These notes will present some basic concepts important for equine clinical pathology. These notes are meant to provide a basis for continued professional development and expertise in equine medicine. Your feedback regarding the content, presentation and approach to clinical pathology is appreciated. Please let me know which things were of the most benefit to you and how these notes could be improved for the future, as well as what additional aspects you may want to address in other continuing professional development courses (kathy-freeman@talktalk.net or kathy-freeman@idexx.com)

Objectives for this course:

1. Provide exposure to concepts of importance in equine haematology, clinical chemistry, coagulation, acid-base/blood gas and endocrinology.
2. Increase awareness of technical aspects of haematology and clinical chemistry.
5. Provide opportunity for participation, questioning and exchange of information via presentation and discussion of notes and clinical case material. Emphasis on interaction and cooperative learning rather than standard lecture format.
EQUINE HAEMATOLOGY

Haematologic evaluation is a part of the minimum database that should be collected for a variety of conditions and presentations. While a definitive diagnosis is seldom possible on the basis of haematology alone, correlation with the results of clinical examination, presentation, history and results of other evaluations often provides valuable information for formulation of clinical diagnoses and prognoses. Serial haematologic evaluations may be of benefit in monitoring the progress of a condition and/or response to treatment.

Haematologic Evaluation

Haematologic evaluation consists of 3 components:

1. Automated analysis
2. Manual methods
3. Microscopic evaluation

Depending on the laboratory and type of equipment, the Full Blood Count (FBC) and differential count may utilize one or more of these components.

Automated Analyses

The automated analyses usually include direct measurements and indirect measurements (usually calculations).

Direct measurements usually include:
- Haemoglobin (Hgb) determination
- Red Blood Cell Count (RBC)
- White Blood Cell (Leukocyte) Count (WBC)
- Platelet Count
- MCV (some instruments)

Indirect measurements include calculation of:
- Haematocrit (some instruments)
- Mean Cell (Corpuscular) Haemoglobin (MCH)
- Mean Cell (Corpuscular) Haemoglobin Concentration (MCHC)
- Mean Cell (Corpuscular) volume (MCV) (some instruments)

Some types of instruments measure MCV and Haematocrit directly and others do this by calculation. Many instruments originally used for human blood evaluations have been adapted for use in animals and require special adjustments or modifications for animals.

NOTE: If considering inhouse testing equipment, documentation of validation of the equipment for horses and a set of comparative testing using a laboratory as a ‘gold standard’ comparison is
recommended. Evaluation of precision (repeatability) and accuracy for equine specimens over a wide range of haematologic values that may be encountered in health and disease is recommended.

Haemoglobin is usually measured spectrophotometrically following lysis of erythrocytes; this measurement is based on absorption of light at a particular wavelength. Any interference with light passage through the specimen (erythrocytes resistant to lysis, large numbers of Heinz bodies or increased turbidity due to lipaemia) may result in errors in haemoglobin measurement. The most common problem is artifactual elevation of haemoglobin.

RBC in horses may be problematic because of the small size and tendency for RBCs to form rouleaus. The donkey is worse in this respect.

WBC in horses is not usually problematic. However, the automated differential cell counts are often not reliable. A scan of the peripheral blood smear is always recommended to confirm whether or not the automated differential cell count is likely to ‘fit’ and whether there may be increased numbers of band cells, toxic changes or other morphologic abnormalities (not detected on automated differential cell counts).

Platelet counts in horses may be problematic due to small fibrin clots or platelet clumping that may result in falsely decreased platelet counts, whether automated or manual. Correlation with the peripheral blood smear evaluation for platelet clumping and visual platelet estimate is recommended as an essential part of nonstatistical quality control for platelet counts.

**Calculated Variables (See Table 1 for Summary of Calculations)**

Manual Packed Cell Volume (PCV) or microhaematocrit is one of the most accurate, reliable and reproducible techniques for evaluation of the erythron.

Important features are standardization of spinning time and recognition of time necessary for maximum packing. The manual PCV may be slightly higher (1-2%) than calculated haematocrit due to trapped plasma within the erythrocytes column.

Usually manual PCV and calculated haematocrit agree within +/- 4-5% but occasionally a larger difference (6-8%) may be seen without obvious explanation (such as haemolysis).

**Calculated Haematocrit (Htc)**

\[
Htc = \frac{MCV \times RBC \times 10^{12}/l}{1,000}
\]

Ex: 45 x 8.9/1,000 = 0.4005 or 45.05%

Calculated Haematocrit is subject to inaccuracies in cell size measurement and/or RBC counting. This is the method used by most large commercial laboratory analyzers for determination of calculated haematocrit.
Mean Cell Volume (MCV)

\[
MCV = \frac{PCV (l/l) \times 1,000}{RBC (x \times 10^{12}/l)}
\]

Ex: \[0.4005 \times 1,000 / 8.9 = 45\]

Haematocrit may be used in the place of PCV in this calculation.

MCV is sensitive to inaccuracies in determination of PCV or haematocrit and/or RBC counting. Elevations of MCV are not consistently found in responding anaemia in horses since immature cells are not routinely released from the bone marrow, but may be one of the few indicators a bone marrow response, when present. Occasionally increased MCV may be seen with myelodysplasia or myeloproliferative disease (rare in the horse). Decreased MCV is rare, but may be seen with iron deficiency due to chronic blood loss. Excessive EDTA (so called ‘short sample’) is usually not a problem with equine specimens since blood is usually relatively easy to collect. However, a high concentration may result in erythrocyte shrinkage, presenting a decrease in MCV and an increase in MCHC.

Mean Cell Haemoglobin (MCH)

\[
MCH = \frac{Hbg (g/l)}{RBC (x \times 10^{12}/l)}
\]

Ex: \[135 / 8.9 = 15.0\]

MCH is sensitive to inaccuracies in RBC counting and/or haemoglobin determination. It is the least useful calculated parameter for evaluation of the equine erythron. Increases in MCH and MCHC may be the result of haemolysis (pathological or as a result of specimen collection or handling or ageing) and reflects elevated haemoglobin values relative to numbers of erythrocytes.

Mean Cell Haemoglobin Concentration (MCHC)

\[
MCHC = \frac{Hbg (g/l)}{Htc (l/l)} \times 10
\]

Ex: \[135 / 0.4005 \times 10 = 135 / 4.005 = 33.71\]

PCV can be used in the place of Htc in this calculation.

MCHC is sensitive to inaccuracies in haemoglobin and/or PCV or Haematocrit determination. Increases in MCH and MCHC may be the result of haemolysis (pathological or as a result of specimen collection or handling or ageing) and reflects elevated haemoglobin values relative to numbers of erythrocytes.

Manual counts for RBC and/or WBC may be done using a haemocytomaeter or using the Unopette systems that contain a pre-measured volume of diluents in a plastic reservoir.

Errors inherent in manual cell counts include:

- Random settling of cells within the counting chamber (error of the field)
- Estimation of cell count from a very small volume of sample (sample error)
- Variation associated with filling of separate chambers for counts (error of the chamber) and inaccuracies of pipetting (error of the pipette).
Use of trained personnel with accurate pipettes and pipetting technique and standard techniques is recommended to help minimize variation. Use of the Unopette system eliminates error associated with dilution that may occur with standard haemocytometer pipettes since a capillary action tube is part of the system.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Formula</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated haematocrit (Htc)</td>
<td>l/l or %</td>
<td>$\text{MCV} \times \text{RBC} \times 10^{12}$/l divided by 1,000</td>
<td>May be measured directly by spun PCV. To convert l/l to %, multiply by 100.</td>
</tr>
<tr>
<td>Mean Cell Volume (MCV)</td>
<td>Femtoliters (fl)</td>
<td>$\text{Htc} \times 1,000$ divided by RBC ($\times 10^{12}$/l)</td>
<td>May be measured directly on some analyzers. PCV can be used instead of Htc in this calculation.</td>
</tr>
<tr>
<td>Mean Cell Haemoglobin (MCH)</td>
<td>Picograms (pg)</td>
<td>$\text{Hbg}$ divided by RBC ($\times 10^{12}$/l)</td>
<td></td>
</tr>
<tr>
<td>Mean Cell Haemoglobin Concentration (MCHC)</td>
<td>g/l or %</td>
<td>$\text{Hbg}$ divided by Htc (l/l) $\times 10$</td>
<td>PCV can be used instead of Htc in this calculation</td>
</tr>
</tbody>
</table>

**Tips for Evaluation of Haematologic Data**

The following are tips for evaluation of haematologic data. By consistently evaluation these variables you will more likely to be able to spot errors, artefacts and/or problems that may need repeat testing or additional evaluation.

**Evaluation of Haemoglobin/Haematocrit Match**

RBC, haemoglobin and haematocrit should be proportional. If a mismatch is present, this may indicate a problem either in measurement, with the specimen or within the animal! A quick way to
determine haemoglobin/haematcrit matching is to remember that haemoglobin x 0.03 should give you the haematocrit +/- 0.02.

Example:

If the haemoglobin is 24 g/l, you would expect the haematocrit to 0.372 +/- 0.02 or between 0.352 and 0.392. If the haematocrit falls outside this range, the following situations and/or actions should be considered:

- For a single specimen with unexpected findings, re-run this patient specimen through the automated analyser to determine if this is a repeatable finding. If this is a sampling error or other malfunction associated with the initial analysis and it is corrected in the repeat analysis, the repeat values are accepted without additional problem-solving actions.
- If a rerun does not result in acceptable data or there are problems with multiple patient specimens, do a spun PCV (microhaematocrit) to determine if it more closely matches the haemoglobin.
- If haematocrit is lower than expected for the amount of haemoglobin, determine if haemolysis may be present (will show up as red to pink discolouration of the plasma column of the microhaematocrit tube. These cases often also will have an increase in MCHC that exceeds reference interval and the maximum physiologic concentration for haemoglobin. Since this calculation uses both Hbg and Haematocrit it often reflects any mismatch that is present.
- If haemolysis is detected, determine if this haemolysis is likely to be iatrogenic (poor needle stick, improper handling, specimen aging) or of pathologic significance (associated with anaemia). If no haemolysis is present, the possibility of inaccuracies in RBC counting or MCV determination may be contributing.
- If the haematocrit is higher than expected for the amount of haemoglobin, consider inaccuracies in RBC and MCV determination or error in haemoglobin analysis.

Correlation of automated or manual platelet count and platelet estimate

A platelet estimate that is ‘adequate’ does not necessarily mean that the automated or manual platelet count will be within normal limits. Usually an estimate that is ‘adequate’ means that there is not a high probability of spontaneous haemorrhage due to thrombocytopenia. Therefore, slight to moderate decreases in automated or manual platelet count may occur without a decreased platelet estimate.

Experienced personnel usually have good correlation in platelet estimates and automated or manual platelet counts. If platelet clumping is detected in the peripheral blood smear, this may make platelet estimation difficult or impossible, depending on the degree of clumping and numbers of platelets involved.

A ‘Rule of Thumb’ for estimation of platelet numbers is:

Estimation from average number of platelets per high power field (oil immersion):

< 3-6 platelets on average – supports thrombocytopenia.
Between 10-25 platelets on average – suggests platelet count within normal limits
>25 platelets on average – supports an elevation in platelet count

Correlation of automated or manual WBC with WBC in peripheral smear

As with platelets, usually experienced evaluators can quickly and easily determine if correlation is present. Errors in WBC estimation may occur if the majority of leukocytes or a subpopulation of leukocytes has been carried out to the featheredge of the smear and not represented in the body of the smear. Occasionally leukocyte clumping may occur (particularly lymphocytes) and may make estimation difficult.

A ‘Rule of Thumb’ for estimation of WBC count is:
Average number of leukocytes in a high dry (40x) field x 2-3 = estimated WBC (x 10^9/l)

Microscopic Evaluation of the Peripheral Blood Smear

Microscopic evaluation of the peripheral blood smear is one of the most important parts of the haematologic evaluation. A quick scan of the film can be used as part of quality control to determine if there is good agreement with the automated analyses. Some large commercial haematologic analysers may give automated differential cell counts of acceptable accuracy in many cases. However, even with large commercial analysers, important features may not be identified in all cases. Smaller bench-top analysers cannot be relied upon to give accurate automated differentials in horses, particularly if the animal is ill or there are abnormalities in WBC or leukocyte proportions.

A good quality binocular microscope with high quality light source with adjustable intensity is a ‘MUST’. Learn how to adjust your microscope so that the best illumination is obtained for the magnification that you are using. Periodic (annual) professional cleaning may be of benefit if the microscope is used frequently. If you are not thoroughly familiar with your microscope and its adjustment, tuition may be available from repair or cleaning professionals, trained technicians, haematologists or pathologists. Know your equipment, its limitations and how to make optimal use of it!

Variables and features that can be determined by microscopic examination of a peripheral blood smear include:

- WBC estimate
- Platelet estimate
- Morphology of
  - Leukocytes
  - Erythrocytes
  - Platelets

In order to obtain maximal information from the peripheral blood smear, it should be a good preparation!
How can you prepare a good peripheral blood smear?

- Use good quality, CLEAN glass slides. Slides with a FROSTED END to mark with the animal identification are preferred since they provide the ability for unique identification and prevent mix-up of smears. This type of slide is usually of a grade that is good quality. Slides should be free of film, dust or oily coating. Keep slides in a closed box.
- Use blood that has been well mixed by repeated inversion or gentle mechanical mixing. If blood is not properly mixed, leukocytes and platelets may settle out and will not be accurately represented in the smear.
  - Note: If blood has settled and has not been on a mechanical rocker, a few quick inversions does NOT provide adequate mixing. Take the time to mix properly!
- Anticoagulants for whole blood that are suitable for morphologic evaluations are EDTA (preferred for automated analysers) and sodium citrate. It is possible to evaluate smears made from heparinised blood, but the staining quality will be poor. Routine use of heparinised blood for morphologic evaluation is not recommended for eqine patients!
- Use a spreader slide with a clean end for making smears. It is helpful if the corners of the spreader slide have been bevelled so that the drop of blood used to make a smear is not carried to the edges of the long sides of the slide. A spreader slide can easily be made by breaking off the corners of a slide with a pair of haemostats. It may be helpful to smooth the edge with a file.
- Dry smears rapidly. This will result in the best morphology. If smears are not rapidly dried, cells may round up and condense, making evaluation of their features more difficult or impossible. A small hairdryer (hand-held or on a stand) or a heat bar can be used to help make drying rapid.
- Have the person(s) in your practice that will be responsible for making blood films PRACTICE MAKING SMEARS. They should make smears frequently enough so that they retain expertise with the technique.
- Make smears from freshly collected blood so that the features reflect those of in vivo conditions and not those associated with artefact such as sample aging, degeneration or ongoing metabolism that occur in vitro.

