BEST USE OF CLINICAL PATHOLOGY TESTING
IN EQUINE PRACTICE

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INTRODUCTION

The potential value of hematology and plasma chemistries in equine practice is well documented by numerous case series reports and experimental studies. The maximal use of hematology and chemistries are to help with the diagnosis, clinical management and/or prognosis of the ill or poorly performing horse. In cases where the diagnosis can be arrived at from the history, signalment, and clinical exam, and the prognosis can be accurately predicted, hematology/chemistry testing may not be the best use of the client’s financial resources. During the past few years several point of care instruments (mostly for blood gases, lactate, glucose and chemistries) have become available for use in equine practice. These instruments allow almost immediate stall side results for many analyte measurements. Additionally, at least 3 companies have marketed small, automated bench instruments for equine hematology and chemistry measurements. These instruments have proven reliable and provide quick and valuable laboratory information without using a reference laboratory. The stall side testing and small bench top testing facilitates an improved quality of care for horses and in many practices are a sound investment. The information on hematology and plasma/serum chemistries in this handout refers mostly to adult horses. Responses in foals may differ and will be mentioned in the presentation.

HEMATOCRIT

Abnormally high values (spurious polycythemia) are most commonly seen in horses with abdominal pain and/or colitis or rhabdomyolysis. The high values are caused by intravascular volume depletion and splenic contraction. A retrospective study on equine abdominal pain found horses with HCT (PCV)> 50% are at increased risk of death. HCT and plasma protein concentration should be used along with clinical findings such as mucus membrane color, heart rate, blood lactate, response to treatment and peritoneal fluid analysis in predicting prognosis for abdominal pain.

Monitoring of HCT% and plasma protein concentration is routine for most equine medicine cases and will frequently provide both diagnostic and prognostic information. Along with clinical findings and measurement of BUN, PCV often correlates with intravascular volume depletion and acute dehydration involving loss of extracellular fluids (eg. diarrhea, sweating, etc.). When the PCV is abnormally high and plasma protein abnormally low, one should consider intestinal disease and dehydration. PCV and plasma protein are minimally affected by water deprivation in healthy horses since most of the water loss is intracellular loss! On rare occasion, horses have absolute polycythemia, most commonly associated with neuroendocrine neoplasia or hepatic
disease. Race horses may normally have resting HCT between 35-44% but should be increased immediately following race.

**LOW HCT**

A moderately low HCT (21-26%) is most commonly a result of a chronic inflammatory disease. In many cases, plasma protein will be elevated indicating increased globulin production from chronic antigenic stimulation and/or elevated acute phase proteins! If the inflammatory disease involves the bowel (encysted small strongyles or inflammatory bowel disease), the total protein is often abnormally low.

Lower hematocrits (<20%) may occur from hemolytic or hemorrhagic disorders. For hemolytic diseases, the HCT%, along with heart rate, clinical signs, pVO$_2$, blood lactate, and persistence of the hemolytic process can be used to determine the need for transfusion. There is no single HCT number that serves as a “transfusion trigger” with a range from 9-20%. Low HCT will not be seen with acute hemorrhage; in fact, animals may die from acute hypoxia/hypotension caused by acute hemorrhage but have a normal HCT. Regarding crystalloid therapy in the anemic patient, this therapy is indicated in most horses if there is evidence of dehydration/poor perfusion. Although the fluid therapy will decrease the PCV, it will not decrease the total number of RBCs and may improve oxygen delivery!

Severe nonregenerative anemias are rarely seen, but may result from adverse reaction to erythropoietin injections (red cell aplasia), Fell pony syndrome, and rarely from cytotoxic drug reactions or neoplasia. MCV would be expected to be low in these cases.

When blood is spun in a micro hematocrit tube, the plasma should always be examined for icterus, hemolysis and lipemia!!! Lipemia is common in sick ponies and miniature horses and can be rapidly fatal due to hepatic lipidosis. Myoglobin, due to its small size and rapid filtration in the glomerulus rarely causes discolored plasma. The size of the buffy coat can provide an idea of the white blood cell count.

