HOW TO READ A BLOOD PANEL IN ONE EASY LESSON

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The basic purpose of measuring blood chemistry is to assess the current status of the body---how well different substrates are being produced and utilized, whether by-products are being efficiently eliminated, whether the different organ systems are doing their job at the right time and at the correct rate. Blood panels will also often give indicators that something isn't quite right long before outward signs appear. Does the horse have an infection or inflammation brewing somewhere? Is he dehydrated? Are the muscles or kidneys having trouble keeping up with the workload? Is the liver damaged?

While this guide should *in no way* replace the services of a veterinarian, or provides a complete explanation of each parameter, it may help the owner in understanding why each are measured and what abnormal results might suggest in endurance horses during and after strenuous exercise.

MUSCLE ENZYMES - CPK, LDH and SGOT/AST. Levels of specific enzymes help indicate the presence of muscle injury or disease, its severity and progression. Measured blood levels of enzymes, along with the observation of other clinical signs, such as lameness, pain or dark urine, help tell the veterinarian whether, when and to what extent muscle damage has occurred. It is extremely important to consider whether any increased enzyme levels were measured before, during or after exercise; as well as whether any other stressful events (a ride last weekend, an unplanned midnight gallop through camp, even recent vaccinations) may have contributed to chemistry results. High---even very high---enzyme levels after a long ride are not *necessarily* the voice of doom in predicting muscle damage. In some cases, however, obtaining a consultation with your veterinarian and possibly a follow-up blood panel to measure subsequent increases or decreases is often a good idea. This is especially true if concurrent signs of muscle damage are present, such as muscle stiffness or pain, signs of colic or dark urine during or after exercise.

CPK - refers to creatine kinase (also called CK), a muscle enzyme produced during exercise. While horses suffering from exertional rhabdomyolysis (tying up) will demonstrate increased levels, other studies have shown that prolonged endurance exercise *can* result in very high levels (> 30,000 IU/liter) without signs of clinical muscle damage[(1)]. Distance and intensity of exercise are significant factors, so that one horse who completes 100 miles at the back of the pack may have higher CK levels than an equally fit horse who finishes a top-ten 50, with neither suffering clinical damage. An elevated level during or following an endurance ride (or other stressful event) indicates the horse has had a long, hard day, but should not necessarily be interpreted as "muscle

damage" without considering other clinical signs such as muscle pain, stiffness or dark-colored urine. Elevated levels in a resting horse that has not exercised intensively for several days, however, may indicate disease such as infection, dehydration, electrolyte imbalances or chronic rhabdomyolysis[2].

AST/SGOT - refers to aspartate aminotransferase (the SGOT refers to an earlier term for the same enzyme), an enzyme released by both skeletal and cardiac muscle, as well as the liver as the result of protein metabolism. As with CK, AST levels may rise significantly as a result of prolonged exercise without necessarily indicating damage[1,3]. AST levels rise more slowly, and remain in the blood for a longer period, than do CK levels. Elevated ASTs in a horse with normal CK would suggest that the horse has undergone intense muscular stress sometime during the prior week. High AST and CK levels in a horse that has not recently exercised at an intense or prolonged level may indicate an ongoing disease process occurring in the muscles. High AST levels in a horse that has not exercised recently, without a concurrent increase in CK levels, may be indicative of liver disease [2,4].

LDH - refers to lactate dehydrogenase, yet another enzyme released by both cardiac and skeletal muscle cells during stress. Although LDH levels are used to diagnose cardiac disease, elevated levels without other characteristic signs of heart trouble are almost certainly due to its release from skeletal muscle[2]. As with the other muscle enzymes, increased levels may only indicate that the horse has undergone an intense and prolonged bout of exercise, without necessarily indicating damage. Interpretation of enzyme results should include consideration of other clinical signs such as muscle pain or myoglobin in the urine, as well as the horse's immediate and past clinical history. Likewise, clinical signs similar to tying-up without concurrent increases in enzyme levels may be indicative of other diseases such as laminitis, colic or kidney disease.

LIVER FUNCTION - GGTP, BILIRUBIN and ALKALINE PHOSPHATASE.