What are the macroscopic features of a good smear of peripheral blood?

- The smear should extend form one-half to three-quarters of the length of the slide.
- A feathered edge should be present at the end of the smear.
- The smear should be free of ‘holes,’ lines or other defects.

A good peripheral blood smear also should be well stained!

What are the microscopic features of a well-stained peripheral blood film?
• The erythrocytes should be orange-pink with sufficient differentiation of purple hues in leukocytes to provide contrast. Although recognition of polychromasia is not an aspect of concern in equine films because immature cells are not released from the bone marrow, proper staining will result in better contrast and recognition of all cell types.
• Equine platelets often stain very palely, but should be visible upon careful examination. Inadequate staining may result in lack of recognition and erroneous estimates of platelet counts.
• Equine leukocytes should have distinct purple/blue nuclei and recognizable cytoplasmic borders. The nuclear staining density should be sufficient to recognize chromatin clumping and presence of nucleoli. Eosinophil granules should be pink/orange and easily distinguishable on low to intermediate magnification.

What are good practices with regard to staining of peripheral blood smears?

• Personnel within the practice who routinely examine peripheral blood smears should be responsible for evaluating stain quality and make appropriate adjustments should staining artefacts or precipitation occur. The quality of evaluation is dependent on the quality of the preparations and their staining!
• Make sure that whatever stain you use is properly maintained and that jars are periodically cleaned to prevent bacterial or fungal growth and/or build up of stain precipitate. Stains should be kept covered and protected from exposure to light in order to prolong their life and effectiveness. Recognition of refractile artifacts that may indicate exhaustion of methanol and accumulation of water within the alcohol step of staining indicate that that portion of the stain should be discarded and replaced with fresh methanol solution (usually the first step in a multi-jar staining process such as DiffQuick).

Standardization of timing of staining so that slides routinely have the same staining quality will help ensure consistency of interpretation.

Systematic Evaluation of the Peripheral Blood Smear

Just as a thorough, systematic physical examination of the entire animal is important, a system for evaluation of the peripheral blood smear will be of benefit in assuring that maximum information is obtained with a minimum of time.

With practice, a scan of the peripheral blood film can be done quite rapidly and correlated with the results of automated analyses (2-3 minutes).

I find that examination of the peripheral blood film is the part of the haematologic examination that is most often omitted, even though it may provide very important information! It is important as a component of quality control by providing correlation with
automated analyses. Some types of information are not available by other means. Omitting making smears or failing to give them a quick scan is FALSE ECONOMY if you are going to do any haematology at all!

**Sequence for evaluation of the peripheral blood smear:**

- Assess macroscopic features of the smear to determine if adequate quality is likely.
- Assess smear at low magnification with scan of feather edge for platelet clumps, abnormal cells or features, large numbers of smudged or ruptured cells or large numbers of leukocytes carried out to the edge. At this stage stain quality can usually be evaluated, but may require intermediate or high magnification.
- Obtain general impression of density of erythrocyts and leukocytes at low magnification.
- Determine area optimal for cell estimates and morphologic evaluation. This is the area of the smear in which erthrocyts are spread out without overlapping or edges are just touching. Quickly estimate leukcytes numbers (decreased, within normal limits, increased) and types (predominantly mature neutrophils? Band cells present ? abnormal features). This is done on high dry (40x) or 50x or 100x oil magnifications.
- Assess morphology of erythrocytes, leukocytes and platelets. Usually requires 50x or 100x oil magnification. Estimate platelet numbers (decreased, adequate or increased).

For specimens with few or no abnormalities the above assessment can be done within 1-2 minutes by experienced personnel. If marked abnormalities are present, the assessment may take longer, depending on the degree and type of abnormality.

**Guidelines for estimating WBC:**

For experienced evaluators, a general impression of cell density as increased, decreased, or within normal limits can be obtained with a rapid scan of the smear. If a more quantitativae evaluation is desired, the following ‘Rule of Thumb’ is applied:

Estimation of leukocytes in the blood film:
Average number of leukocytes per high dry (40x) field x 2-3 = estimated WBC (x 10^9/l)

Other methods can be used, based on determination of number of RBC, but sually the above method and correlation with automated or manual counts is adequate for determining if clinically significant variation from a normal WBC is present. #

**Features to note in peripheral blood smears:**

**Evaluation of Anaemia and Marrow Response to Anaemia**
The peripheral blood smear is of little or no value in evaluation of equine anaemia since immature erythrocytes are not usually released into peripheral blood. Occasionally nucleated RBCs (nRBC) or a few erythrocytes with Howell-Jolly bodies (nuclear fragments) may be seen in responding anaemia.

Sequential haematocrit determinations and/or bone marrow evaluation provides the best approach to evaluation of anaemia and whether or not there is an effective or ineffective bone marrow response to anaemia in the horse.

*Morphologic Abnormalities of Diagnostic Significance:*

These include:

- **Detection of erythrocyte-associated parasites**, such as *Babesia* spp. Absence of parasitic agents does not rule out infection since they may be few or have cyclic appearance in peripheral blood.

- **Agglutination.** Be sure to differentiate from rouleaux formation (a normal finding in equine blood). Rouleaux forms a ‘stack of coins’, while agglutination produces overlapping erythrocytes in clumps. Agglutination indicates a probable immune-mediated mechanism for anaemia. It may be idiopathic, related to underlying viral or parasitic infection, related to drug administration (with possible hapten formation). Heparin administration may cause agglutination and sequestration of erythrocytes, but is usually reflected by marked ‘pseudo-anaemia’ with decreased haemoglobin and haematocrit.

- **Heinz Bodies or Eccentrocytes.** Indicators of oxidative damage to haemoglobin. New Methylene Blue (NMB) preparations are helpful for identification of Heinz bodies. Consider:
  - Phenothiazine toxicity.
  - Red maple leaf toxicity
  - Onion toxicity

- **Schistocytes.** May indicate micro-angiopathic disease. Consider
  - Chronic disseminated intravascular coagulation (DIC)
  - Advanced liver disease with possible intravascular haemolysis
  - Localised thrombosis
  - Vasculitis

- **Hypochromasia with Microcytosis.** Supports iron deficiency. Rare in horses, but may be seen in foals since milk is ‘iron-poor.’ Also consider with
  - Chronic gastrointestinal ulceration (stress-related or with gastric squamous cell carcinoma.
  - Coagulopathy
  - Severe chronic parasitism (external or internal)
Leukocyte Morphology

- Toxic changes. In the horse, usually reflected by increased cytoplasmic basophilia and increased granularity of neutrophils. Toxic changes like cytoplasmic vacuolation or Dohle bodies, which are seen in other species, are rare. Toxic changes are graded (usually 1 to 4+) based on the degree of change occurring within the cell and the number of cells affected indicated as few, moderate, or many. Usually knowledge of the presence or absence of toxic neutrophils is more important than the degree of toxicity.

REMEMBER:
Toxic changes may be difficult to differentiate from changes in morphology due to specimen aging. Prompt preparation of a blood film from freshly collected blood is important in providing a smear that reflects true in vivo status!

- Left shift. Increased number of band cells. Equine band cells may have some irregular spicules or indentations. Slightly less chromatin clumping than mature neutrophils is present in band cells. If indentation does not exceed 50% of the width of the nucleus or class ‘U-shaped’ nuclei are present, I classify these cells as band cells. If technicians trained on human material read smears, they will likely over-call band cells. Automated differential cell counts do not recognize band cells.

In human medicine, there have been studies that suggest treatment will not differ based on the presence or absence of a left shift (band cells). I feel that there is benefit in knowing if a left shift is present. The equine leukocyte response to inflammation or infection is not as marked as that in the dog or cat and increases in WBC to greater than 25-30 x 10⁹/l are considered marked elevations. Therefore, knowledge of whether a left shift is present may be of value in determining a peracute or acute situation and in planning for initial therapy, monitoring and/or client education.

Platelet Morphology.

- Platelet clumping and estimation of platelet numbers. Most important aspect of platelet evaluation.

REMEMBER: Equine platelets are poorly granular and stain very palely. Good stain quality and careful evaluation to make sure you are recognizing platelets is important!

1. Is smear of adequate quality?
   - Yes
   - No

2. Is stain quality acceptable?
   - Yes
   - No
     - Trouble shooting required to rectify problem
     - Stain another smear

   Nuclear and cytoplasmic stains distinct

3. Are there platelet clumps, large numbers of smudged or rupture cells, abnormally large numbers of leukocytes carried out to the featheredge of the smear?
   - No
   - Yes
     - Consider possible impact relative to other analyses and ability to evaluate morphology. If extreme, make another smear.

4. Is there an adequate portion of the smear where cells are spread out without overlapping within which to do estimates and morphologic evaluation?
   - Yes
   - No
     - Make another smear

5. Do estimates and features correlate well with results of automated analyses and your findings at clinical examination?
   - Yes
   - No
     - Consider manual differential cell count.

6. Are there features that provide additional information for interpretation (i.e. toxic changes in leukocytes, left shift, nRBC, large form platelets, etc.)
   - Yes
   - No
     - Record all additional features
     - Determine

1. Interpretation
2. Diagnosis or differential diagnoses
3. Plan for additional testing and client education.
The Equine Leukogram

Changes in the equine leukogram rarely constitute a primary diagnosis, but may provide clues as to underlying processes.

1. Leukocytosis

   Interpretation depends on the degree and types of cells involved.

   A. Stress leukogram.
      The classic stress leukogram has slight to moderate leukocytosis (WBC usually < or = 25 (x 10⁹/l) with neutrophilia and eosinopaenia. Monocytosis may or may not be present. Band cells or less mature leukocytes are NOT part of the stress leukogram and indicate release of immature cells from the bone marrow. The presence of band cells is consistent with an acute inflammatory response. Occasionally a marked increase in WBC is seen with stress alone, but deserves additional consideration and investigation to rule out other causes of inflammation before it is attributed to stress alone.

   B. Regenerative left shift
      Presence of increased numbers of band cells with mature neutrophils greater than the number of band cells. Usually a reflection of acute inflammation.

   C. Degenerative left shift
      Presence of increased numbers of band cells with band cells and other immature myeloid cells outnumbering mature neutrophils. May see myelocytes an dmetacmyelocytes in addition to band cells. May indicate severe inflammatory condition. Potential confusion with myeloid leukaemia in which numbers of atypical immature myeloid appear in the peripheral circulation.

   D. Lymphocytosis
      May occur with antigenic stimulation or a as a reactige process when leukocytosis is present or in association with lymphoid neoplasia (lymphoid leukaemia, spill over from lymphoma).

   E. Monocytosis
      Uncommon as a sole abnormality of the leukogram. May occur with stress leukogram or in conditions in which necrosis or chronic inflammation is present.

   F. Eosinophilia and/or Basophilia
      Uncommon as sole abnormalities of hte leukogram. Considerations should include parasitic disease, allergy/hypersensitivity, dermatologic conditions, reaction to foreign material or antigenic stimulation, mastocytosis/mast cell neoplasia or myeloproliferative disease/leukaemia of these cell lines.

   G. Leukocytosis with Mature Neutrophilia
      This is the most common finding with leukocytosis in the horse and is often associated with inflammation and/or stress.
2. **Leukopaenia**

Leukopaenia may involve any or all of the cell lines. Mild decreases in otherwise healthy horses may be of little or no significance. Primary considerations for leukopaenia include conditions resulting in immigration of leukocytes to a site of inflammation or decreased production (marrow problem).

A. A classic condition associated with leukopaenia is Salmonellosis. There is typically diarrhoea with marked leukocyte migration into the intestine, decreased peripheral and increased faecal leukocytes. There may or may not be a degenerative left shift.

B. Myelophthisic diseases typically result in leukopaenia in conjunction with anemia and/or thrombocytopaenia.

3. **Myeloid Neoplasia**

A. May present with leukocytosis, leukopaenia or WBC that is within normal limits. Abnormal myeloid cells are present in the peripheral blood in most cases. May involve granulocytes, monocytes and/or eosinophils or have features of both myeloid and monocytoid (myelomonocytic leukaemia). No age predilection is apparent. Reports range from 10 months to 9 years old. Reported clinical signs include weight loss, oedema, anaemia, mucosal petechiae, fever, peripheral lymphadenopathy haemorrhagic diathesis and oral ulceration.

B. Diagnosis confirmed by bone marrow aspirate and/or biopsy of multiple organs. May have infiltration of a variety of organs, including lymph nodes, spleen and liver.

C. Usually cellular morphology allows differentiation from malignant lymphoid cells, but cytochemical typing or flow cytometry may be needed to determine the cell lineage involved.

4. **Lymphoid Leukaemia**

A. Atypical lymphoid cells in peripheral blood are estimated to occur in 30-50% of horses with lymphoma. Are usually present in small numbers.

B. Leukogram alterations are highly variable, but peripheral lymphocytosis with large numbers of circulating neoplastic lymphocytes is unusual and usually associated with bone marrow infiltration. Acute lymphoid leukaemias may occur as a separate entity from lymphoma and result in peripheral lymphocytosis with atypical and immature cells.

C. Most common clinical abnormalities are chronic weight loss, ventral oedema and regional lymphadenopathy.

D. May see immune-mediated haemolytic anaemia, coagulopathy, thrombocytopaenia and/or paraneoplastic hypercalcaemia.

E. Common forms of lymphoma in the horse are generalised, intestinal, mediastinal or cutaneous.
Age of onset is usually 5-10 years, although has been documented at birth or into old age. Diagnosis by histologic and/or cytologic evaluation of affected tissues, fluids or organs.

F. Chronic lymphocytic leukaemia may result in large numbers of small lymphocytes in peripheral blood. These cells appear mature and well-differentiated.

G. A special type of lymphoid neoplasia is multiple myeloma, a tumour of plasma cells. Rare in horses. Neoplastic proliferation of plasma cells in bone marrow, spleen and lymph nodes is common. Focal osteolysis, a common finding in the dog, may or may not be present in horses. Diagnosis by identification of plasmacytosis in bone marrow or soft tissue with invasion of surrounding tissue and monoclonal gammopathy.
EQUINE PLATELETS

In addition to platelet numbers, platelet function is important to consider. Nonsteroidal anti-inflammatory drugs may result in decreased platelet function. Decreased platelet function also has been reported with promazine tranquillisers, in association with uremia, liver disease, anaemia, some malignancies and a variety of immune-mediated conditions. Hereditary problems with decreased platelet function are suspected to occur, but have not been documented.