**PLATELET COUNT**

Platelet counts can help determine the severity of an illness and can provide a laboratory clue for neoplasia. Thrombocytopenia can be a laboratory clue for diseases such as anaplasmosis! Horses with severe systemic inflammation/coagulopathy may have thrombocytopenia (usually in the 40-70,000 range), increased D-dimers and low anti-thrombin III levels; fibrinogen is often normal or sometimes even high. Horses with marked thrombocytopenia (< 20,000) usually have drug-induced or neoplasia related (immune) thrombocytopenia. In the horse, another cause of “reportedly” low platelet count is pseudothrombocytopenia which is a result of platelet clumping and can be seen on a blood smear. If the platelet count is low and there is no clinical evidence to support the finding, submission of a sample in a citrate tube is recommended. Some of the small bench analyzers may underestimate platelet counts.
LEUKOGRAMS

The neutrophil count and morphology are one of the most valuable laboratory tests in equine internal medicine. High neutrophil counts (neutrophilia) can be used, along with acute phase protein measurements, (e.g., high serum amyloid, and low serum iron and high fibrinogen and globulin concentrations) to help determine if an inflammatory disease is present and, to some extent, the duration of the inflammatory response. Common causes of a mature neutrophilia include inflammatory diseases without endotoxemia or severe systemic inflammation, excitement and either physiologic stress or corticosteroid administration. If band neutrophils and/or toxic changes are present, this is highly suggestive of a bacterial infection and supports some degree of systemic inflammation. One mechanism for this is the causative relationship between sepsis, increased TNF, and early release of neutrophils into circulation. Increased adhesion activity associated with sepsis results in margination of neutrophils and resulting neutropenia. Although there is no evidence-based publication to confirm such, this author believes that most horses that develop laminitis secondary to systemic inflammatory illness undergo either a “left shift”, and/or have toxic changes in neutrophils 12-36 hours prior to the clinical signs.

Neutropenia with a left shift and/or toxic changes is frequently observed in acute bacterial infections and/or endotoxemia. In horses with acute colitis and/or severe systemic inflammation, a change from neutropenia to neutrophilia is commonly observed during the first 3-4 days of the illness. Although this is generally thought to be a favorable response (less margination of neutrophils and less systemic inflammatory mediators), I am not aware of published data to support this. Persistent neutropenia may be seen in some horses in association with drug administration, (e.g., TMP-S, NSAIDs). Acute viral infections may cause severe neutropenia with variable lymphocyte counts, but left shift and toxic changes are usually absent. Lymphocyte, monocyte, and eosinophil counts are of less value to the equine practitioner although some diseases (eg. EHV-5) associated pulmonary fibrosis have a consistent lymphopenia (along with elevated fibrinogen). Foals with EHV-1 infection are persistently leucopenia (low number of both neutrophils and lymphocytes). Premature foals with normal or high neutrophil counts generally have a better prognosis than premature foals with neutropenia. It should be noted that some healthy foals have neutrophil counts below the normal range for that age foal. Remember most normal laboratory ranges are age sensitive and based upon 95% of the normal population being within that range. On that rare occasion when eosinophilia is present, diseases of either the skin or intestines should be considered.

An important analyte that should be measured when inflammation is suspected is plasma fibrinogen. Plasma fibrinogen is an acute phase protein and a component of the coagulation pathway. Increases in fibrinogen may occur with either local or systemic inflammatory responses with elevations occurring as quickly as 24-36 hours. Although this measurement is of clinical value, other measurements, such as abnormal neutrophil numbers, low serum iron, and increased serum amyloid occur more quickly following inflammation and should be used along with plasma fibrinogen in determining presence...
or absence of inflammation and response to therapy. Certain diseases tend to have very high plasma fibrinogen concentrations, (eg. abdominal abscess, septic physisis).

Total plasma protein is an important measurement in clinical practice. Horses with inflammation, either acute or chronic, often have elevations in plasma protein with the greatest elevations occurring in horses with chronic infection (abscission). Abnormally low total protein concentration is most commonly associated with enteric loss or hemorrhage (PCV should be decreased concomitantly when there is subacute or chronic hemorrhage). Protein-losing nephropathy and “third spacing” of protein, (peritonitis) is rare in the horse. Additionally, liver disease in the horse rarely causes low total protein; albumin may be decreased, but this is often offset by increased globulins! The plasma protein concentration can be used as a guide to when colloid therapy is needed. In critically ill adult horses, P.P. < 4.5 may be an indication for colloid therapy?

