The University of Guelph research team (Dr. Mike Lindinger, veterinary student Megan Monaghan, and students Jessica Culbert and Nava Manierka-Quaile), arrived at the Biltmore Estate in the evening of May 3rd after a pleasant 10 hour drive. We travelled from Guelph, which is about 60 miles west of Toronto in Ontario, to Fort Erie where we pick up Dr. Art King (renowned endurance veterinarian), past Buffalo, Cincinnati and on south to Asheville, NC. Along the way we were regaled with stories of past endurance events by Dr. King who is a great story teller and doesn’t forget anything.

On arriving in the early evening at Asheville we immediately teamed up with Susan Mercer who joined us from VacuMed, the company marketing the Equistat hydration monitor in North America. We were treated with hotel rooms for our first night – a treat because the next two nights would be on-site in a tent where we would only visit the sleeping bags for 4-6 hours between midnight and 4 - 6 am. After breakfast on the 4th we drove onto the Estate and meandered our way to the marshalling area, made camp, settled in and set up our equipment in close proximity to the start and to the Abaxis trailer. This was convenient because the always cheerful Abaxis folks gave us a bit of trailer space so that we could process our blood samples (for subsequent magnesium analysis of plasma and red blood cells) along side. Our ride hosts, Cheryl and Stagg Newman, Anne Ayala, and their team, took great care of us, making sure that we were well fed and watered. Our demanding schedule, however, meant that
we were never well rested – but this is normal for everyone at these endurance rides!

Our team had been invited to participate in the performance profiling endeavor put together by APEX (A Partnership for Endurance Xcellence) and Abaxis Animal Health. Performance profiling is essentially field research with the collection and subsequent interpretation of data within the context of horse performance. A main goal of Performance Profiling is to provide useful, practical information that riders can use to improve their horse’s health (pre-ride, during the ride and after the ride) and improve ride performance. Another goal is to provide high quality scientific data from horses competing in endurance rides to the endurance community in order to improve our understanding of the physiology of endurance competition.

Our main purposes at the ride was to field test the Equistat hydration monitor using horses that enrolled for performance profiling. Another main purpose was to facilitate the sampling of blood from these horses for biochemical and hematological analyses performed by Abaxis. Dr. Lindinger subsequently put together individualized reports on each profiled horse that included hydration, blood biochemistry and hematology data and recommendations.

Performance profiling was an option provided to the riders/owners prior to the start of the ride. About 10 horses were included from each of the 50 mile, 75 mile and 100 mile distances. The way things worked out, the top three riders at each distance were not part of the profiling.

Friday morning was sunny, but a good chill was in the air as the fog burned off the surrounding meadows. We set up our research station, talked with lots of folks about the Equistat, performance profiling and made some additional recruits. The Equistat is a portable hydration monitoring device that uses bioelectrical impedance analysis technology to assess total body water, intracellular water (water within cells) and extracellular water (water outside of cells) and body fat. It was developed by Dr. Lindinger at the University of Guelph over a period of several years using data collected from research horses of various breeds and Standardbred race horses. The device had not yet been tried on endurance horses under field conditions.

The BIA procedure comprises placing electrode straps on the left forelimb and hindlimb, just above the knee and hock, connecting wire leads to the ‘blue’ box, and obtaining the measurements – the entire procedure takes about 2 minutes. Body weights, blood samples and Equistat measures were obtained from the horses on Friday afternoon and evening, on Saturday at different points during the ride, at the end of the ride, 2 hours after ride completion and a final sample.
on Sunday morning. Each of the three ride distances comprised a series of loops, with each loop returning back to the starting point. This made the work of sampling the horses relatively simple and allowed the research team to stay together.

The weather was simply brilliant for the entire event and did not conspire to pose limitations on horse and rider performance. Mornings dawned sunny and cool, with high but not overwhelming humidity. By mid-morning most of the humidity had burned off and a slight breeze managed to offset the radiant heat load. Although it had rained earlier in the week, the trail conditions were good. Despite the fine weather, many horses still struggled with trying to maintain adequate hydration. All horses that were profiled became dehydrated to some extent, and a majority of these horses started the ride somewhat dehydrated — this was likely due to the dehydration associated with prolonged transport to the ride location in the days preceding the ride. A few horses had to withdraw as a result of hydration and metabolic concerns, and some horses also came up lame due to joint, muscle, tendon or ligament strains. The veterinarians were fantastic and enthusiastic.