A. Thrombocytopaenia

Decreased platelet count may be the result of
- Platelet destruction
- Platelet consumption
- Sequestration
- Blood loss
- Decreased production

Be sure to determine that thrombocytopaenia is not a result of platelet clumping (review peripheral blood smear!). Platelet counts of < 50 x 10^9/l may result in spontaneous haemorrhage. Bleeding typical of thrombocytopaenia includes petechia, epistaxis, urinary tract haemorrhage and/or gastrointestinal haemorrhage. Sometimes retinal haemorrhage is detected. Extensive haemorrhage due to trauma, wounds or coagulation abnormalities may result in decreased platelet counts because of loss from the body or presence of bleeding into body cavities that may be associated with these conditions. Thrombocytopaenia is typically present with disseminated intravascular coagulation (DIC) because of increased consumption of platelets.

B. Thrombocytosis

Uncommon finding. May be associated with general marrow stimulation, chronic inflammation, malignancy, haemolysis, iron deficiency or rebound phenomenon if there has been previous thrombocytopaenia, or with neoplastic proliferation (myeloproliferative disorder, essential thrombocytosis). May occur with increased release due to splenic contraction.
THE EQUINE ERYTHRON

Classification of abnormalities may be according to:

1. Bone marrow response: Responding vs. Nonresponding Anaemia. Identification of a nonresponding anaemia requires at least two blood samples at least 5-7 days apart with evidence of static or progressively worsening haemoglobin and haematocrit/PCV. Identification of a responding anaemia requires at least 2 blood samples at least 5-7 days apart with evidence of increasing haemoglobin and haematocrit/PCV.

   Use of this classification is limited by the lack of release of immature erythroid cells into the peripheral blood. If an apparently non-responding anaemia is demonstrated, sequential evaluations of haematocrit/PCV and/or bone marrow aspirates are used to determine if the marrow is likely to be capable of a response, whether there may be ongoing destruction of erythrocytes and what other factors or conditions, if any, may be contributing.

   Guidelines for evaluation of a response: In a severely anaemic horse with a good bone marrow response, the haematocrit may increase about 1-2% (0.01 – 0.02 l/l) per day. As anaemia becomes less severe, the rate of increase declines, such that several weeks to as much as 90 days may be required for the haematocrit to return to within normal limits.

   Evidence of erythroid hyperplasia on microscopic evaluation of the bone marrow is an indication of the marrow’s ability to respond to anaemia, but does not determine if this response will be effective in returning haemoglobin and haematocrit to within normal limits.

2. Underlying general mechanism: Physiologic vs. Pathologic
3. Underlying pathologic mechanism: Blood loss, haemolysis or production abnormality.
4. Duration: Peracute, acute, subacute or chronic.
5. Mean corpuscular indices:
   a. Macrocytic, normochromic or macrocytic, hypochromic
   b. Normocytic, normochromic or normocytic, hypochromic
   c. Microcytic, hypochromic or microcytic, normochromic
6. Aetiologic mechanism: Toxic, infectious, idiopathic, traumatic, neoplastic or paraneoplastic,
7. Specific aetiology: Babesia equi, equine infectious anaemia virus, etc.

Evaluation of Elevated Haematocrit:

Primary concerns are whether this is a RELATIVE ERYTHROCYTOSIS (common) or an ABSOLUTE ERYTHROCYTOSIS (uncommon). Relative erythrocytosis is usually the result of decreased plasma volume (dehydration). Absolute erythrocytosis is an increase in circulating red blood cell mass without change in plasma volume. Ensure elevations in haematocrit have haemoglobin and RBC counts that are in proportion to the elevation in the haematocrit to rule out artefactual or specimen problems.

Is total protein elevated?
   If so, consider dehydration as most likely underlying cause. Is this abnormality corrected following fluid therapy?

If dehydration is not likely,
Consider possible splenic contraction. Has there been recent exercise, excitement or nervousness?

*Is there a possible endotoxaemia or other disease causing this finding?*

*Has the sample been properly handled, without likelihood of sample dessication?*

  Has the sample been spun without the stopper on the tube? Has it been stored next to a radiator or heater or on the dashboard of a vehicle resulting in evaporation of fluid components?

  Has there been a delay prior to analysis, such as prolonged transit or other conditions that may compromise specimen quality?

If the above conditions are not likely, *is the elevation a consistent, repeatable finding?*

Is it repeatable on the same specimen and on another specimen? If so, consider the following differential diagnoses:

1. Absolute erythrocytosis due to physiologically appropriate mechanisms?
   a. Chronic tissue hypoxia – document low arterial pO2?
   b. High altitude disease
   c. Cardiac insufficiency with right to left shunting (congenital or acquired)
   d. Chronic pulmonary disease (resulting in hypoxia) with increased erythrocyte production
   e. Other chronic organ disease or insufficiency

2. Abnormal increase in erythropoietin production
   a. Erythropoietin administration
   b. Abnormal endogenous erythropoietin production
   c. Neoplasia – renal, hepatic, adrenal, ovarian, other tumours
   d. Androgenic steroid administration
   e. Adrenal disorder

3. Abnormal bone marrow proliferation
   a. Primary erythrocytosis – myeloproliferative disorder restricted to the erythroid cell line
   b. Polycythaemia vera – myeloproliferative disorder involving erythroid, myeloid and megakaryocytic lines.

**Evaluation of Anaemia**

Formulation of differential diagnoses for equine anaemia should be based on thorough documentation of anaemia and consideration of all aspects of the history, physical examination and results of other testing.

- Remember: foal haematologic values differ from adults that breeds of horses (‘hot-blooded’ vs ‘cold-blooded’) breeds may differ.
- Determine if your laboratory has reference intervals based on age and/or breed.
- Find out the characteristics of the population used to determine reference values and the number of animals and statistical techniques used.
- As a general rule, haematologic reference intervals and cutoff values/medical decision limits from the equine literature can be used as an aid in interpretation of haematologic data generated from most analysers used in large commercial laboratories.
- Reference interval validation and/or generation of reference intervals within your practice may be helpful if you deal with specific populations or types of horses for which ‘generic’ references intervals may not apply.
Anaemia associated with blood loss

Peracute blood loss may result in splenic contraction, releasing stored erythrocyts into the circulation and masking blood loss for at least several hours following haemorrhage. Look for a decrease in total protein as an initial indicator at approximately 4-6 hours post haemorrhage.

Look for reduced haematocrit at approximately 12-124 hours post haemorrhage. Continued decline with fluid fluxes may continue for up to 72 hours following a single episode of haemorrhage with marked blood loss.

Chronic blood loss results in slowly developing anaemia when blood loss exceeds the bone marrow erythropoietic capacity.

Internal haemorrhage may result in anaemia, if severe, prolonged or repeated. May be difficult to determine if there is haemorrhage within a body cavity (pleural, abdominal, intra-articular). Repeat taps may be needed to have confidence that bloody nature is due to true haemorrhage and is not iatrogenic. Appearance of hte specimen at the time of collection (red throughout collection, haemolysed appearance different from fresh blood) or appearance at microscopic evaluation (lack of platelets, presence of haemosiderin, erythropagocytosis in specimen immediately added to fixative or in smears prepared immediately following collection) support internal haemorrhage.

Haemorrhage due to abnormalities of haemostasis can result in haemorrhage at a variety of sites, depending on the underlying cause, trauma, environmental or individual factors. Considerations should include:

- Thrombocytopenia
- Warfarin toxicosis
- Mouldy sweet clover toxicity
- Hameophilia A or other inherited coagulopathies

Table 2. Considerations for Chronic Blood Loss Resulting in Anaemia in the Horse

<table>
<thead>
<tr>
<th>Site of Blood Loss</th>
<th>Differential Diagnoses</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>External</td>
<td>Repeated trauma</td>
<td>Physical examination very important!</td>
</tr>
<tr>
<td></td>
<td>Parasites (including ticks, lice)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ulcers/sores</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Parasitism</td>
<td>Faecal occult blood test is sensitive but not specific.</td>
</tr>
<tr>
<td></td>
<td>Gastric or duodenal ulcers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NSAID toxicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gastric squamous cell carcinoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swallowed blood from pulmonary bleeding</td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>Guttural pouch mycosis</td>
<td>Endoscopic and cytologic evaluation may be helpful.</td>
</tr>
<tr>
<td></td>
<td>Ethmoid haematoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fungal rhinitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nasal amyloidosis with bleeding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe pneumonia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung abscess</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pulmonary neoplasia</td>
<td></td>
</tr>
<tr>
<td>Exercise-induced pulmonary haemorrhage</td>
<td>Microscopic evaluation of urine required since may not have macroscopically visible abnormality. Endoscopy of the urinary system is sometimes helpful.</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Urogenital</td>
<td>Urinary cystisis Vascular abnormality/rupture, esp. post-foaling Pyelonephritis Trauma Urolithiasis</td>
<td></td>
</tr>
</tbody>
</table>

**Haemolytic Anaemia in the Horse**

May or may not be accompanied by icterus/hyperbilirubinaemia. Expect total serum or plasma protein to be normal or increased compared to anaemia from blood loss, in which decreased total protein is more common.

May be accompanied by leukocytosis with neutrophilia +/- regenerative left shift.

Types of haemolytic anaemia in the horse include:

**Immune-mediated Haemolytic Anaemia (IMHA)**

May or may not show auto-agglutination. Auto-agglutination may be macroscopic and/or microscopic. Sometimes microscopic auto-agglutination is recognized when macroscopic auto-agglutination is not detected. Auto-agglutination supports an immune-mediated mechanism for anaemia.

True auto-agglutination should be differentiated from rouleaux, the ‘stack of coins’ appearance which is an expected finding in equine blood, and from nonspecific clumping. A Saline Agglutination Test is helpful in making this distinction:

- One drop of EDTA blood is mixed with 1-2 drops of isotonic saline on a slide and viewed against a white background (piece of paper) and under the microscope.
- Immune-mediated erythrocyte agglutination will persist, whereas rouleaux and nonspecific clumping will disperse.

Confirmation of immune-mediated basis for anaemia in the absence of auto-agglutination is by a Coombs’ Test.

- If auto-agglutination is present macroscopically, a Coombs’ test cannot be evaluated since a positive test is based on absence of agglutination in the initial sample and presence of agglutination following addition of antiserum to equine IgG, IgM and complement.
- False negative Coombs’ tests may occur immediately after haemolytic crises, if corticosteroids have been administered, or if the test is not conducted with
appropriate dilutions and controls or in some cases without obvious reasons. A negative Coombs’ test does not rule out the possibility of IMHA.

Of special importance in the horse is Neonatal Isoerythrolysis (NI) caused by blood group incompatibility between the dam and foal.

- Maternal antibodies against foal erythrocyts absorbed from colostrums – results in destruction of foal erythrocytes.
- Diagnosis may be presumptive in neonates with lethargy, anaemia, and icterus during the first 4 days of life.
- Confirm with haemolytic cross-match test or Jaundiced foal Agglutination Test
- Best prevented by knowledge of blood typing and prevention of colostral ingestion in foals at risk for this condition.

Jaundiced Foal Agglutination Test:
1. Set up 7 clear tubes, each with 1 ml of saline. Label consecutively as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and BLANK.
2. Make serial dilutions of mares plasma or colostrums by adding 1 ml to tube 1 and amixing with pipette and then removing 1 ml from this tube into the next tube, mix and remove 1 ml to the third tube and so on. Discard 1 ml from the sixth tube (labelled 1:64).
3. Add one drop of the foal’s whole EDTA blood to each of the 7 tubes and mix by agitation or gentle rocking.
4. Centrifuge tubes for 2-3 minutes at speeds suitable for separation of plasma/serum
5. Pour or pipette off supernatant from each tube, taking care not to disturb the erythrocytes in the bottom of the tube. Observe the status of the red cell button.

Interpretation:
Complete agglutination causes cells to remain firmly packed in a button at the bottom of the tube and they will not move when the tube is tilted. Negative samples show free movement of cells when the tube is tilted.

Positive reactions are taken to be > 1:16 dilution - in other words, if the 1:1 or higher dilutions form firmly fixed buttons, the foal is likely a Neonatal Isoerythrolysis case. The results are less certain with colostrums with more false positives.

Confirm suspected NI cases based on this test by blood typing of the sire and dam.

Equine Infectious Anaemia (EIA)

- Equine retroviral infection.
- Thrombocytopenia is often the first indicator of infection and is a consistent finding.
- In additional to anaemia, there may be leukopenia, lymphocytosis, and/or monocytosis.
- Hypergammaglobulinaemia is often present.
• There may be periods in chronic carriers and affected individuals in which all haematologic variables are within normal limits.

**Idiopathic Immune-mediated Haemolytic Anaemia**

• IMHA of unknown aetiology
• A ‘diagnosis of exclusion’ – rule out other possible causes to the best of your ability before assigning it to an ‘idiopathic’ category.

**Secondary IMHA**

• May be secondary to infections, especially *Clostridium perfringens*, injection site abscess or purpura haemorrhagica, drug administration (especially penicillin), as a paraneoplastic condition (especially lymphoma), or miscellaneous conditions (such as protein-losing enteropathy).

**Oxidant-Induced Haemolytic Anaemia in the Horse**

Oxidized haemoglobin results in the formation of Heinz bodies and/or eccentrocytes. Heinz bodies are aggregates of oxidized haemoglobin and are visible in peripheral blood smears as small membrane projections or ‘pale areas’ within erythrocytes. Sometimes distinctive eccentrocytes, in which haemoglobin is located to one side of the erythrocytes with maintenance of a thin erythrocyte membrane at the opposite side, are present.

**Evaluation of a New Methylene Blue (NMB) stain of EDTA blood may be helpful in identifying Heinz bodies.**

• One drop of NMB is mixed with 1 drop of EDTA blood on a glass slide and covered with a cover slip
• When examined microscopically, Heinz bodies are blue-green granules located near or protruding from the red cell membrane

Icterus and haemoglobinuria are common. Renal failure may be a sequel to severe haemoglobinuria.