Serum or plasma chemistries are used to detect electrolyte abnormalities, organ system disease, inflammatory markers, and some metabolic diseases. Hyponatremia is common with diarrheal and some urinary system diseases. The finding of hyponatremia, hypochloremia and neutropenia in a colicky/febrile horse or foal is suggestive of colitis/enteritis. The degree of hyponatremia in foals is important to establish as too rapid correction of the hyponatremia may result in neurologic signs and irreversible CNS disease. This author believes that too rapid correction of hyponatremia should be considered but the condition may not be as common in foals as is reported in children. I don’t hesitate to increase the Na 10-15 mEq/L in a couple of hours if the foal is severely dehydrated and the hyponatremia is believed to be of less than 3 days duration. Hyponatremia and hypochloremia are also present with acute or chronic renal failure, ruptured bladder and severe myopathy/myositis. These finding may also be present with severe edema, peritonitis and or body cavity effusion. Another cause of marked hyponatremia and hypochloremia in foals < 10 days of age is hydroureter; an interesting and as yet unpublished syndrome. With ruptured bladder (in foals) and severe muscle disease, the serum potassium is often elevated and in foals with ruptured bladder it may be a serious management problem. With acute or chronic renal failure plasma potassium concentration is variable, generally either normal or high. When it is high and does not drop with fluid therapy (most fluid therapy causes a drop in K concentration), oliguric or anuric renal failure should be considered. Of course, another cause of hyperkalemia in Quarter horses is HYPP. The “dash board” effect on chemistries is not as dramatic in horses as some other species with glucose (decreasing) being the only consistently dramatic finding when samples are carried in the truck all day before submission. Increases in K and phosphorus may occur when the sample is hemolyzed. Low potassium is most common in foal diarrhea, often reaching a life threatening level! Although anorexic horses may maintain normal serum potassium, their total body potassium is always low. Only 1.5% of body potassium is in plasma and for every 1 mEq/l decline in K below normal range, a 10-15% loss of total body K (this could be equal to 225 grams for a 500 kg horse) should be considered! Large K deficits are best replaced by oral administration. Hypernatremia is rarely a problem in equine medicine except in the treatment of neonatal foals with high Na containing fluids. Both sick and healthy foals maintain higher levels of aldosterone than do adult horses and even prolonged use of
Bicarbonate measurement is very useful in determining the metabolic acid/base status of the horse or foal and the need for either enhanced fluid therapy (perfusion) or less commonly bicarbonate administration. Low bicarbonate is by definition a metabolic acidosis (if the pH is also low then there is a metabolic acidosis with acidemia). The most common cause of a metabolic acidosis is titration of HCO₃ by lactic acid or other strong anions that are not routinely measured. L-lactic acid can be indirectly measured by measuring L-lactate. The point of care measurement of lactate is of great value in the immediate evaluation of critical care horses and foals. The magnitude of the elevation in lactate is not as important prognostically as the lactate measurement following aggressive treatment, (if lactate concentration does not go down following resuscitation with fluids etc. then the prognosis is often guarded or worse). A lactate value above 6.0 mEq/l has been associated with outcome in horses with large colon volvulus. Other strong anions such as sulfates (most commonly elevated by renal dysfunction) may also accumulations and can cause a metabolic acidosis. The anion gap [(sodium + potassium) - (chloride + bicarbonate)] is usually elevated (normal, 15 mEq/L) if there is an increase in lactate or sulfates and this magnitude often gives us a clue as to the severity of the disease and the required therapy. The anion gap may not be increased in spite of increased in unmeasured anions if albumin values are low such as commonly occurs in horses with colitis! Hypoalbuminemia can cause a mixed metabolic acidosis (increased lactic acid/lactate) and mild metabolic alkalosis (increased bicarbonate) with a normal calculated anion gap. The important clinical message is fluids (crystalloids and ideally colloids) are still needed to combat both the lactic acidosis (poor perfusion most likely) and the hypoalbuminemia in those horses.