They did a great job ensuring that horses continuing the ride were fit to do so. Some vets worked closely with ourselves and the Abaxis team to closely monitor blood parameters and hydration on a few horses that ran into trouble. Sunday morning saw us all tired but very happy with how well BIA sampling, blood sampling and data collection went. The riders and horses that we worked with were fantastic, cooperative and chatty. With mixed feelings (hard to believe it was over already!) we left the ride site in late Sunday morning, arriving back home in the wee hours.

Our preliminary results on the Equistat hydration monitor show that the device was able to adequately detect the level of hydration in most horses prior to the start of the ride (Friday and Saturday morning) as well as after the ride on Sunday morning. The data collected during the ride were highly variable and not correlated with actual hydration (as estimated from changes in body weight). This may be due to fluids and electrolyte balance to not being steady state at the vet checks. The timing of the measurements may have been too close to when horses arrived, and the measurement of fluid balance could also be affected by eating and drinking.

The main results from the measurements of body weight and blood markers showed that many horses arrived at the site dehydrated, and that most of these also started the ride somewhat dehydrated as well. One conclusion from this is that there is a need to better assess and ensure hydration prior to the start of the ride. All horses in the performance profiling study lost hydration during the ride, with the greatest losses occurring during the first loop, when horses are going the fastest. Some horses continued to lose hydration throughout the ride, while some horses showed significant recovery of hydration during the last half of the ride. One conclusion from this is that there is a need to implement effective strategies for better maintaining hydration, and thus health and performance, during the ride. The main indicators from the blood results that contributed to the assessment of hydration status included plasma proteins and hematocrit. Cell markers of muscle and liver injury were also very useful, with all horses showing some increase associated with the intensity and duration of exercise. Those horses that had high levels of dehydration also tended to have elevated concentration of cell markers in the blood.
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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.3 Heat precipitation is based on the property of fibrinogen to precipitate at 56-58°C. Heat precipitation is considered semi-quantitative while the results from 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 assay2 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.2 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.1,2 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.4 Care must be taken when attempting to compare values between different methods and analyzers, especially at elevated fibrinogen concentrations, as medical evaluation of severity may be different between different methods and analyzers. 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.5

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 test 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 upmost 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

**Figure 1.**

![Graph showing the comparison between VSpro vs. UC Davis Reference Lab (Stago) with the equation y = 0.3974x + 0.3545 and R² = 0.9715.](image-url)

**Reference:**

A 3-day-old filly was presented for lethargy. Foaling was uneventful and the foal was observed nursing shortly after birth.

Physical examination was performed and blood drawn for a CBC and IgG levels at approximately 24 hours of age. All were found to be within normal limits. The foal displayed normal activity until the time of examination.

Physical examination revealed the foal to be in good body condition, lethargic with slightly enlarged abdominal contours. The foal postured to urinate during examination and produced a small volume of urine; posturing appeared prolonged. She had an increased body temperature (102.8°F/39°C), was tachycardic (76 bpm), and had normal respirations. Mucous membranes and sclera were Ballottement of the abdomen was normal.

Blood was drawn for a CBC and Equine Profile Plus. An arterial sample was also obtained for i-STAT® blood gas analysis. The CBC showed a decreased WBC compared to an initial CBC. The Equine Profile Plus revealed hyperkalemia, hyponatremia, and hypochloremia. The BUN was normal, but the creatinine levels were mildly elevated. The GGT and ALP were elevated, but this is a normal finding in newborn foals. The arterial blood sample showed a mild metabolic acidosis.

Based on the physical examination and supported by laboratory data, the working diagnosis was uroperitoneum due to ruptured bladder. Similar abnormalities may also be observed in foals which are septic, suffering from renal or gastrointestinal disease, or urinary obstruction. All of these problems may occur simultaneously.

Paracentesis was then performed and yielded >500 mL of a clear fluid, which had a protein concentration <2.5 g/dL that had an odor similar to urine and confirmed our working diagnosis.

Initial treatment consisted of glucose, calcium gluconate and sodium bicarbonate followed by saline, which was aimed at stabilizing the foal and correcting the electrolyte and acid-base abnormalities. A Foley catheter was then placed in the abdomen to remove the excess peritoneal fluid; another 3 liters were drained.

After the metabolic abnormalities were corrected, the foal became much more alert and active. The foal was then anesthetized, placed in dorsal recumbency, and underwent celiotomy to repair the suspected bladder defect. A 5 cm tear was found on the dorsal aspect of the urinary bladder. Recovery was uneventful and the foal made a complete recovery.

In this case, the ability to perform both field and in-house testing with the i-STAT and the VetScan allowed prompt medical and surgical intervention resulting in complete recovery of this foal.