**Differential diagnoses of Heinz Body anaemia are:**

1. Phenothiazine toxicity. Uncommon due to reduced use of this as anthelminthic. May be present in rumnent supplements or some salt blocks. May be some individuals that are more sensitive than others to development of toxicity.
2. Onion Toxicity. Uncommon. A disulfide in wild or domestic onions is responsible for oxidative damage. Usually distinctive onion odour oof breath, urine and faeces.
3. Red Maple Leaf Toxicity. Ingestion of dried red maple leaves (not fresh or viable leaves).

**Parasite-Induced Haemolytic Anaemia**

1. *Babesia caballi* and *Theileria equi* (formerly *Babesia equi*)
   • Intra-erythrocytic protozoan parasites transmitted by ticks.
   • May be visible in peripheral blood smears during febrile periods, but may be absent at the time of haemolytic crisis.
• Infection may result in death or chronic disease.
• Following recovery from clinical Babesiosis, horses may become unapparent carriers for an indeterminate period unless treated.
• Stress may induce clinical disease in carriers.
• Imidocarb diproprinate is effective in eliminating Babesia caballi infection, but is only 50-60% effective in eliminating Theileria equi. The more effective drug diminozene acaeturate (3.5 mg/kg) is very dangerous but will effectively sterilise the blood.

**Haemolytic Anaemia Associated with Other Conditions**

Haemolytic anaemia may occur with chronic disseminated intravascular coagulation (DIC). The potential for haemolysis exists with any microangiopathic process, including thrombosis or fibrinoid degeneration of small vessels.

Intravascular haemolysis may occur in some horses with terminal hepatic or renal failure.

**ANAEMIA DUE TO DECREASED ERYTHROCYTE PRODUCTION**

This type of anaemia is associated with bone marrow inability to produce erythrocytes. This may be a relative or absolute inability. Conditions that are associated with decreased erythrocyte production include:

1. **Iron Deficiency**
   • Iron is necessary for production of haemoglobin. Usually a problem of foals rather than adult horses. Usually associated with chronic blood loss. Initial normocytic normochromic anaemia with decreased marrow iron, progressing to microcytic, hypochromic anaemia. Smaller cells deficient in haemoglobin are the result of increased divisions of erythrocytes in an attempt to produce a critical intracellular concentration of haemoglobin.
   • Serum ferritin assay may help make this diagnosis. Serum ferritin < 45 ng/ml is suggestive of iron deficiency in horses, but assay is species-specific, not widely available and may be falsely increased by concurrent inflammation. Usual confirmation by evaluation of the bone marrow aspirate for iron and finding a predominance of rubricytes and metarubricytes with ragged cellular boundaries. Diagnosis of an underlying condition and response to elimination of the underlying condition and treatment with iron supplements is also useful.

2. **Anaemia of chronic disease**
   • Most common form of equine anaemia.
   • Usually a low-grade anaemia (haematocrit usually not less than 20%)
   • Bone marrow iron is normal to increased.
   • Investigation to identify underlying chronic disease is indicated in ANY equine anaemia.
   • A special case of anaemia of chronic disease is that associated with renal disease. Anaemia is thought to be associated with defective marrow response to erythropoietin since decreased erythropoietin levels have not been demonstrated consistently. There may be reduced erythrocyte lifespan.
3. Anaemia of Myelophthesic Disease

- Myelophthesic diseases replace or destroy the normal bone marrow environment. Because these diseases do not selectively target erythroid cells, pancytopenia is often present.
- Neoplastic infiltration or inflammatory infiltration are reported causes of equine myelophthesic disease.
- Myeloid neoplasia is the most commonly reported cause. Peripheral leukaemia may or may not be present. When myeloid neoplasia involves the bone marrow but atypical cells are not visible in peripheral blood, this is termed ‘aleukaemic leukaemia.’

4. Aplastic or Hypoplastic Anaemia

- This refers to failure of bone marrow stem cells to differentiate due to toxic damage, intrinsic damage, immune-mediated damage or interruption of interactions within the bone marrow environment necessary for normal cellular production.
- This is an uncommon finding in horses.
- Usually idiopathic, although rare cases have been reported with phenylbutazone administration.
- Other causes – drugs, toxins, ionizing radiation, immune-mediated destruction at the marrow level – have been suggested. Diagnosis is based on finding peripheral anaemia and/or pancytopenia with severe bone marrow hypoplasia/aplasia and replacement of marrow by fat. Because it may be difficult to judge if bone marrow aspirates of low cellularity are truly representative bone marrow core biopsies from at least 2 sites are recommended for this diagnosis.

Miscellaneous Tests Related to Haematology

Blood Transfusions

There are at least 8 blood groups in horse relating to surface antigens on the erythrocyte. There are many other proteins also related to the RBCs. It is likely that no two horses will have identical blood types and be totally compatible with each other for transfusions. A Test for Blood Compatability is recommended prior to any transfusion, but the absence of incompatibility does not negate the need for careful monitoring of the transfusions. Initial flow rates should be very slow. Most reactions will reflect haemolysis rather than agglutination and can be difficult to detect. Almost all transfusions will have some ‘reactions’ but the extent will dictate the efficiency of the transfusion.

If you are unable to do a Test for Blood Compatability (as outlined below), obtain blood from a middle-aged gelding who has not had any previous blood treatments. Universal donor horses with neither Qa or Aa antibodies can be identified for repeated use. In large practices it may be convenient to identify one or more of these animals. Male ponies are often A and Q negative. Mares that have had foals should be avoided.

The normal lifespan of equine erythrocytes is 120-135 days, but transfused cells (even if compatible) will only last 2-3 days at most. Transfusion, therefore, is a short-term measure to be taken while an underlying problem is addressed. A bone marrow response should be apparent at the time of transfusion or, if an acute blood loss,
should develop within 4-7 days...as you can see, a single blood transfusion is barely adequate or not adequate to bridge the gap needed to develop a bone marrow response and see evidence in the peripheral blood.

Blood transfusion is indicated if the haematocrit is < or = 10-12% and falling. The risks associated with blood transfusion are high. If the recipient of a blood transfusion is mare, she will be more likely to produce a foal with Neonatal Isoerythrolysis. The administration of plasma expanders and crystalloid products with amino acids and glucose are often a better choice than blood transfusion and may help the patient to recover enough to mount a regenerative response.

Commercial blood collection bags are available and contain the correct levels of ACD anticoagulant (10 ml anticoagulant per 100 ml of blood). Most commercial bags have wide bore needed which need to be cut off and the tube is then inserted into a three-way stopcock attached to a 10-12 g canula which has been inserted into the jugular vein using aseptic technique. The catheter should be flushed with heparin saline after insertion. The bag should be lowered to ground level. Repeated agitation during collection is important and it may be helpful to flush the catheter and tube with a small amount of ACD solution from time to time to avoid clotting. Collection into open glass bottles is NOT acceptable! There is too high a risk of infection and damage to RBCs from the glass contact. Glass also activates platelets and Factor XII and causes lysis.

A 450 kg adult horse can safely donate up to 15 L of blood. A pony can usually donate 6-7 liters without harm. Blood should be used immediately but can be stored for up to 4-6 days at 4 degrees C.

Blood is administered via a 10 or 12 g intravenous canula (with a three-way stopcock, which is flushed from time to time). Initial flow rates should be very slow – 1 liter over the first 15-20 minutes). If no reaction is seen the flow rate can be doubled for the next 15 minutes and if that is safe, the flow rate can be maximized via the catheter. A suggested theoretical maximum rate is 40 ml/kg per hour.

Transfusion reactions include: restlessness, shock, colic, pyrexia, defecation/diarrhoea, cardiac arrhythmia, urticaria, pruritis, icterus, haemoglobinuria. Nonimmunological reactions may include overload resulting in pulmonary oedema, hypocalcaemia (anticoagulant excess), hyperkalaemia (lysis of RBCs releases K into circulation), haemolysis (bad collection, preparation or storage of blood), microaggregation (in brain, kidneys or other organs), or infection (over following 2-4 days – endocarditis, pneumonia).

If a reaction is seen during transfusion, it probably means that another donor will not be any better!

**Test for Blood Compatibility (Major and Minor Cross Match Test)**

Should be done prior to any blood transfusions.

1. Obtain 10 ml clotted blood (red top Vacutainer) and 10 ml of citrated blood (yellow top Vacutainer – acid-citrate-dextrose anticoagulant)
2. Major cross match: Recipients serum with washed donor red cells
   Minor cross match: Recipient’s washed red cells with donor serum

**Method:**
1. Obtain blood from recipient and prospective donor(s), as indicated above. Identify the samples carefully.
2. Place 5 ml of saline in 2 sterile centrifuge tubes marked ‘recipient’ and ‘donor A/B/C, etc., respectively.
3. Push a glass rod into the clotted blood in the tube and transfer the rod to the appropriate sample tubes to make a weak suspension of red cells from the donor(s) and recipient.
4. Spin both the clotted blood and cell suspension in a centrifuge for 2-3 minutes.
5. Remove the suspension samples and pour off the supernatant. Resuspend the cells in 5 ml of clean saline and repeat the spinning.
6. Repeat the washing procedure on the suspension of red cells at least 5 times and after the last wash, re-suspend the cells in a drop or two of saline only (to give a slightly more concentrated suspension of red cells).
7. The serum should be separated following centrifugation of the clotted samples.
8. Place two slides on a sheet of white paper. The slide on the left should be placed under a label on the sheet of paper as ‘donor serum’ and the slide on the right should be placed under a label on the sheet of paper as ‘recipient serum’.
9. Each slide will contain one CONTROL reaction area and one TEST reaction area. Slide is divided into two halves.
10. A drop of donor serum should be added to each reaction area on the left slide (Donor) and a drop of recipient’s serum should be added to each reaction area on the right slide (Recipient).
11. Then add 1 drop of washed red cells to each of the reaction areas. The control area on the Donor (left) slide should have donor erythrocytes added. The test area on the Donor (left) slide should have recipient erythrocytes added. The test area on the Recipient (right) slide should have donor erythrocytes added and the Control area on the Recipient (right) slide should have recipient cells added.
12. Then carefully mix the cells in the serum drops with a toothpick. Use a fresh toothpick for each reaction area.
13. Incubate for 10-15 minutes in a humid environment (such as a Petri dish containing a moistened paper towel on the bottom at room temperature.
14. Interpretation is as follows:
   a. The control areas on both should contain even sedimentation of erythrocytes in a mat-like deposit, without clumping (haemagglutination). If this is not present, a mistake has been made in setting up the slides and you should start over.
   b. If there is even sedimentation of red cells in a mat-like deposit in ALL of the reaction areas, without clumping (haemagglutination), then this is a FULLY COMPATIBLE CROSS MATCH.
   c. If there is even sedimentation of erythrocytes in a mat-like deposit except in donor serum with recipients cells (Test area of left slide) there is uneven clumping of erythrocytes, then this is an INCOMPATIBLE MAJOR CROSS MATCH.
   d. If there is even sedimentation of erythrocytes in a mat-like deposit, except in recipient serum with donor cells (Test area of right slide) there is uneven clumping of erythrocytes, then this is an INCOMPATIBLE MINOR CROSS MATCH.
   e. If there is even sedimentation of erythrocytes into a mat-like deposit in controls only and uneven clumping (haemagglutination) in the test areas of BOTH slides, then this is a TOTALY INCOMPATIBLE CROSS MATCH (INCOMPATIBLE MAJOR AND MINOR CROSS MATCHES).

Bone Marrow Collection in the Horse

A bone marrow aspirate and core biopsy may be helpful in investigation of
- Severe unexplained anaemia
- Persistent or unexplained leukopaenia
- Persistent or unexplained thrombocytopenia
- Suspicion of leukaemia or lymphoma
Sites for collection include:

1. Wing of ileum – difficult site but accessible, harvest is often poor
2. Rib – difficult site, harvest is often poor
3. Sternum – awkward access, but harvest is more reliable and easier

Technique:
The horse should be restrained by a competent handler to prevent movement during collection. Sedation may be needed for nervous or excitable patients.

1. Locate the site to be used on the ventral midline just behind the point of the elbow, with the horse standing normally.
2. A sterile bone marrow biopsy needle or 18 g spinal needle (stylet is important to prevent needle being blocked by a plug of bone) is needed.
3. Clip and surgically prepare the site
4. Instill 3-5 ml of local anaesthetic into skin and deeper tissues down to the bone. Be careful not too use too much as the area swells and makes the technique too difficult.
5. Make a small stab incision through the skin with an 11 scalp blade
6. Insert collection needle vertically up to the cortex of the sternum, rotate and drive the needle forward (take care not to break it!). A sudden reduction in resistance means the cortex has been penetrated. Remove the stylet then.
7. Draw a small amount of EDTA solution from a blood tube into a 20 ml syringe. If only dry EDTA is present in a blood tube, fill the tube to the line with sterile water or saline to make an appropriate EDTA solution. The EDTA solution in the syringe will help prevent clotting of the bone marrow specimen. If EDTA is not available, citrate anticoagulant form a blood collection bag or citrate tube is also suitable.
8. Attach the syringe to the collection needle and apply firm suction with a pumping action until marrow becomes apparent in the hub of the syringe. Do not keep applying suction once material is seen. Remove the syringe and squirt the marrow specimen into a petri dish containing a small amount of EDTA solution.
9. Examine the specimen by gently rotating the petri dish over a white paper with strong indirect adjacent lighting. Fat from the marrow will appear as glistening droplets. Marrow particles are visible as small, dull flecks. If no marrow particles are seen, advance the needle a small amount and re-collect. You may need to re-introduce the needle at an adjacent site. If you do this, be sure to replace the stylet for the initial insertion.
10. When bone marrow particles are identified, transfer one or several to a clean dry labelled slide. If you transfer a lot of fluid with it, this can be wicked away using a tissue. Then place a second slide on top of the particle. Apply a small amount of pressure with your fingers to get the particle to spread between the slides, then slide them apart, keeping the surfaces of the slides in contact. This should make marrow particle smears on BOTH of the slides.
11. If marrow particles are numerous, make at least 6-8 slides since not all slides will be of the same quality and there may be slight differences in marrow representation.
12. To collect a bone marrow core biopsy, the collection needle is advanced into the bone marrow and then withdrawn. The core biopsy will be present within the needle. Tease the biopsy out of the needle retrograde using a paper clip or small wire. Do NOT push it through the small end of the needle – this will cause rupture of cells and distortion. Place the core biopsy in 10% buffered formalin, as you would for any tissue specimen. Collection of bone marrow core biopsies from at least two sites is recommended.
13. A small suture at the site of the stab incision may be used. If there is oozing or bleeding from the site, application of a pressure bandage is recommended. Complications associated with bone marrow collections are few. Careful monitoring of the site is recommended over the next 5-7 days to make sure there is not continued trauma to the area or development of infection.
EVALUATION OF THE HAEMOSTASIS

The processes of haemostasis involve complex interactions of the blood vessel wall, endothelial cells, platelets, coagulation factors and anticoagulant proteins. The fibrinolytic systems is activated simultaneously with coagulation and prevents tissue ischaemia that may result from prolonged presence of fibrin clots. With normal function there is a balance of coagulation and fibrinolysis that helps maintain vascular integrity and blood flow to all body systems.