Extremely severe and sometimes persistent metabolic acidosis is most common in foals with diarrhea. Many of these cases require bicarbonate therapy in order to correct the acidosis suggesting excessive loss of bicarbonate or d-lactate production rather than titration with L-lactic acid. Metabolic acidosis may also be caused by excessive administration of chloride. When administering bicarbonate to foals, serum potassium must be monitored very carefully!! A common cause of hyperchloremic metabolic acidosis with a normal anion gap is large volume of NaCl treatment. Hypochloremic metabolic alkalosis with elevated anion gap is common with excessive sweating, (e.g., myopathy, exhaustion), diarrhea and renal failure; severe cases may develop metabolic acidosis. Bicarbonate should rarely if ever be administered to these horses. Some knowledge of the importance of strong ions (Na, K, Cl, albumin) is important for understanding metabolic acidosis/alkalosis but I personally do not believe calculation of a strong ion difference is necessary in clinical cases.

The most common causes of decreased plasma calcium are hypoproteinemia, acute gastrointestinal disorders and/or endotoxemia. Ionized calcium is often normal in horses with low albumin and in most cases no calcium therapy is recommended. Ionized calcium can be quickly measured using an I-STAT. With acute gastrointestinal disease and low ionized calcium treatment with diluted calcium may be used in hopes of
improving intestinal motility and cardiac contractility. Lactation tetany, synchronous diaphragmatic flutter and tetany associated with acute gastrointestinal disease should be treated with both calcium and magnesium as both are low in these cases. Magnesium and phosphorus are commonly abnormal in sick horses but their importance in many cases is not known. Low serum magnesium is common in many horses with systemic inflammatory disease unless they are severely azotemic. I generally do not treat with magnesium unless there are muscle tremors etc. Hypermagnesemia may occur from administration of excessive magnesium sulfate to azotemic (dehydrated) horses in which case paralysis can occur. Phosphorus is often very low in critically ill horses but specific treatment is rarely given.

Blood glucose determination, measured within 1 hour or on separated plasma samples should be routine in sick foals as hypoglycemia is common. Although sick horses (other than foals) rarely have hypoglycemia, they may have a nutritional need for glucose supplementation, especially pregnant mares! High blood glucose is common in “colicky” horses and horses with primary hyperammonemia (where glucose of >250 mg/dl, lactate > 10 mEq/L and correspondingly low bicarbonate are expected). There is some correlation between magnitude of the hyperglycemia horses with colic and outcome. Persistently high blood glucose is also present in many horses with Cushing syndrome, but is rare with equine metabolic syndrome unless they have received grain/molasses within the hour! Horses with hyperlipidemia syndrome and triglycerides > 1500 mg/dl have been reported to have a poor prognosis although I have found that it is not as important how high the triglycerides are initially but instead, do they decrease significantly with the initial treatments? This concept is similar to using blood lactate or cardiac troponin I as prognostic measurements in horses with shock. In some cases the triglycerides decrease remarkably fast and the miniature horses/ponies quickly recovers! I have seen ponies go from > 2000 mg/dl to less than 100 within 24 hours. Fasting plasma insulin and seasonal ACTH concentrations have been excellent predictors of metabolic diseases in the horse. Plasma leptin might be a future test to add to the metabolic profile testing. Dynamic challenge test for both Cushing’s disease and equine metabolic syndrome are thought to be better than baseline testing.

Muscle disease is best detected by elevations in CK and/or AST (if it has been 2-7 days since the possible muscle disturbance. Poor performing horses, especially fillies, that have abnormally high AST as the only abnormality on a full chemistry panel should be strongly considered to have a myopathy. Horses suspected to have chronic intermittent rhabdomyolysis should be jogged for 10-15 minutes and a CK sample taken 3 hours later to help confirm the myopathy. QHs with PSSM often have very high CK and AST when clinically affected, while draft horses and Warmbloods may have only modest elevations in some cases. Horses with PSSM often have resting CK and AST in high normal or slightly increased range.