GGTP - refers to gamma glutamyltransferase, an enzyme involved with liver function. GGTP levels, along with bilirubin, are used to indicate the presence of liver damage or disease. As seen in the muscle enzyme discussion, some enzyme levels may increase for variety of reasons, but GGTP is indicative of liver function only---not cardiac or skeletal muscle activity. Therefore, increases above the normal range of 3-30 IU/liter during or after an endurance ride, possibly concurrent with increases in bilirubin and alkaline phosphatase, are not due simply to prolonged exercise, but may indicate liver disease such as an obstruction of the bile duct[5]. If your lab report indicates significantly increased levels, a follow-up examination by your regular veterinarian is *strongly* recommended.

Bilirubin - a breakdown product of hemoglobin, the molecule which transports oxygen in blood. High levels may be an indication of liver dysfunction, or may related to hemolysis, the process by which red blood cells are destroyed. As many different processes may cause hemolysis, including toxicity, drugs, immune deficiency and

infectious disease[6], increased bilirubin levels must be considered along with other factors such as GGTP and alkaline phosphatase.

Alkaline phosphatase - an enzyme used to help identify liver dysfunction. Although AP levels alone do not define hepatic problems, when supported by other clinical signs, may help confirm a diagnosis, especially when bile duct obstruction is suspected[6]. AP is also essential in bone remodeling, the process by which bone tissue is continually responding to the stresses of exercise. Increased serum levels are a general indication of osteoblast activity, the cells involved in building new bone tissue[7]. Therefore, AP levels will normally be higher in young animals during rapid growth. A follow-up examination is highly recommended for mature animals with high levels of GGTP and/or bilirubin accompanying serum increases of AP.

ELECTROLYTES - SODIUM, POTASSIUM, CHLORIDE, CALCIUM and MAGNESIUM.

Electrolytes are a critical element in cellular metabolism, muscle contraction, nerve transmission and enzyme reactions. Imbalances or deficits lead to impaired athletic performance at best, and life-threatening metabolic disruption or death at worst. It is important to realize that the body has no mechanism for storing "extra reserves" of electrolytes. Therefore, while electrolytes are closely regulated by the body, much is lost in the sweat, urine and feces during exercise and hence are an important parameter in monitoring a horse's ongoing status.

Sodium, chloride, potassium - the electrolyte ions lost to the greatest extent in sweat production, although diarrhea, kidney dysfunction and other pathologies can also be a cause of electrolyte imbalance. Sodium is a primary ion in the body involved in virtually every metabolic process from glucose transport to neural transmission. The body does not store reserves of these electrolytes in tissue (as is the case with some minerals such as calcium), therefore losses during exercise which are not replaced through supplementation or other dietary intake will result in a progressive depletion. Assuming baseline level were within normal ranges, measurements of high serum levels of sodium or chloride during an endurance ride usually only reflect recent intake before the kidneys have filtered out and disposed of excess ions in the urine. Low levels indicate depletion and are often a predisposing factor, along with dehydration, in fatigue, muscle cramps, colic, synchronous diaphragmatic flutter ("thumps"), diarrhea and other symptoms of exhausted horse syndrome[8]. Even seemingly normal or high-normal levels may in reality be lower, but appear higher due to concentration secondary to dehydration as measured by total protein and albumin levels. Therefore, levels at the lower end of the normal range should be evaluated relative to concurrent dehydration.

High serum levels of potassium during an endurance ride are generally not a concern. These increases often reflect nothing more serious than a delay between blood collection (when potassium is actively sequestered inside cells) and sample measurement (after potassium has had time to "leak" from inside the cells out into the plasma or serum). Decreased levels may be indicative of depletion, changes in acid-base status, fecal losses or renal disease.