The following chart provides a simplified summary of the major components of haemostasis:

Table 3. Simple Summary of Components of Haemostasis

<table>
<thead>
<tr>
<th>Component</th>
<th>Constituents</th>
<th>Functions</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood vessel wall</td>
<td>Endothelium, collagen, substances that activate or inhibit haemostasis</td>
<td>Vasoconstriction, Scaffold for fibrin clot formation, Regulation of the clotting process</td>
<td>Important in primary haemostasis (initial stages), Functions and reactions associated with vascular injury</td>
</tr>
<tr>
<td>Platelets</td>
<td>Variety of chemical mediators</td>
<td>Adherence, activation, aggregation and release reactions</td>
<td>Forms initial platelet plug at site of vascular injury, leading to fibrin meshwork and thrombus formation by coagulation factors</td>
</tr>
<tr>
<td>Coagulation Factors</td>
<td>Pro-coagulant (inactive) forms converted to active forms</td>
<td>Formation of fibrin meshwork and thrombus</td>
<td>Prevention of haemorrhage into pervascular tissues and maintenance of vascular integrity</td>
</tr>
<tr>
<td>Anticoagulant proteins</td>
<td>Variety of factors</td>
<td>Localise the coagulation process to the site of injury, Maintain blood flow</td>
<td>Prevent generalized thrombosis, Maintain blood flow</td>
</tr>
</tbody>
</table>
Coagulation Factors have traditionally been divided into the Intrinsic, Extrinsic and Common Pathways. This division is helpful for categorization of conditions, but are highly artificial since there are multiple links between pathways. However a simplified representation of Secondary Coagulation is useful in helping to provide a framework for categorization of processes:

Intrinsic System

- This is a very complex system and I usually view it as the network of factors activated by coagulation within (intrinsic to) the blood vessel lumen. Because we do not want intravascular coagulation to proceed without a good reason or without sufficient control, this system includes a variety of complex factors and interactions.

Extrinsic System

- This is primarily regulated by tissue factors that occur within the blood vessel wall and are, therefore, outside (extrinsic to) the blood vessel lumen.

Common Pathway

- Intrinsic and Extrinsic Systems are linked by Factor X to form the Common Pathway.

Anticoagulant Proteins consist of two primary groups:

1. Inhibitors of serine proteases – antithrombin III and Heparin Cofactor II are important examples.
2. Protein C system

Fibrinolytic System

- A key component is Plasmin (generated by activation of plasminogen).

**Laboratory Tests Commonly Used to Evaluate Coagulation**

1. Activated Partial Thromboplatin Time (APTT)
   a. Evaluates Intrinsic System and Common Pathway.
   b. Some disease or conditions that may result in prolongation of APTT include
      i. Liver disease. Many of the factors of the intrinsic system are synthesized in the liver.
      ii. Rodenticide or warfarin toxicity. Vitamin K dependent factors are affected.
      iii. Disseminated Intravascular Coagulation (DIC)
      iv. Haemophilia A (Factor VIII deficiency)
      v. Haemophilia B (Factor IX deficiency)
2. Prothrombin Time (PT)
   a. Some diseases or conditions that may result in prolongation of PT include
      i. DIC
      ii. Rodenticide or Warfarin Toxicity
      iii. Liver disease. In early liver disease, PT may be elevated without concurrent elevation of APTT.

3. Thrombin Time or Thrombin Clot Time (TCT)
   a. Depends on normal function of fibrinogen.
   b. Increases are seen in hypofibrinogenaemia and dysfibrinogenaemia.
   c. Thrombin clot time is typically elevate d with DIC, may or may not be elevated with warfarin or rodenticide toxicity or with Haemophilia or their cases of severe haemorrhage.

4. Fibrinogen Degradation Products (FDP) and D-dimer (DD)
   a. FDP requires special tube for collection with relatively short shelf life compared to other blood collection tubes. Methods are the same across species so may have source of tubes in local hospital or by request from veterinary testing laboratory.
   b. Confirmatory tests for DIC. Recognize products produced by the fibrinolytic system.
   c. D-dimer specific for fibrinolysis and may be more sensitive than FDP for diagnosis of thromboembolic disease.

5. Von Willebrand Factor Assay
   a. A factor produced by endothelial cells and which is necessary for normal platelet function.
   b. Deficiency may result in abnormal platelet function. May be acquired or inherited deficiency.
   c. Has been rarely reported or investigated in the horse.

6. Specific Factor Assays
   a. May be needed to determine if single or multiple factor deficiencies are present.
      i. Factor VII deficiency is classic: Haemophilia A.
         - Sex-linked disease with expression of bleeding in affected males.
      ii. Other specific factor deficiencies have been reported in the horse.

Other tests that are not widely available but which have been reported to be useful in monitoring haemostasis or disease processes that result in abnormalities of haemostasis include Antithrombin III and Protein C assays.

**Tips for Coagulation Testing**

1. If you do not do coagulation testing frequently, consider submission of a ‘control’ specimen. This should be collected form an individual without suspected haemostasis abnormality, matched for age, sex and management conditions, if possible. A specimen from a healthy control is helpful in controlling for pre-analytical variables such as specimen collection, anticoagulant efficacy, handling and transport of specimens. If the patient AND control specimen are abnormal, the possibility of artifactual elevation due to these variables is likely and consideration should be given to likely underlying problems. If a problem is thought to be likely, recollection is recommended. Be sure to submit another control with the recollection.
An elevation of >25% in the patient sample compared to the control sample is likely to be of significance. Differences of less than this amount are less likely to represent a true difference. Reference intervals established by the laboratory specific for horses should be used as an aid in identifying abnormalities.

Collection and Handling of Specimens for Haemostasis Studies

It is advisable to submit an EDTA sample for platelets and haematocrit determination at the same time as citrate anticoagulant sample for tests for secondary coagulation. Anaemia, haemoconcentration or thrombocytopenia may result in altered concentrations of coagulation factors, causing anomalous results.

Proper collection and handling are of paramount importance in obtaining an accurate result. Specimens should be collected in a manner that minimizes stress and results in a ‘clean’ collection with minimal numbers of needle sticks, rapid blood flow, anticoagulation and avoidance of haemolysis. This may be accomplished by a variety of means, depending on the size and conformation of the animal and its behaviour and reactions.

Use only plastic syringes and tubes.

Collection of a minimum of 3.0 ml of blood is recommended to ensure than an adequate volume of plasma is obtained. Collection of 5.0 ml of blood is preferred since tests are routinely run in duplicate.

Step 1. Specimen Collection

A. Option 1. Vacutainer Collection

Use at least a 3.0 ml blue top plastic Vacutainer tube containing 3.8% Trisodium citrate anticoagulant with a 20-22 gauge needle. Draw blood directly into the tube. Do NOT draw blood into a dry syringe and then transfer it to the Vacutainer tube. Be sure to fill the tue as much as the vacuum will allow to ensure the correct ratio of anticoagulant and blood. Mix gently but thoroughly to prevent formation of a clot that may result in artifactual results.

B. Option 2. Needle and Syringe Collection.

This option can be used if Vacutainer tubes are not available. Use a plastic syringe and 20-22 gauge needle. Draw the blood into a plastic syringe in which there is 3.8% trisodium citrate anticoagulant. Anticoagulant may be drawn out of the blood collection tube. Observe the ratio of 1 part anticoagulant with 9 parts blood. For example, for a final volume of 3 ml, place 0.3 ml of anticoagulant in the syringe and collect 2.7 ml of blood to fill the syringe to the 3.0 ml level.

<table>
<thead>
<tr>
<th>Volume of Anticoagulant in the Syringe</th>
<th>Final Volume of Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 ml</td>
<td>Draw up to 3.0 ml mark</td>
</tr>
<tr>
<td>0.4 ml</td>
<td>Draw up to 4.0 ml mark</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>Draw up to 5.0 ml mark</td>
</tr>
<tr>
<td>0.6 ml</td>
<td>Draw up to 6.0 ml mark</td>
</tr>
</tbody>
</table>

Do NOT squirt blood through a needle following collection. This may result in haemolysis.
Remove the needle from the syringe and gently expel blood down the side of a clean, sterile, plastic tube that can be used for centrifugation. Usually a minimum volume of 3.0 ml of blood is recommended to ensure an adequate volume of plasma for testing. Submission of at least 1.0 ml of separated plasma is requested.

C. Option 3. Butterfly Catheter Collection
For small patients or those in which jugular vein is not easily accessible for collection. A butterfly catheter can be used to help ensure good blood flow and prevent clot formation. The blood may be drawn through the catheter directly into anticoagulant in a syringe or a blue top tube. The catheter tube is usually of sufficient length to allow gentle mixing of blood and anticoagulant by hand rocking or rotation as the specimen is collected.

DO NOT COLLECT SPECIMENS THOROUGH INDWELLING CATHETERS THAT HAVE BEEN PLACED FOR OTHER PURPOSES.

Step 2. Mixing of blood and anticoagulant.
A. Gentle but thorough mixing of blood and anticoagulant is needed to help prevent clot formation and to ensure that there is no activation of coagulation factors.

IF A CLOT IS PRESENT, THE SPECIMEN SHOULD NOT BE SUBMITTED. RECOLLECT.

Step 3. Centrifugation and separation of plasma.
A. Prompt centrifugation and separation of plasma from blood cells is required. Usually centrifugation at 2000-3000 rpm for 10-15 minutes is sufficient. Attention to proper balancing of the centrifuge is important since vibration due to inadequate balancing may result in haemolysis.

IF HAEMOLYSIS IS PRESENT, THE SPECIMEN SHOULD NOT BE SUBMITTED. REPEAT SAMPLINGS IS ESSENTIAL.

B. Carefully pipette the plasma, taking care that erythrocytes are not aspirated. Use a plastic pipette and transfer plasma to a clean plain plastic tube that does NOT contain additional anticoagulant.

Step 4. Specimen Identification.
A. Be sure to label the tube with date of collection, animal and owner name, name of the clinical and contents (citrated plasma).

PROPER SPECIMEN IDENTIFICATION IS IMPORTANT IN ENSURING THAT THE CORRECT TEST IS RUN AND THAT RESULTS ARE ATTRIBUTED TO THE CORRECT PATIENT.

Step 5. Freeze plasma.
A. Immediately freeze the plasma specimen.

Step 6. Submit frozen specimen.
A. Submit the FROZEN specimen to the laboratory by overnight or courier delivery. Ice packs or dry ice should be included with the specimen to ensure that it arrives frozen.
EQUINE CLINICAL CHEMISTRY

Clinical chemistry evaluation includes a variety of tests. Selection of tests, combinations of tests or proliﬁes will depend on your assessment of the clinical condition and your differential diagnoses.

I am a proponent of the minimum data base approach to clinical problems. This approach requires that you critically consider various types of presentations or situations in which laboratory data may be of beneﬁt and determine what laboratory tests you consider essential for the situation. Since some clinical presentations n the horse are not speciﬁc as to underling casue (i.e., chronic weight loss, poor performance, lethargy, partial or complete anorexia), a systematic approach to workup of these cases is of beneﬁt in assuring that common and uncommon differential diagnoses are considered. Laboratory tests may provide valuable information or ‘clues’ as to underlying conditions or may be helpful in ruling out particular differentials. Prior consideration of what you require as a minimum data base will be helpful in assuring that the case is properly worked up, that maximum information is obtained for the money spent and provides a basis for educating the client about various conditions, their diagnosis, prognosis, treatment and/or monitoring. This is a personal decision and will depend on your practice philosophy, ability ot educate clients and experience in diagnosiso f conditions based on variables other than laboratory results.

Financial considerations often have a role in determining whether or not laboratory work is conducted and how much laboratory work is done. Often it is false economy to select only a few tests. Evaluation of a more comprehensive selection may have allowed more rapid recognition of abnormalities and contributed to a more rapid diagnosis while minimizing animal suffering, maximizing value for money spent, enabling a correct diagnosis and/or prognosis and determining the best course of treatment. My preference is to u sually start with a comprehensive proﬁle with aplan to use only selected tests or clinical response to moitor the condition or response to therapy once a clinical diagnosis has been made. However, we all have to deal with clients, who may be reluctant to understand these factors, even when we attempt to educate them about their importance! So, educate yourself about the use of laboratory testing. Consult with a clinical pathologist or experienced internist if you have questions or concerns about the appropriate tests to run, the likelihood of obtaining useful information or the correct specimen(s) to submit.

This section will discuss the laboratory evaluation of various systems.

Evaluation of the Liver

The liver is considered separately from the gastrointestinal system.
Tests that are commonly available and useful for evaluation of the liver include

- Alkaline phosphatise (ALP)
- Aspartate Aminotransferase (AST)
- Gamma-Glutamyl Transferase (GGT)
- Total Protein (TP)
- Albumin (Alb)
- Total Bilirubin (Tbili)
- Direct and Indirect Bilirubin (DBili, IBili)
• Bile Acids
• Glutamate Dehydrogenase (GLDH)
• Idiotal Dehydrogenase (IDH – formerly Sorbitol Dehydrogenase – SDH)
• Arginase (Arg)
• LDH, Isoenzyme 5

Serum levels of both GLDH and IDH are considered liver-specific and are expected to be elevated with acute hepatocellular damage.

Other tests may be of benefit depending on the disease or stage of disease affecting the liver; whether additional organ systems may be involved (hepato-renal syndrome, haemolytic hepatencephalopathy, etc.).

Absence of elevations hepatic enzymes does not completely rule out the possibility of chronic liver disease with little active hepatocellular damage. Sometimes low albumin may be the sole finding with hepatic failure and, rarely, there may be chronic hepatic disease without abnormalities in laboratory data.