Renal function tests include BUN and creatinine. Differentiating prerenal azotemia from primary renal dysfunction is best done by combining history, clinical exam, PCV/P.P. and other chemistry values, urinalysis (specific gravity) and speed of return to normal values following fluid therapy. The BUN:creatinine ratio may also
provide some information that is helpful in separating pre-renal azotemia from primary renal failure. Thoroughbreds generally have lower creatinine concentration than Quarter Horses or Warmbloods, and this should be considered when evaluating renal function, particularly if the horse is being treated with nephrotoxic drugs; ie. a creatinine of 1.8 mg/dl (still within published normal range for the horse) in a Thoroughbred being treated with an aminoglycosides might be highly significant (decrease in GFR and toxicity). Marked elevations in serum creatinine in a horse with a severe myopathy is a result of a decreased GFR and not abnormal release of creatinine from damaged muscles. Newborn foals with serum/plasma creatinine values > 2.2 mg/dl generally suggest that placentitis was present!

Liver disease is detected by measuring liver enzymes in the serum. These enzymes may be from hepatocellular origin (SDH, AST, GLDH) or biliary (GGT, ALP) in origin and are often helpful in determining the location of most severe pathology in the liver. Consideration of half-life and liver specificity of the enzymes should also be duly noted in the evaluation. GGT is the most sensitive enzyme for detecting serious liver disease in horses but it test disease and not function. Its decline often lags behind the other enzymes during recovery from hepatic injury and greatest elevations occur with biliary diseases. ALP is of limited value in the horse, commonly increased above reference adult values in growing foals and in a variety of organ disorders in all ages. Liver function test include direct bilirubin concentration, indirect bilirubin (not specific for liver as hemolysis and fasting also cause elevations), prothrombin and partial thromboplastin times, ammonia, urea, fibrinogen and total bile acids. Bile acids are increased prior to the other function test in the progression of most equine hepatic diseases. Bile acids are increased (10-22 µmols/l) due to anorexia and some normal foals < 6 weeks of age have higher concentrations than the normal adult range. Only serum bile acids and blood ammonia are elevated in the rare cases of portosystemic shunts in a foal. Measurement of blood ammonia is important in horses with liver failure, hyperammonemia associated with gastrointestinal disease or in some Morgan foals with acute cerebral signs and in some foals with meconium impaction. To my knowledge, only one of the veterinary bench machines measures ammonia; we use that equipment to measure approx. 40 samples per year. If blood is separated immediately after collection and plasma (EDTA or heparin)/serum frozen the blood ammonia can be measured at an outside laboratory the following day. Blood ammonia increases if left at room temperature.

Elevations in GGT without other hepatic enzyme/function abnormalities are also associated with poor performance in race horses and some have speculated this relationship is related to overtraining. GGT elevations do not occur with pancreatic disease; serum and peritoneal fluid amylase and lipase are increased with acute pancreatitis.

POINT-OF-CARE DIAGNOSTICS

Definition: “Point-of-care testing” is diagnostic testing performed at or near the patient. These analyses are important and, in many cases, essential in evaluating the emergency or critical care equine patient. Point-of-care devices are oftentimes portable and are used
Practice Tip: The primary advantage of point-of-care testing is the ability to obtain immediate results, allowing adjustments in patient treatment and minimizing the need to send and await sample results from a clinical pathology laboratory. Other benefits include:

- Convenience.
- Lower cost compared with purchase.
- Less maintenance of laboratory-based diagnostic equipment.

This allows more frequent monitoring of critically ill patients and provides trends on the changing clinical picture. Sample volumes are generally tiny, which is important in the monitoring of smaller patients. Information gained from point-of-care testing devices is best used when the operator has a complete understanding of the:

- Methodology of the device.
- Use instructions.
- Species-specific precision.
- Accuracy.

ADVANTAGES OF POINT-OF-CARE TESTING

- Immediate access, if cartridges are at room temperature (store test cartridges in the refrigerator to increase the shelf life to 1 to 2 years. Most cartridges must be kept at room temperature for 4 hours before use. Therefore, keep one or two of each test set at room temperature if the shelf life is only 2 weeks).
- Store boxes of cartridges in the refrigerator, where the shelf life is 1 to 2 years.
- Most cartridges must be kept at room temperature for 4 hours before use.
- Therefore, keep one or two of each test set at room temperature if the shelf life is only 2 weeks.
- User-friendly equipment—both point-of-care and small benchtop equipment.
- Small amounts of blood needed (often only 1 to 3 drops) for point-of-care testing.
- Results within 30 seconds to 10 minutes.