Calcium, (total and ionized) - one of the most highly regulated ions in the body, and essential for muscle contraction. Normally, adequate serum levels of ionized calcium (the physiologically active form) will be maintained by mobilizing reserve stores in bone. However, new supplies may not be able to keep up with sweat or urine losses, especially during prolonged exercise under hot conditions, resulting in a progressive depletion of available serum calcium. The availability of ionized calcium can also be affected by changes in acid-base status (ie, after a long sprint or during hot weather when the horse 'pants' excessively to cool himself). It should be noted that low serum ionized calcium is not an indication that the horse is lacking in total calcium in bone stores, simply that the body may be unable to mobilize calcium from bone into the bloodstream quickly enough[7]. As depletion of ionized calcium, as well as for other electrolytes, progresses, the muscle cells lose their ability to contract and relax, resulting in thumps, muscle cramps and poor gut motility[8,9]. Therefore, as with other electrolytes, progressively decreasing levels of ionized calcium throughout a ride may provide hints to explain muscle fatigue, metabolic failure or poor recoveries, as well as avenues of management by which performance may be improved. High serum levels of total calcium are unusual, but if measured in conjunction with abnormal levels of other electrolytes and phosphorus, could possibly be indicative of kidney disease.

Magnesium – Lost only in trace amounts in the sweat during exercise, magnesium is a relatively minor factor in evaluating electrolyte and mineral status in endurance horses. Deficiencies may contribute to development of cramps, thumps and tying up. Theories (as yet unproven in clinical data) suggest that magnesium also plays a role in regulating temperament and excitability. Like calcium, blood levels of magnesium do not reflect total body stores. A dietary deficiency is unlikely. Most deficiencies are likely to be secondary to interference of calcium in high-calcium rations (such as those high in alfalfa).

KIDNEY FUNCTION AND HYDRATION STATUS - BUN, CREATININE, PHOSPHORUS, TOTAL PROTEIN, ALBUMIN AND HEMATOCRIT.

BUN and creatinine - BUN refers to blood urea nitrogen, a nitrogenous waste by-product of protein metabolism, which is filtered out and excreted almost exclusively by the kidneys. Creatinine is a normal by-product of muscle metabolism that is also cleared exclusively by the kidneys. Both of these substances are normally present in measurable quantities in the blood, and increased levels, usually in relationship to each other, are used to assess kidney function. However, it is important not to automatically assume kidney damage when evaluating increased levels. Increased BUN alone may simply be in response to a recent meal high in protein, such as a rich alfalfa hay, and the body's attempts to excrete excess nitrogen[9]. BUN also normally increases to some extent in fasting horses that have not eaten well during the previous time period (such as during sickness or times of stress such as a long trailer ride). Increased creatinine levels may indicate the onset of exertional rhabdomyolysis (tying-up), but may also occur during dehydration. The two substances are often related to each other by dividing BUN levels by the creatinine level. A ratio of approximately 10:1 is normal, while ratios of 20-30:1 are often seen in horses after a high-protein meal. A normal ratio, increased levels of

both BUN and creatinine, and increased levels of albumin and total protein would generally indicate dehydration. Increased BUN/creatinine, with normal total protein levels might indicate impaired kidney function[11].

Phosphorus - Unlike sodium, chloride and potassium, very little phosphorus is lost through sweating and thus does not require specific supplementation during a ride. Like calcium, serum phosphorus levels are a poor indicator of the total phosphorus content of the body[7]. High serum phosphorus is often used to help diagnose renal (kidney) failure in mature horses. In young horses, high serum phosphorus is generally nothing more than an indication of rapid bone growth.

Total protein, albumin - Measuring the amount of protein in the serum provides an index of hydration status, as well as indices of infection, inflammation, increased protein loss or decreased protein production. **Increased albumin invariably indicates dehydration.** Increased total protein levels are also usually due to dehydration, although can also be due to increased levels of globulins (antibodies to fight infection). Low protein levels are unusual, but if detected, should be further investigated to rule out possible sites of protein loss, such as via the kidneys or gastrointestinal tract due to inflammation or parasites[11].

Hematocrit - a measurement of the relative amount of red blood cells present in a blood sample. After blood is drawn, a small tube is filled and centrifuged to separate the heavier blood cells from the lighter white blood cells and the even lighter fluid (plasma or serum) portion. A higher than normal reading generally indicates dehydration (same number of cells in less plasma volume) or may be due to splenic contraction secondary to excitement or the demands of exercise. A low reading *may* indicate anemia, though not invariably [4]. Highly fit athletic horses may normally have a slightly lower hematocrit at rest due to an overall more efficient cardiovascular system. Evaluation of true anemia in horses requires several blood samples over a 24-hour period.