**Table 4. Selected Enzymes Useful in Evaluation of Equine Hepatic Disease**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Distribution</th>
<th>Interpretation</th>
<th>Alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>Hepatobiliary system Bone Intestine Kidney Placenta Leukocytes</td>
<td>Elevatd in growing foals and young horses compared to adults. May be induced by drugs (glucocorticoids, anticonvulsants, vitamins)</td>
<td>Common causes of elevations: Cholestasis, GI disease. May see lesser elevations with GI disease or haemolysis.</td>
</tr>
<tr>
<td>AST</td>
<td>Hepatocytes Skeletal muscle</td>
<td>Half life relatively long; elevations may persist for up to 2 weeks following single hepatic insult. May be within normal limits in chronic hepatic disease.</td>
<td>Common causes of elevations active hepatocellular damage, muscle damage. May see some elevation with haemolysis.</td>
</tr>
<tr>
<td>GGT</td>
<td>Hepatobiliary system Pancreas (possibly) GGT of renal tubular origin – present in urine but not serum</td>
<td>Half life approx. 3 days, stable for 2 days in serum at room temp. Elevated in foals compared to adults.</td>
<td>Elevated with biliary damage or cholestasis. Pancreatitis may cause elevations – difficult to verify.</td>
</tr>
<tr>
<td>IDH (SDH)</td>
<td>Hepatocellular cystosolic enzyme</td>
<td>Short half life (approximately 4 hours) Should be analysed within 12 hours of collection or 48 hours if serum separated and refrigerated.</td>
<td>Elevated with active hepatocellular damage.</td>
</tr>
<tr>
<td>GLDH</td>
<td>Highest concentration hepatocytes</td>
<td>Considered to be liver specific in the horse.</td>
<td>Elevated with hepatocellular damage.</td>
</tr>
<tr>
<td>------</td>
<td>---------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------</td>
</tr>
</tbody>
</table>

Guidelines for Interpretation of Elevated Bilirubin in the Horse:

1. Bilirubin is sensitive to sunlight. If exposed to sunlight, bilirubin may rapidly degrade and will be artifactually decreased
   a. Consider this possibility if you have an icteric horse with bilirubin reported to be within normal limits. If protected from sunlight, bilirubin is stable for approximately 48 hours at room temp.
2. Increased Direct (Conjugated) Bilirubin is a reliable indicator of hepatic disease in the horse.
   a. Rule of thumb: When Direct (conjugated) Bilirubin levels exceed 30% of total bilirubin levels, cholestatic disease should be the primary consideration.
3. Indirect (Unconjugated) Bilirubin may be increased in neonates compared to adults.
   a. Increases in unconjugated bilirubin may occur with haemolysis, intestinal obstruction, cardiac insufficiency, drug administration (steroids, heparin, halothane anaesthesia).
   b. Levels up to approximately 140 umol/l (between 3-4 x the upper limit of reference interval) have been seen in horses attributable to anorexia alone.

Interpretation of Bile Acids in the Horse

Bile acids assay is most useful when there is evidence of hepatic disease clinically and/or biochemically. It may be difficult to interpret if there is mild elevation as part of a ‘screen’ when nonspecific clinical signs are present or when there is no biochemical support for hepatocellular or hepatothelial compromise. Moderate to marked elevations usually accompany portosystemic shunts, but do not indicate underlying cause or prognosis.

1. Serum bile acids assay may be elevated if fasting or anorexia of greater than 14 hours. Fasting longer than 3 days has been reported to increase serum bile acids up to 3 x above baseline values.
2. Elevations in bile acids are considered highly sensitive for the presence of hepatic dysfunction and are abnormal within 24-48 hours of onset of hepatic disease. They are not specific as to underlying cause.
3. A value less than 20 umol/l is a good predictor of the ABSENCE of hepatic disease.
4. Bile acids greater than 50 umol/l in horses with prrolizadine toxicosis (Ragwort /Lantana /Echium lycopsis) have been associated with a poor prognosis.

Liver biopsy may be needed to specifically characterize the morphologic process involving the liver and determine the most likely underlying cause and prognosis.
A tru-cut biopsy may be representative if diffuse hepatic disease is present, but may not adequately represent focal or focally variable disease processes.

Evaluation of the Kidney

Tests that are commonly available and useful for evaluation of the kidney include
- Creatinine
• Urea
• Total protein
• Albumin
• Urine protein
• Serum electrolytes
• Urinary creatinine clearance ratios (Urinary fractional excretion of electrolytes)
• Urine GGT
• Urinalysis

Renal biopsy may be needed to determine:
   The morphologic process involving the kidney, the chronicity and severity of the disease, and prognosis.

Elevations in urea and creatinine reflect decreased glomerular filtration rate. This may be associated with pre-renal, renal or post-renal conditions. Evaluation of urine specific gravity and urinalysis with ancillary tests to rule out concurrent disease that may affect the kidney provides the basis for determination of whether or not renal disease is present.

It is estimated that 65-70% of the nephrons must be dysfunctional before elevations of creatinine and urea and decreased urine specific gravity are detected in association with renal disease.

Urea may be low in foals because of low muscle mass; it is often low in horses with liver failure. Some variation in creatinine may occur with breed. Some newborn foals from mares with placentitis may have elevated creatinine but still have normal renal function. Urine specific gravity in foals is usually very low (< 1.005).

**Differential Diagnoses for Polyuria/Polydipsia (PUPD)**

PUPD may be difficult to document in the horse since water intake, frequency of urination and urine volume may be influenced by diet, stabling conditions, environmental temperature and work requirements. It is discussed in this section since renal disease is a concern in many cases with PUPD. A single random urine collection with a specific gravity less than 1.020 suggests decreased concentrating ability. If urine specific gravity is < 1.008, this suggests dilution of urine that may occur because of increased water intake.

Conditions or diseases that should be considered in investigation of possible PUPD include:

<table>
<thead>
<tr>
<th>Condition or Disease</th>
<th>Potential Laboratory Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal Disease</td>
<td>Urea; creatinine; urine specific gravity;</td>
</tr>
<tr>
<td></td>
<td>Urinalysis; urine reatinine clearance ratios</td>
</tr>
<tr>
<td>Psychogenic or primary polydipsia</td>
<td>Exclude other causes of PUPD;</td>
</tr>
<tr>
<td></td>
<td>Gradual water restriction followed by water deprivation test;</td>
</tr>
<tr>
<td></td>
<td>Clinical response to change in environment;</td>
</tr>
<tr>
<td></td>
<td>Response to companion or other attempts to decrease boredom</td>
</tr>
<tr>
<td>Diabetes insipidus</td>
<td>Exclude other causes of PUPD;</td>
</tr>
<tr>
<td></td>
<td>Gradual water restriction followed by water deprivation test;</td>
</tr>
<tr>
<td></td>
<td>Response to exogenous vasopressin administration</td>
</tr>
</tbody>
</table>
Neurogenic (central) and nephrogenic (peripheral) forms have been described in horses.

Diabetes mellitus: May be associated with Cushings’ syndrome (PPID-pituitary adenoma) or as a separate syndrome without Cushings’ Syndrome. Dexamethasone Suppression Test; TRH stimulation test; Serum glucose; Exclude other causes of PUPD.

Sepsis or Endotoxaemia: Usually fever, abdominal pain and weight loss also present; Exclude other causes of PUPD; less common than psychogenic water drinking.

Excessive Salt Eating: Urine creatinine clearance ratios; review history for access to salt and behaviour.

Pituitary Adenoma/ Equine Cushings Disease/ Posterior Pars Intermedia Disease (PPID): Exclude other causes of PUPD; Dexamethasone Suppression Test; TRH Stimulation Test; Serum glucose; Endogenous ACTH Assay (not in Fall/Winter); Insulin Assay.

Protocol s for Dynamic Testing for Equine Cushings Disease are presented in the Endocrine Testing section of these notes.

**Evaluation of Muscle**

Commonly available enzyme analyses that are helpful in evaluation of suspected muscular problems include:
- Asparate Aminotransferase (AS)
- Creatine Kinase (CK)
- Serum electrolytes
- Urine creatinine clearance ratios (some cases)

Urine myoglobin determination may be helpful if urinary discoloration is present and the identification or differentiation from haemoglobinuria is in doubt. If urine is discoloured, evaluation of PCV and serum appearance is helpful in determining whether haemoglobinuria or myoglobinuria is more likely. If haemolysis is present in the serum (discoloured pink/red serum) and there is concurrent anaemia, hemoglobinuria is most likely. If the serum is clear and anaemia is not present, myoglobinuria should be the primary consideration. Myoglobin will spill over into the urine before it causes serum discoloration.

Urine creatinine clearance ratios are not consistently helpful in evaluation muscle problems, but may detect electrolyte imbalances or abnormalities that have been reported to occur in association with Typing Up Syndrome (Azoturia) when serum electrolyte values are within normal limits. Additional tests that may be of benefit in investigation for an underlying cause once a diagnosis of a muscular problem is established include glutathione peroxidise levels (as a reflection of serum Selenium) and Vitamin E assay.

A Submaximal Exercise Test may be helpful in establishing a diagnosis in some individuals, although a normal Submaximal Exercise Test does not rule the possibility of periodic azoturia/tying up.
Protocol for Submaximal Exercise Test

1. Collect pre-exercise serum specimen for CK and AST determinations
   a. Expect CK = within reference interval (Reference intervals may differ slightly amongst laboratories, Ex: 150-300 iu/l)
   b. Expect AST = within reference interval (Reference intervals may differ slightly amongst laboratories, Ex: 250-350 iu/l)

2. Collect post-exercise (regular moderate exercise) specimen for CK and AST determination at 2-4 hours post-exercise.
   a. Should not see more than a doubling of resting CK levels
   b. Should see little or no change in AST

3. Collect serum specimen 24 hours post-exercise for CK and AST determinations.
   a. Should see return of CK to baseline levels
   b. Expect no more than 50% increase in AST
   c. Should see no clinical signs of stiffness or muscle pain

If these criteria are not fulfilled, then the results of the test are abnormal and support a muscle problem.

Evaluation of the Gastrointestinal Tract

Also see Evaluation of the Liver.

There are few clinical chemistry tests specific for the gastrointestinal tract. Intestinal ALP may reflect irritation of the GI tract, but absence of elevations does not rule out low-grade or focal abnormality. False positive results also may occur. Glucose absorption test may be of benefit in investigation of malabsorption of carbohydrates, but may not reflect malabsorption of other categories of nutrients. Faecal parasite examinations and/or cultures and/or PCR can provide diagnostic information if findings are positive, but negative findings do not eliminate these types of disease. Response to therapy may be useful in making a presumptive diagnosis. Although biopsy of the GI tract may be of benefit, it is often difficult to obtain since it requires exploratory laparotomy.

When clinical signs are not specific or are referable to the GI tract clinical chemistry tests do not indicate involvement of other systems, continued investigation of the GI tract is indicated.

Evaluation of the Respiratory System

There may be significant respiratory disease without alterations in either haematology or clinical chemistry tests. Sometimes haematologic data supporting inflammation or infection is the result of involvement of the respiratory system. Fibrinogen levels may be a valuable tool in detection of respiratory tract inflammation. If a site of inflammation/infection is suspected based on haematologic findings or increased fibrinogen, but there are not significant clinical chemistry abnormalities or abnormalities on physical examination, evaluation of respiratory cytology is recommended. Additional evaluation of the GI system or body cavity involvement (peritonitis, pleuritis, pleuropneumonia, internal abscess, haematoma, granuloma or tumour) should be considered.

Evaluation of the Skeletal System
Haematologic and clinical chemistry abnormalities when there is skeletal abnormality are few. Bone growth in young animals or abnormal turnover in adults may be associated with increase in ALP, but these are usually relatively mild. Increases in urine creatinine clearance ratio of phosphorus (urinary fractional excretion of phosphorus) have been reported with metabolic bone disease/developmental orthopaedic disease in foals and young horses and in older horses with a history of shifting lameness or multiple joint involvement. Supplementation with calcium carbonate in order to achieve a balanced Calcium: Phosphorus ratio in the diet is helpful in these cases.

**Evaluation of the Reproductive System**

Haematological and clinical chemistry abnormalities may or may be present with conditions affecting the reproductive system. Usually any abnormalities are mild and nonspecific. Pyometra in the are may be present with haematology that is within normal limits. Clinical signs referable to the reproductive system usually help in localizing the problem. Laboratory testing may be of benefit in helping to ensure that there is not involvement of other systems or multiple concurrent problems.

**Patterns of Serum Proteins**

When a particular pattern in total protein, albumin and globulins is present, it may help determine the most likely underlying cause.

<table>
<thead>
<tr>
<th>Protein Alterations</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated TP with elevated albumin and normal or elevated globulins</td>
<td>• Dehydration</td>
</tr>
<tr>
<td>Elevated TP with normal or decreased albumin and elevated globulins</td>
<td>• Dehydration</td>
</tr>
<tr>
<td></td>
<td>• Dehydration with decreased production or selective loss of albumin –common with chronic liver disease, may see with glomerulonephritis</td>
</tr>
<tr>
<td></td>
<td>• Response to immune stimulation or inflammation. If moderate to marked increase in globulins, consider serum protein electrophoresi for evaluation of possible monoclonal or polyclonal gammopathy or alpha or beta globulin increases suggestive of acute phase reactant protein increases.</td>
</tr>
<tr>
<td>Decreased TP with decreased albumin and decreased globulins</td>
<td>• Typical of protein-losing enteropathy</td>
</tr>
<tr>
<td>Normal or decreased TP with normal albumin and decreased globulins</td>
<td>• Immunosuppression; partial or complete failure of passive transfer of antibodies in the neonate</td>
</tr>
</tbody>
</table>
Serum Protein Electrophoresis may be of benefit in evaluation of cases with elevated globulins. Findings may contribute support for particular diagnoses or other laboraory findings or may provide important information contributing to diagnosis. When monoclonal gammopathy is present, lymphoproliferative disease is the primary concern.

Elevations in alpha globulins and beta globulins are often associated with reactive or inflammatory processes. A post-albumin ‘shoulder’ has been reported in equine cases with chronic hepatic disease. Elevations in beta globulins are typically seen with chronic hepatic disease and are often present with chronic parasitism. Poly clonal gammopathy can be seen with any chronic immune-stimulatory condition (chronic bacterial or viral infection [esp. Equine Infectious Anaemia, EIA], chronic abscessation, skin disease, hepatic insufficiency with exposure to bacteria or endotoxins from the gut). Monoclonal gammopathy is classically seen with multiple myeloma, but may occur with other lymphoid malignancy and, rarely, with infections. Hypogammaglobinaemia may be seen with selective or combined immunodeficiency, immune suppression, immune dysfunction or partial or complete failure of passive transfer of antibodies in the neonate.