DISADVANTAGES OF POINT-OF-CARE TESTING

- Quality control can be an issue if storage, testing temperature, and cartridge expiration date are not adhered to. Some values are consistently incorrect. With the i-STAT 1 portable clinical analyzers, there is an inconsistent underestimation of hematocrit (Hct).
- Tests must be conducted at temperatures of approximately 18° to 30° C (64° to 86° F). Although this temperature range is recommended, samples tested at temperatures as low as 50° did not result in noticeable erroneous values.

LABORATORY TESTS

Blood Gases and Blood Chemistries
• The i-STAT 1 is marketed by Abaxis and measures the same analytes as the previous i-STAT, with the addition of cardiac troponin I (cTn-I), an expanded Chem 8, and activated clotting time (ACT) and prothrombin time.

• This system has been extensively used during the past several years with accurate and uniform results, except for Hct, which is sometimes falsely low. Hemoglobin (Hgb) is also measured.

**Glucose**

• A veterinary glucometer, AlphaTRAK, is validated for healthy and critically ill horses and foals. This glucometer uses 0.3 µL of whole blood with results in 25 seconds. Assure Chronimed, Inc. and Accu-Chem are two other instruments used to measure blood glucose. These test instruments appear to be accurate at predicting severity of hypoglycemia but may not have the same accuracy in reporting the level of hyperglycemia. Glucose also can be measured with the i-STAT.

**Lactate**

• Increases in blood lactate are used in equine critical care as a marker of disease severity and prognosis.

• Lactate measurement can be performed with a point-of-care device – Accutrend - validated in critically ill horses. This lactate meter uses less than 20 µL of whole blood or plasma and provides results within 60 seconds.

• Lactate can also be measured quickly (1 minute), accurately, and inexpensively using the Lactate Pro.

• Whole blood or cellular samples (i.e., peritoneal fluid) should be measured within 30 minutes, or the lactate may increase from sample storage.

• It is recommended that plasma lactate be used more reliably to evaluate trends in serial lactate measurements.

• Transient elevations in lactate are commonly seen in early life in neonatal foals; however, decreases to adult levels are expected by 24 to 72 hours.

• *Elevations in lactate are present in most critically ill horses; the initial value and the change in lactate concentration following appropriate treatment/resuscitation can both indicate prognosis!*

**Cardiac Troponin**

• Elevation of cardiac specific troponin I (cTn-I) is an indicator of myocardial injury, and changes in the protein provide diagnostic and prognostic information.

• Cardiac injury in horses can be secondary to:
  • Myocarditis.
  • Sepsis.
  • Rattlesnake envenomation.
  • Cantharidin intoxication.
- Other causes—primary or secondary to multiple organ dysfunction syndrome (MODS).
- A point-of-care analyzer capable of measuring cTn-I is validated in horses, both in horses with normal values and in those with experimental monensin intoxication resulting in cardiac disease.

**Bench Chemistry Analyzers**

A number of small, easy-to-use bench chemistry analyzers are available for equine practice and use heparinized whole blood, serum, or plasma. All analyzers provide results within minutes.

**Complete Blood Cell Count (CBC)**

There are automated systems for determining equine total white blood cell count and differential counts, red cell count and indexes, and platelet count.

- Fibrinogen can be measured by automated system using the VetScan VSpro in less than 15 minutes and is portable for out of the hospital use. It can also measure prothrombin time (PT) and partial thromboplastin time (PTT).
- Another method for measuring fibrinogen is using microhematocrit tubes and using the refractometer. Two tubes of the same blood sample are tested: one after spinning; and the other after spinning, heating for 2 minutes at 58° C, and spinning again. *The difference between the two is the fibrinogen.* Example: If the first tube reads 7.2 g/dL and the heated tube reads 7.0 g/dL, this equates to 200 mg/dL of fibrinogen.
- Serum amyloid A (SAA): An acute phase hepatic derived protein can be measured at referral laboratories or with appoint of care instrument.
  - The advantages of SAA are:
    - Very low values in normal horses/foals (0 to 30 mg/L).
    - Dramatic elevations (often 100×) within 6 hours, peaking at 24 to 36 hours after acute local or systemic inflammation.
    - Samples are very stable at room temperature or when refrigerated for several days.
    - SAA increases with tissue trauma (e.g., postsurgery), but SAA is *not* supposed to increase with recurrent airway obstruction (RAO).