ACID-BASE STATUS - pH, TCO_2, HCO_3 and Base Excess. Kidneys, adrenal glands, lungs and special regions in the brain all work together in an amazingly complex system to maintain the internal chemistry within acceptable limits. There are many causes of acid-base disturbance, but in the exercising endurance horse, acid-base changes generally indicate that an exercising horse is working somewhat beyond his immediate capacity. Depending on the type and extent of changes, it may mean that the horse needs to slow down, cool off, or may be indicative of major metabolic changes. Significant changes in a resting horse that has not undergone recent exercise may indicate a disease process.

pH - Most people are somewhat familiar with the concept that pH is an indication of a solution's acidity. Lower pH indicates a more acidic solution; the higher the pH, the less acidic (and therefore more alkaline or "basic"). The normal pH range of blood is between 7.32 - 7.44[12]. Most systems in the body only operate efficiently within this narrow pH range. If blood pH is either too low or too high, the horse's condition is referred to as "acidosis" or "alkalosis", respectively. Under endurance conditions, low pH (acidosis) is a good indication that the horse has recently been exercising beyond his

aerobic capacity, and lactic acid of anaerobic muscle metabolism is accumulating faster than the body can recycle it. The lower the pH, the further the horse has been pushed beyond his limits, and the longer it will take for him to recover. As with all other blood parameters, it's important to look at the total picture---if the horse has recently raced into the finish line, pH levels may be temporarily somewhat decreased due to the release of lactic acid from hard-working muscles. Or it may indicate nothing more than muscles that have not yet warmed up and fully shifted into aerobic metabolism. However, a low blood pH observed along with other acid-base indicators, elevated muscle enzymes, muscle stiffness and other clinical signs help the veterinarian identify ongoing disease processes, such as tying-up or exhausted horse syndrome.

Blood pH level higher than normal often indicates that an endurance horse is overheated and is panting to help with excess heat dissipation. During rapid breathing or "hyperventilating", the body will lose significant amounts of carbon dioxide, which in turn raises blood pH (more alkalotic or basic). Increases in pH have other effects in the body, such as decreasing the availability of physiologically active calcium.

The normal **TCO_2** (total carbon dioxide) concentration is 28 mEq/liter, and also contributes to the 'big picture' of acid-base status. TCO_2 levels of 20-27 mEq/liter indicate a mild acidosis as described above; TCO_2 of less than 20 mEq/liter indicate severe, possibly life-threatening, acidosis [12].

HCO_3 refers to bicarbonate, a buffer released by the kidneys to help prevent changes in the acid-base balance. A normal value is between 24-30 mEq/liter. Although the pathways within the body for regulating bicarbonate within the body are far too complex for these few pages, low levels during endurance exercise would contribute to a diagnosis of metabolic acidosis. Levels slightly above normal indicate mild alkalosis, and might be expected in horses exercising under hot conditions[12].

Base Excess (BE) is the mathematical sum of several of the above positively and negatively-charged ions that contribute to acid-base status. Changes in base excess evaluate "unmeasured anions", usually lactic acid secondary to strenuous anaerobic exercise, inflammation, dehydration or infection. A base excess of zero indicates no abnormal changes in acid-base status. A positive base excess in an endurance horse generally indicates alkalosis secondary to heat and panting. A negative base excess increasingly indicates acidosis, usually secondary to strenuous, anaerobic exercise. Dehydration may be a contributing factor to a negative BE.