Quantitation of various immunoglobulins (IgG, IgM) by radioimmunodiffusion may be of benefit for additional evaluation in cases in which hypogammaglobulinaemia has been identified.

Caution: When evaluating electrophoresis, be sure you understand the orientation! Albumin may be located to the right or the left of the desnitometric tracing, depending on the type of setup that is used. Do not mistake the albumin peak for the gammaglobulin peak (on opposite ends)! Evaluation of electrophoresis should be done at laboratories familiar with the species of interest since peaks and features vary amongst species. Reference to a ‘normal’ electrophoresis pattern may be helpful in determining alterations from normal and in defining alpha, beta and gamma globulin fractions.

**Determination and Interpretation of Fibrinogen Levels**

Elevations in fibrinogen are an accurate reflection of underlying inflammation in the horse. The interpretation of fibrinogen levels relative to total protein is important in determining the significance of fibrinogen that exceeds the upper limit of reference interval. Decreased fibrinogen is most commonly seen with plasma protein loss (haemorrhage, severe exudation with ulceration) or consumptive processes such as localised thrombosis or disseminated intravascular coagulation (DIC).

Fibrinogen determination may be by heat precipitation method, calculated based on clot formation by automated coagulation analysers, calculated from values obtained for plasma and serum viscosity, quantified following thrombin clot induction and drying of the clot, or by specific coagulation factor assay (Factor II) or other methods.

The heat precipitation method is relatively easy to perform in practice. It requires a microhaematocrit centrifuge and refractometer with a TP scale. It is based on the difference in plasma protein readings prior to and after heating the plasma to precipitate fibrinogen. It is done using EDTA or heparinised plasma.

Procedure for Heat Precipitation Fibrinogen:

1. Fill a minimum of 4 microhaematocrit tubes with anticoagulated whole blood and spin in the microhaematocrit centrifuge for 3-5 minutes to provide separation of plasma and cellular layers.
2. Score and break the tube just above the packed cell layer and to isolate the plasma and take a fractometer protein reading of the protein in the plasma.
3. Heat the remaining tubes (minimum 2) in a water bath or heat block at 56 degrees C for 4 minutes.
4. Following heating, spin these tubes in the microhaematocrit centrifuge again. This will result in packing of precipitated fibrinogen in the tube at the top of the cellular layer.
5. Score above the precipitate layer and break the tube to isolate the plasma. Take a refractometer protein reading of the heated plasma.
6. The difference between the pre- and post-heating protein readings is an estimate of the fibrinogen level.

Note: This method is NOT suitable for evaluation of DECREASED fibrinogen.

Example of calculations:
Pre-heating refractometer plasma protein = 70 g/l
Post-heating refractometer plasma protein = 65 g/l
Difference = 5 g/l
Reference interval (2.0 – 4.0 g/l)

Interpretation:
Although 5 g/l is above the reference interval, the most accurate interpretation of fibrinogen levels is when they are considered relative to the total serum or plasma protein.

- A total protein: fibrinogen ratio of > 15 is consistent with normal fibrinogen concentration or dehydration.
- A total protein: fibrinogen ratio of < 10 supports significant fibrinogen elevation.
- A total protein: fibrinogen ratio between 10-15 is a ‘grey zone’ that may or may not indicate an elevation in fibrinogen; inflammation may or may not be present.

Fibrinogen levels < 1.0 g/l are considered decreased. Repeat testing to be sure that fibrinogen has been precipitated and correlation with clinical evaluation and other laboratory tests is needed to determine if this is an accurate result. The accuracy of reading of the refractometer is important since you can only reliably recognize differences of 0.5 – 1.0 g/l (on or between the lines of the refractometer protein scale).

Special Conditions in the Horse Related to Immune Function

There are several conditions in the horse related to immune function that are of special concern.

1. Severe Combined Immunodeficiency (SCID)

A highly fatal inherited disease primary of Arabian foals (also reported in the Appaloosa). Foals delivered to heterozygous parents with have a 25% probability of inheriting the homogzygous recessive (affected) condition, and a 50% probability of being heterozygous carriers. There is a 25% chance of inheritance of a homozygous normal genotype. Mating a heterzygous carrier with anormal homozygous parent results in a 50% chance of carrier status, but will not be affected clinically. This may be important when discussing he problem with owners.

Autosomal recessive gene causes loss of both B and T lymphocytes. Total lack of endogenous humoral or cell mediated responses. Affected foals are also deficient in gamma-interferon which inhibits viral and bacterial and fungal replication, so foals rapidly succumb to overwhelming infections. The complement system is not affected, but does not function normally in the absence of T and B lymphocytes.

Usually born normally and suckle normally. Normal passive transfer with normal health until this falls below protective levels or until ‘foreign’ infection is encountered. Some succumb to overwhelming infections due to
failure of gamma-interferon. Repeated infections and response to antibiotics are common. More severely affected by mild infections (such as adenovirus, influenza virus). Death usually due to viral or bacterial pneumonia and/or enteritis. Death invariably occurs at < 5-6 months of age. Early deaths (2-4 days) usually due to failure of passive transfer of antibodies (FPT).

Diagnosis is based on

- History of dam and sire – breeding lines
- Recurrent infections
- Persistent lymphopaenia (Lymphocyte count that remains < 1.0 x 10^9/l for longer than 48-72 hours). Negative intradermal phytohaemagglutinin test.
- Absence of IgM in foals over 4 weeks of age (using radioimmunodiffusion) – remember that prior to 4 weeks of age, maternal IgM may be significant.
- Generalised lymphoid hyperplasia
- Blood sample from foal at birth show < 0.5 x 10^9 lymphocytes/ l). Beware since low lymphocyte counts also may be found in EHV affected foals.
- Hyperfibrinogenaemia (not diagnostic on its own – seen in many conditions!).
- Remember that alpha and beta globulins may be normal or elevated and that gamma globulins form colostrums are more persistent than IgM.
- Final diagnosis by histologic examination of tissues.

Differential Diagnoses include neonatal septicaemia (often younger), Transient IgM deficiency, Acquired Immunodeficiency Syndrome (adult horses only), Equine Herpesvirus-1-Associated Immunodeficiency Syndrome (susceptibility to infection in first 2 weeks of life after EHV-1 infection of dam and viable birth), bacterial pneumonia (Rhodococcus equi, Strep equi/zooepidemicus), or complex disorder of Fell ponies related to low blood Selenium concentration and immune deficits.

There is no effective treatment. It is a 100% fatal condition and early diagnosis is important (stops waste of money and effort, decreases suffering). Positive diagnosis also condemns the sire and dam as carriers of this condition.

2. Transient Hypogammaglobinaemia

Very rare. Delayed temporary or impaired temporary onset of autologous antibody production. Results in a variable duration of susceptibility to disease – the window between fading passive IgG and production of natural antibodies – may vary between 6 weeks and 6 months of age.

Recurrent infections are common (especially respiratory).

Diagnosis based on decreased immunoglobulins with normal lymphocyte counts and normal responses to vaccinations and normal cellular immunity.

Differential diagnoses include agammaglobulinaemia (no globulins at all) and CID (low lymphocytes counts present).

Antibiotic and disease control treatment may be effective until immunity develops. Plasma transfusions/commercial immunoglobulin transfusions may be helpful.

3. Agammaglobulinaemia
Rare. Thoroughbred, Standardbred and Quarterhorses reported to be affected. Failure of production of immunoglobulins. All classes are low or absent, depending on the status of passive transfer of antibodies. Absence of B-lymphocytes and low/absent responses to immunization. Cellular immunity often in tact. Possible X-chromosome linkage – inherited disease so far only reported in males.

Normal passive transfer of antibodies is expected. Recurrent multi-system, serious infections after passive protection wanes. Death at 2-18 months of age. Normal lymphocyte counts, absence of serum IgM, IgA and low levels of colostrum-derived IgG and IgG (T) which wane over 2-6 weeks. Normal lymphocyte responses to phytohaemoagglutinin test.

Diagnosis based on low or undetectable IgG, IgM, and IgG(T) measured with Radioimmunodiffusion, with normal cellular functions. Maternally derived IgM and IgA fade most rapidly, so these prove the earliest diagnosis.

Differential diagnoses include CID, transient hypogammaglobulinaemia, Specific IgM Deficiency.

Plasma transfusions are used for treatment. None survive past 18 months.

4. Selective IgM Deficiency

Low concentrations of IgM with other globulins within normal limits. Normal cellular responses and normal lymphocytes counts. Possible genetic basis. May occur in older horses with lymphoma.

Severe multiple life-threatening infections (pneumonia and septicaemia primarily) in foals of 6-9 months of age, death before 8-10 months. Failure to thrive and chronic debility. Also detected in horses 3-5 years of age, usually associated with lymphoma or other internal neoplasia. Lymphocytes may have strong suppressor activity and down-regulate B-lymphocyte function and differentiation, as well as decreased IgM synthesis.

Diagnosed by demonstration of low IgM levels. Pre-suckle blood sample with no IgM is suspicious – foals produce their own IgM in utero and milk contains low levels, so for first 3 weeks after suckling the condition may be masked.

Differential diagnoses include SCID, but normal lymphocyte counts and normal cellular responses are present. Other immunoglobulins are within normal limits. Foals with FPT have normal levels of IgM (manufactured by immune system before birth).

One horse has been reported to recover spontaneously (possible Transient Selective IgM Deficiency), but all others died.

5. Fell Pony Immunodeficiency Disorder

This condition is an hereditary failure of cellular immunity, not related to failure of passive transfer of antibodies.

Severe multiple life-threatening infections occur in foals aged 6-9 months. Failure to thrive, chronic debility, severe nonregenerative anaemia are often seen. Concurrent selenium deficiency is a common finding. Often
steatitis is present. Secondary viral infections, including adenovirus (cough and nasal discharge), pancreatitis, intestinal bacaterial or fungal infections often result in diarrhoea.

Foals may have normal/mildly depressed lymphocytes count. Demonstrate low IgM levels. Presuckle blood sample with no IgM is suspicious.

It closely resembles SCID, but normal lymphocyte counts and normal cellular responses are seen. Other immunoglobulins are with normal limits. May be similar to selective IgM Deficiency. Foals with FPT have normal levels of IgM (manufactured by immune system before birth).

Plasma transfusions, antibiotics, Selenium/Vit E supplementation parenterally.

A few cases appear to recover but fail to thrive as normal.

Interpretation of Electrolytes

Electrolytes that are most commonly measured are

- Sodium (Na)
- Potassium (K)
- Chloride (Cl)
- Calcium (Ca)
- Phosphorus (Phos)

Bicarbonate (Total CO2) may be available in some laboratories. An advantage of TCO2 is the ability to calculate and use the Anion Gap (AG). TCO2 measurements may be of limited value when collected at high altitude in an open system (not Vacutainer) but is usually relatively stable in specimens handled for routine chemistry.

Summary of Anion Gap:

- The Anion gap is the difference between the major cations and major anions.
- \( \text{AG} = (\text{Na} + \text{K}) - (\text{Cl} + \text{TCO}_2) \)
- \( \text{AG} \) reference interval = (10-20 mmol/l)

When AG is either decreased or increased, this may indicate alterations in acid-base or electrolyte status that are important for differential diagnoses. Young foals have been reported to have a larger AG than adults and there may be some differences in AG related to aging in horses. But, AG can still be used in conjunction with other tests and evaluations as a diagnostic aid.

### Table 5. Alterations in Anion Gap and Diagnostic Considerations

<table>
<thead>
<tr>
<th>Alteration in Anion Gap</th>
<th>Considerations</th>
</tr>
</thead>
</table>
| Decreased AG            | • Increased cationic protein, such as IgG (may occur with gammopathies)  
                          | • Hypoalbuminaemia |
Hyperchloraemic acidosis
Overhydration
Error in measurement of electrolytes

Increased AG
Metabolic acidosis (esp. With increased lactic acid and/or ketones)
Uraemia
Intoxication or poisoning

Changes in Electrolytes and Diagnostic Considerations

**Hyponatraemia**

Usually loss of sodium-containing fluid. Most common cases are GI loss through reflux, diarrhoea or diuretic therapy. Sometimes may result from renal disease, loss via sweating, or burns or exudative wounds involving a large surface area.

‘Third spacing’ may result in decreased sodium. This is the result of accumulation of sodium-containing fluid within body cavities. A classic example is marked hyponatraemia seen with ruptured urinary bladder in the neonatal foal.

Other considerations for hyponatraemia should include
- Repeated pleural drainage in horses with chronic pleuritis/pleuropneumonia
- Ascites formation due to congestive heart failure
- Chronic hepatic insufficiency or nephritic syndrome

It may occur with chronic renal disease or psychogenic water drinking if accompanied by decreased renal function or absence of access to salt. Hypoadrenocorticism with decreased mineralocorticoid production can result in decreased sodium as a result of urinary loss and may be the result of prolonged exogenous steroid administration resulting in adrenal atrophy and/or chronic ‘stress’ with ‘adrenal exhaustion.’ Increased urinary loss of Na (increased urinary Na creatinine clearance ratio, increased urinary fractional excretion of Na) in the face of low serum Na is diagnostic for decreased aldosterone (mineralocorticoid) production. Potassium may or may not be increased in the horse with hypoaldosteronism.

Artefactual decreases in sodium may occur with hyperlipidaemia with some methods of determination (indirect ISE). This may also occur with marked hyperproteinaemia or hyperglycaemia that result in decreased serum water. In hyperlipidaemic syndrome in ponies or with severe metabolic disturbances there may be true hyponatraemia.

**Hypernatraemia**

Hypernatraemia occurs if there has been a loss of serum water relative to electrolytes. This may occur with reflux or diarrhoea, extensive burns, use of diuretics, renal disease, diabetes insipidus or inadequate water intake (water deprivation, exhaustion and/or reduced thirst mechanism).
**Hypokalaemia**

Decreased potassium may be the result of GI or renal loss. Reflux or diarrhoea, diuretic use or renal disease or total body depletion associated with a combination of decreased intake and increased loss. Marked catecholamine release may result in internal shifts in K resulting in hypokalaemia, as may alkalosis.

Rapid bicarbonate administration may result in hypokalaemia with shifts from the extracellular to intracellular compartment.

