitivity and precision allows for recognition of hypofibrinogenemia and detection of mild to moderate increases in fibrinogen concentration formerly lost in the low pre-
cision (+/- 200 mg/dl) heat precipitation method.

There are many different clotting rate assay analyzers that have different detection (optical vs. mechanical) and activation (Clauss vs. prothrombin) methodologies. Currently, there are no commercially available equine fibrinogen controls, and human fibrinogen concentration standards are not produced with values greater than 500 mg/dL (5 g/L). Given the different fibrinogen measurement methods, different methodologies used within analyzers, and the lack of high level standards, there can be significant biases in measured concentrations between methods and analyzers, especially at moderately to markedly increased concentrations. Care must be taken when attempting to compare values between different methods and analyzers, especially at elevated fibrinogen concentrations. Patient case management utilizing the same type of analyzer and method is highly recommended to avoid this issue.

The VetScan VSpro Analyzer utilizes a modified Clauss clotting rate assay to determine fibrinogen concentration. Capillary action moves the sample within the cartridge and allows for mixing with coagulation activators. The activation of coagulation converts soluble fibrinogen into insoluble fibrin, and the rate of increased turbidity within the sample is measured optically by the analyzer. The rate of increased turbidity is proportional to the fibrinogen concentration within the sample. The VSpro fibrinogen method was calibrated to known fibrinogen standards and also to a validated and accepted clotting rate assay analyzer.

Methodology differences are a very important consideration in the areas of patient evaluation and result interpretation. An example of the differences between two clotting rate assays, one using an optical method and one using a mechanical method, is shown below: VetScan VSpro Equine Fibrinogen reference interval (normal range): 150 - 400 mg/dL STA Stago Equine Fibrinogen reference interval (normal range): 127 - 224 mg/dL Agreement (r) = 0.926 (excellent)

Above, the VSpro reference interval is wider and has different reference limits at both the low and high end of the ranges, and the statistical agreement between the methods is excellent. This confirms the slight positive bias of the VSpro method versus the Stago method, and emphasizes that care needs to be taken when trying to directly compare the values obtained by these, or any two methods. For example, a practitioner that is accustomed to the values obtained by the Stago, and not aware of the bias, may become alarmed by the values generated by the VSpro and vice versa. This is most likely to happen at elevated fibrinogen concentration values, as a value of 1000 mg/dL (10.0 g/L) generated by the VSpro is only 2.5 times the upper reference limit, but would be >4 times the upper reference limit of the Stago. These fold differences may yield different medical decision limits to the practitioner, especially if the practitioner is not made aware of the possible bias between the analyzers. This bias may not only occur between different clotting rate assay analyzers, but also between different methods such as the clottable fibrinogen and heat precipitation assays.

This may be even more shocking to the practitioner that uses the heat precipitation method as a point-of-care test. Since the heat precipitation test is semi-quantitative and highly subject to sample handling and environmental issues, there may be significant differences in values obtained. Once again, comparison of these two completely different methods can create clinical confusion. In addition, the
accuracy and precision of the automated and validated method of the VSpro should be considered superior to the manual method.

Finally, proper collection and handling of samples for fibrinogen analysis, and following manufacturers’ guidelines for analyzer operation are of vital importance for correct fibrinogen measurement. Appropriate anticoagulant selection, filling of blood collection tubes, centrifugation, dilution preparation, consumable handling, and analyzer operation are of utmost importance in generating the most accurate and precise fibrinogen measurement, regardless of the method or analyzer used.

A look at real practice data is shown below to exemplify the topics discussed above. The data was generated at a California equine practice from actual patients. Samples were obtained and split, with one sample run in duplicate on the VSpro and the other half sent to the clinical pathology lab at the University of California, Davis, College of Veterinary Medicine. The results are shown in Figure 1.

**Key points regarding this study**

1. Normal Ranges with the two analyzers are quite different with the VSpro at 1.5 – 4.0 g/L and UC Davis 1.27 – 2.24 g/L.

2. Clinical correlation was excellent.
   a. One patient was considered borderline high on the VSpro and normal from UC Davis.
   b. All other patients with elevated fibrinogen levels were elevated on both instruments.

3. R2 – indicating the level of agreement between the methods – was excellent at 0.9715 (perfect is 1.0)

4. Because of the different methodologies, above normal values seem higher on the VSpro than the UC-Davis instrument. This is due to the higher end bias on the VSpro.

**Conclusions**

- The fibrinogen test performed on the VSpro has been validated as accurate and precise against two instruments commonly used in reference laboratories
- Automated fibrinogen testing is more likely to be accurate and precise than the semi-quantitative method of heat precipitation
- The VSpro methodology has excellent clinical correlation to other methods
- It is vitally important to use the same methodology in monitoring all patients, as values can be significantly different between analyzers even with the same clinical interpretation
- The VSpro analyzer may have a slightly higher bias at the higher end of fibrinogen levels when directly compared to some of the other fibrinogen methods. This is another reason for consistent use of the same method on each patient

**Reference:**