OXYGEN TRANSPORT SYSTEMS - pO2, pCO2, sO2 and Hb. During strenuous exercise, the amount of oxygen inspired is not nearly as important as the amount that actually reaches the tissues. Various forces and barriers have an effect on this delivery system, including infections or obstructions that compromise respiratory function; dehydration, which thickens the blood and forces the heart to work harder to circulate it; or anemia, which results in fewer red blood cells to actually transport oxygen and carbon dioxide.

pO_2 and pCO_2 represent the amount of dissolved oxygen and carbon dioxide circulating in the bloodstream. "Normal" levels of each vary depending on the fitness of the individual horse, but levels of approximately 39 mmHg and 47 mmHg for oxygen and carbon dioxide, respectively, would be considered normal for average, healthy horses. Oxygen levels higher than this might be one indication of a horse that is aerobically very fit. Low levels of pO_2 might indicate some barrier preventing adequate movement of oxygen from the lungs into the bloodstream---for example, respiratory infection, partial paralysis of the larynx (often seen in thoroughbred racehorses) or even horses bred for 'teacup' muzzles and accompanying small nostrils. Decreased oxygen levels would be perfectly normal at high altitudes, where less atmospheric pressure is available to help drive oxygen across respiratory membranes. As intensity of exercise increases, circulating oxygen tends to decrease, while CO_2 tends to increase. pO_2 levels between 30 - 16 mmHg, and pCO_2 levels of 50 -96 mmHg, respectively, as speed increased from a slow trot to a fast gallop would not be abnormal13. Observing relative levels before, during and after a ride gives a good indication of how aerobically stressed the horse was at this intensity of exercise.

Hb refers to hemoglobin, a component of red blood cells which actively binds and transports oxygen from the lungs to the peripheral tissues. Normal levels in a healthy horse are between 10 - 18 g/dl[(4)]. Low hemoglobin levels, along with a low hematocrit, might indicate anemia, a decrease in the number of circulating red blood cells.

sO_2 levels represent a measurement of how much of the available hemoglobin molecules are currently involved in transporting oxygen. For example, an sO_2 level of 78 would indicate that 78% of the available hemoglobin is being utilized to transport oxygen and that the horse is still exercising at less than his maximum aerobic capacity. Horses with low hemoglobin levels could usually be expected to have higher sO_2 levels during exercise (and therefore a reduced aerobic capacity), simply because a larger proportion of available hemoglobin are being utilized to transport oxygen.

IMMUNE FUNCTION - WBC, POLYS, BANDS, LYMPHS, MONOS, EOS and BASO.

Collectively known as white blood cells or leukocytes, measurement of these parameters evaluate the presence of infection, inflammation and ongoing systemic disease processes in the body.

WBC - a total count of all types of white cells (eosinophils, basophils, neutrophils, etc), this count is made so that relative proportions of its subunit cells can be calculated. It also provides a general indication of normal balance between cell production in bone marrow and tissue uptake. Before drawing any conclusions, it is critical to evaluate each subtype cell in relationship to each other.

Segs - refers to the number of segmented, mature neutrophils present per milliliter of blood. Neutrophils in general are the predominant circulating white blood cell whose function is to seek out, ingest and kill invading microorganisms, such as bacteria. Mature neutrophils are referred to as "segmented", immature neutrophils are referred to as

"banded", based upon their appearance under a microscope. The normal range of segmented neutrophils in equine blood is between 2.7 – 6.7 x 10^3/µl (that is, 2700-6700 neutrophils per microliter of blood). A high proportion of segmented neutrophils indicates inflammation, excitement or response to chronic stress. Accompanying levels of band neutrophils and lymphocytes are used to pinpoint a more specific cause of increased neutrophils (see below). A significantly decreased level may indicate severe inflammation (and thus consumption of more neutrophils than can be produced). If concurrent with abnormal levels of other white blood cells, it may indicate bone marrow failure, such as may occur with some drug or chemical toxicity, severe viruses or neoplasia.

Bands - refers to the relative proportion of banded (immature) neutrophils. A range of 0.0 – 0.1 x 10^3/µl is considered normal. Increased levels indicate acute inflammation that has stimulated the bone marrow to release large numbers of neutrophils, including those not yet mature.

Lymphs - refers to the relative proportion of lymphocytes. Unlike neutrophils, which attack a broad spectrum of invading microbes, lymphocytes differentiate into specialized cells that attack and destroy very specific infecting antigens (teaching these "memory cells" which antigens to attack and destroy is the basis of vaccinations). Normal range for the horse if between 1.5 – 5.5 x 10^3/µl. Low lymphocyte levels concurrent with increased band neutrophils and low segmented neutrophils indicate a severe, overwhelming viral or bacterial infection. Low lymphocyte levels concurrent with normal band neutrophil levels, *and* increased segmented neutrophils indicates a stress response (such as during disease or other stressful circumstances). Normal or increased lymphocytes along with normal band neutrophils and increased segmented neutrophils indicate excitement. Extremely high lymphocytes along with evidence of immature lymphocyte cells may indicate neoplasia, such as lymphoma.

Monos - refers to monocytes, an immature stage of macrophage cells. Like neutrophils, macrophages attack and engulf foreign bacteria, but are also the "clean-up cells" which remove dead tissue wherever damage has occurred, such as a healing wound site. The normal range for horses is between 0.0 – 0.8 x 10^3/µl. Large numbers of circulating monocytes are generally an indication of an increased demand for macrophages, as might be the case following injury and tissue destruction.

Eos - refers to eosinophils, yet another type of leukocyte with functions similar to those of neutrophils. Eosinophils have a role in the inflammatory response, such as swelling, redness and pain following injury or during allergic reactions. In addition, they have the major function of parasitic control, in that they attack and damage parasites circulating in the bloodstream (such as strongyles during their migratory phase). Normal ranges are between 0.0 – 0.6 x 10^3/µl. Increased levels may be an indication of infection by internal parasites, of an allergic response or of inflammation in the body, such as gastroenteritis.

Basos – refers to basophils, the last of the large categories of white blood cells. Normal range is between 0 – 0.2 6 x $10^3/\mu l$. Basophils contain the substances histamine and heparin, which are involved in the inflammatory process. Increases in basophils generally accompany increases in eosiniphils and help support diagnosis of inflammation due to allergies, parasites or inflammation.

PLAT – refers to platelets, the third cellular component of blood (along with red and white blood cells). These cells contain a number of biologically active molecules that are critical to the blood clotting process. Low levels may indicate a number of disease processes not necessarily directly related to a bleeding disorder. Chronic or acute blood loss, immune disease, toxemia, liver, spleen or bone marrow disease, or even critically reduced or increased body temperatures can also cause low platelets counts. Any significantly low platelet counts should be further investigated by a veterinarian. High levels are generally clinically insignificant unless the condition persists, in which case it may be indicative of bone marrow neoplastic disease.

GLUCOSE - Blood glucose levels, and the manipulation thereof, is probably one of the most controversial subjects in endurance horse management. While horses exercising at typical endurance speeds rely primarily on the oxidation of fatty acids for energy production, a certain amount of glucose is always required for certain metabolic pathways and by certain vital organs. The brain, for example, is unable to utilize any substrate *other* than glucose. At the same time, the animal body is able to store relatively small amounts in muscle and liver tissue, and its depletion during exercise is a major factor in fatigue. Normal levels for a horse with a "full gas tank" range between 69 - 122 mg/dl. As adrenaline also raises blood glucose levels, levels measured in excited horses might normally be at the high end of the normal range.

In other species, very high glucose levels would often indicate diabetes. However, diabetes is extremely rare in horses, and very high levels of blood glucose would generally indicate recent extreme dietary manipulations. Low levels below the normal range may indicate several conditions---if measured fairly soon after the above mentioned glucose "spike", the result may be an "insulin rebound", wherein large amount of insulin are released to sequester the excess glucose, resulting in dramatically lowered glucose levels. If low glucose levels are measured during or after sustained, strenuous exercise, it is more likely due to glycogen depletion, in which the body is rapidly reaching the end of its available glucose stores.

CORTISOL – Not normally measured in routine blood chemistry panels, cortisol is one of a family of glucocorticoid hormones which regulate metabolism. Cortisol in particular is a "stress hormone" in that it is released in greater amounts during times of stress such as strenuous exercise, disease, pain or fear. It function at high levels is to put the body into emergency status, thus triggering the liver to produce more glucose and facilitate lipolysis (burning of fat for energy) for a "fight or flight" response. However, cortisol also has the potentially deleterious effects of increasing water excretion through the kidneys, increasing hydrochloric acid secretion in the stomach and suppressing the

immune system. The purpose of its measurement during research projects is to evaluate whether high levels adversely affect endurance horses during strenuous exercise.

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