**Hyperkalaemia**

Artifactual elevation in K may result from haemolysis or delay in separation of serum from cells/clot, resulting in shifts of intracellular K into the extracellular fluid. Hyperkalaemia is seen with some renal disease, ruptured urinary bladder, urethral obstruction or hypoadrenocorticism. It may occur with metabolic acidosis, diabetes mellitus, severe tissue damage or necrosis.

Most horses, even with severe rhabdomyolysis, do not develop hyperkalaemia. High intensity exercise in the horse may result in elevations of serum K up to 9-10 mmol/l.

A classic cause of hyperkalaemia in the horse in Hyperkalaemic Periodic Paralysis (HYPP) – seen as an inherited disease in horses with Quarter Horse breeding.

**Hypochloraemia**

Decreases in serum Cl may occur in conjunction with and proportional to decreases in sodium, as a result of over-hydration (fluid administration or decreased renal water clearance). Decreases in chloride that do not have a parallel decrease in Na are seen with metabolic alkalosis (exhaustion syndrome with loss of Cl in sweat), or in compensation for respiratory acidosis.

**Hyperchloraemia**

Increases in chloride that parallel an increase in Na are usually the result of dehydration. Increases may occur with some types of metabolic acidosis or as a compensatory response with respiratory alkalosis.

**Hypophosphataemia**

Decreased serum phosphorus levels may occur with respiratory alkalosis, metabolic acidosis, and catecholamine release or insulin treatment. Decreases in phosphorus are typical of chronic renal disease in the horse or reduced GI absorption.

**Hyperphosphataemia**

Increased serum phosphorus levels may occur with decreased glomerular filtration rate, acute renal disease or hypoparathyroidism. If there is a delay in separation of serum from clot/cells, there may be shifts of phosphorus from intracellular to extracellular fluid, resulting in artifactual elevation of serum phosphorus levels.
**Hypocalcaemia**

Decreased serum calcium can be seen with hypomagnesaemia (required for PTH release from parathyroid gland), oxalate toxicity (chelation of cations by compounds in some plants), rapid IV administration of tetracycline (calcium chelation), frusemide diuretic administration or bicarbonate overload.

It is classically associated with cantharadin (blister beetle) toxicity, acute renal failure and severe rhabdomyolysis. Hypocalcaemia may be seen with colitis or severe colic in association with anorexia. It is commonly seen with chronic renal failure.

Hypocalcaemia may be seen in lactating mares (lactational tetany), may occur with exhaustion in endurance horses (often accompanied by diphargamtic flutter ['thumps']). Decreases in calcium may occur with decreased albumin. This generally does not result in decreases in ionised calcium (the physiologically important calcium fraction).

**Hypercalcaemia**

Increased serum calcium may be seen with secondary hyperparathyroidism, usually in conjunction with hyperphosphateamia and low calcium diet (high grain ration with grass hay). However, in many cases of metabolic bone disease, serum calcium levels will be maintained within normal limits.

Hypercalcaemia with hypophosphateamia occurs with primary hyperparathyroidism.

Hypercalcaemia may be seen as a paraneoplastic syndrome, especially with lymphoma or gastric squamous cell carcinoma, but has been reported with other malignancies. Sometimes hypercalcaemia may occur with chronic renal failure, although hypocalcaemia may be more common.
What laboratory tests are likely to be affected by lipaemia, haemolysis or lack of separation of serum/plasma from the clot/ cells?

Tests that are affected and the degree and direction of alteration in the test result may vary with different types of analysers and methods of biochemical analysis. The following table gives guidelines based on the most commonly used instruments and methods.

<table>
<thead>
<tr>
<th>Test</th>
<th>Haemolysis</th>
<th>Lipaemia</th>
<th>Delayed separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count</td>
<td>Decrease</td>
<td>___</td>
<td>May result in haemolysis</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>Relative increase</td>
<td>Increase</td>
<td>May result in haemolysis</td>
</tr>
<tr>
<td>MCHC</td>
<td>Increase</td>
<td>Increase</td>
<td>Not applicable</td>
</tr>
<tr>
<td>MCV</td>
<td>Decrease</td>
<td>--</td>
<td>Specimen aging may result in cellular swelling and increased MCV</td>
</tr>
<tr>
<td>Total protein</td>
<td>Increase</td>
<td>Increase</td>
<td>Decrease possible</td>
</tr>
<tr>
<td>Albumin</td>
<td>Increase</td>
<td>Increase or decrease</td>
<td>Not applicable</td>
</tr>
<tr>
<td>AST, ALT, CK</td>
<td>Increase</td>
<td>Increase possible</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Phos. K</td>
<td>Increase</td>
<td>Decrease or increase possible</td>
<td>Increase</td>
</tr>
</tbody>
</table>
Reference Intervals

Reference intervals may vary with age and breed or horse. Reference intervals can be ‘verified’ for transference to your laboratory by testing 20 healthy individuals of the desired characteristics (age, breed, gender). If 2 or less of the results obtained fall outside the reference intervals of interest, then these reference intervals can be reliably transferred to your laboratory. If 3 or more of the results obtained fall outside the reference intervals of interest, then test another 20 individuals. If 2 or fewer of the results obtained fall outside the reference intervals of interest, then these reference intervals can be transferred to your laboratory. If 3 or more results fall outside of the reference interval, then it is preferable to use the 40 values that you have now obtained to develop your own reference intervals. There are guidelines in the literature for development of reference intervals that can be followed.

If reference intervals are used without such verification, caution is advised until you have used them sufficiently frequently to determine their validity for use with the population seen in your practice.

### Reference Intervals for Neonatal Thoroughbred Foals < 48 hours old

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Mean</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>X 10¹³/l</td>
<td>9.4</td>
<td>6.9-11.8</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>l/l</td>
<td>0.36</td>
<td>0.30-0.44</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>g/dl</td>
<td>13.2</td>
<td>10.2-15.4</td>
</tr>
<tr>
<td>MCV</td>
<td>fl</td>
<td>38.8</td>
<td>31.7-44.9</td>
</tr>
<tr>
<td>MCH</td>
<td>Pg</td>
<td>14.0</td>
<td>11.2-16.4</td>
</tr>
<tr>
<td>MCHC</td>
<td>g/dl</td>
<td>36.1</td>
<td>31.7-39.4</td>
</tr>
<tr>
<td>WBC</td>
<td>X 10⁹/l</td>
<td>8.8</td>
<td>6.2-12.4</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>X 10⁹/l</td>
<td>6.7</td>
<td>4.1-9.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>X 10⁹/l</td>
<td>1.8</td>
<td>1.0-3.1</td>
</tr>
<tr>
<td>Monocytes</td>
<td>X 10⁹/l</td>
<td>0.19</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>X 10⁹/l</td>
<td>0.1</td>
<td>0.1-0.2</td>
</tr>
<tr>
<td>Platelets</td>
<td>X 10⁹/l</td>
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<td>Total Protein</td>
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<td>Albumin</td>
<td>g/l</td>
<td>31</td>
<td>25-35</td>
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<tr>
<td>Globulin</td>
<td>g/l</td>
<td>24</td>
<td>15-36</td>
</tr>
<tr>
<td>AST</td>
<td>IU/l</td>
<td>157</td>
<td>111-206</td>
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<tr>
<td>CK</td>
<td>IU/l</td>
<td>414</td>
<td>165-761</td>
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<td>GGT</td>
<td>IU/l</td>
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<td>GLDH</td>
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<td>25</td>
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</tr>
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<td>IU/l</td>
<td>3,341</td>
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<td>Urea</td>
<td>mmol/l</td>
<td>5.7</td>
<td>2.9-8.4</td>
</tr>
<tr>
<td>Creatinine</td>
<td>umol/l</td>
<td>133</td>
<td>97-188</td>
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<tr>
<td>Glucose</td>
<td>mmol/l</td>
<td>3.1</td>
<td>2.1-4.1</td>
</tr>
<tr>
<td>Tili</td>
<td>umol/l</td>
<td>55</td>
<td>16-94</td>
</tr>
<tr>
<td>Dbili</td>
<td>umol/l</td>
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<td>6-34</td>
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<td>Ibbili</td>
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<td>10-60</td>
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<tr>
<td>Bile acids</td>
<td>umol/l</td>
<td>-</td>
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<td>Calcium</td>
<td>mmol/l</td>
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<td>2.7-3.2</td>
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<td>Phosphorus</td>
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<td>1.6-2.5</td>
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<td>Magnesium</td>
<td>mmol/l</td>
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<td>0.6-1.1</td>
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<td>Sodium</td>
<td>mmol/l</td>
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<td>Potassium</td>
<td>mmol/l</td>
<td>4.3</td>
<td>3.8-5.0</td>
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<tr>
<td>Chloride</td>
<td>mmol/l</td>
<td>97</td>
<td>90-103</td>
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<tr>
<td><strong>Endocrinology</strong></td>
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<tr>
<td>T4</td>
<td>nmol/l</td>
<td>400</td>
<td>&lt;800</td>
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Reference Intervals for Thoroughbred Foals 3-6 weeks old

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Mean</th>
<th>Reference Interval</th>
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<tbody>
<tr>
<td><strong>Haematology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>$10^{12}$/l</td>
<td>10.1</td>
<td>8.8-11.8</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>l/l</td>
<td>0.34</td>
<td>0.30-0.38</td>
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<tr>
<td>Haemoglobin</td>
<td>g/dl</td>
<td>12.4</td>
<td>10.6-13.6</td>
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<tr>
<td>MCV</td>
<td>fl</td>
<td>34.3</td>
<td>30.6-39.3</td>
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<td>MCH</td>
<td>Pg</td>
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<td>MCHC</td>
<td>g/dl</td>
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<td>34.4-38.9</td>
</tr>
<tr>
<td>WBC</td>
<td>$10^9$/l</td>
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<td>6.9-15.2</td>
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<tr>
<td>Neutrophils</td>
<td>$10^9$/l</td>
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<td>4.1-9.1</td>
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<tr>
<td>Lymphocytes</td>
<td>$10^9$/l</td>
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<td>0.9-5.9</td>
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<tr>
<td>Monocytes</td>
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<tr>
<td>Eosinophils</td>
<td>$10^9$/l</td>
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<td>0.1-0.3</td>
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<td>Platelets</td>
<td>$10^9$/l</td>
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<td>Tbil</td>
<td>umol/l</td>
<td>38</td>
<td>22-54</td>
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<tr>
<td>Dbil</td>
<td>umol/l</td>
<td>12</td>
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<td>Ibil</td>
<td>umol/l</td>
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<tr>
<td>Bile acids</td>
<td>umol/l</td>
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### Reference Intervals for Thoroughbred Adult Horses

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<tr>
<td>Selenium</td>
<td>umol/l</td>
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<td>1.6-5.3</td>
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<td>Copper (serum)</td>
<td>umol/l</td>
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<td><strong>Endocrinology</strong></td>
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</tr>
<tr>
<td>T4</td>
<td>nmol/l</td>
<td>22.7</td>
<td>7.7-42.8</td>
</tr>
<tr>
<td>Cortisol</td>
<td>nmol/l</td>
<td>136</td>
<td>71-240</td>
</tr>
<tr>
<td>Fasting Insulin</td>
<td>U/iu/ml</td>
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</tr>
<tr>
<td><strong>Urinary Fractional Excretion of Electrolytes</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ca</td>
<td>%</td>
<td>6.2</td>
<td>2.6-15.5</td>
</tr>
<tr>
<td>Phos</td>
<td>%</td>
<td>0.3</td>
<td>0.02-0.53</td>
</tr>
<tr>
<td>Na</td>
<td>%</td>
<td>0.09</td>
<td>0.02-1.0</td>
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<tr>
<td>K</td>
<td>%</td>
<td>32.8</td>
<td>15-65</td>
</tr>
<tr>
<td>Cl</td>
<td>%</td>
<td>0.72</td>
<td>0.04-1.6</td>
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<tr>
<td>Mg</td>
<td>%</td>
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<td>3.8-21.9</td>
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## Reference Intervals Non-Thoroughbred Adults

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Mean</th>
<th>Reference Interval</th>
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<tr>
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<tr>
<td>RBC</td>
<td>$X \times 10^{12}/l$</td>
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<td>Haematocrit</td>
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<td>Haemoglobin</td>
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<td>MCV</td>
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<td>$Pg$</td>
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<td>MCHC</td>
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<tr>
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<td>4-16</td>
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<td>Ibili</td>
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<td>9-18</td>
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<td>mmol/l</td>
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<td>mmol/l</td>
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<td>Selenium</td>
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<td>Copper (serum)</td>
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<td>14.0-22.0</td>
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<td>Zinc</td>
<td>umol/l</td>
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<td>10.0-15.0</td>
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**INTRODUCTION TO ACID-BASE AND BLOOD-GAS EVALUATION**
(Notes used with permission of Ron Tyler)

**Blood (Plasma) pH, PCO₂, and PO₂ Determination**

Blood pH, PCO₂, and PO₂ values are determined by measuring their values in the plasma portion of anticoagulated whole blood using blood gas instruments that employ ion specific electrode techniques. Exposure of the sample to air results in erroneously high PO₂ values (up to 150 mm of mercury) and low PCO₂, bicarbonate, and TCO₂ values. Optimally, the sample should be assayed immediately, but acceptable blood gas results usually can be obtained for up to an hour if the sample is immersed in an ice bath. Delayed sample analysis can result in erroneously decreased PO₂ due to aerobic metabolism by leukocytes and platelets and erroneously decreased pH due to glycolysis occurring in leukocytes, erythrocytes, and platelets.

**Blood pH**

Normal arterial blood pH for most domestic animals is around 7.4, with a range of about 7.36 to 7.44. Venous blood pH is slightly lower than arterial blood pH. Enzymatic activities and chemical reactions and interactions change markedly when blood pH departs significantly from normal. Fortunately, there are buffer systems and adaptive mechanisms which dampen changes in blood pH.

**Electrolyte/Bicarbonate Interactions**

Electroneutrality demands that the sum of positive charges and the sum of negative charges be equal. Therefore, loss of a positive charge (a cation, e.g., sodium) must be accompanied by concurrent loss of a negative charge (an anion, e.g., chloride or bicarbonate) or by the simultaneous gain of another positive charge (e.g., potassium or hydrogen).

**Chloride**

Chloride is the major anion of plasma. To maintain electroneutrality, any increase or decrease in sodium concentration must be accompanied by an equimolar increase or decrease in chloride concentration. If sodium increases and chloride does not increase, another anion must enter the plasma to maintain electroneutrality. If the anion that increases in the place of chloride is bicarbonate, a metabolic alkalosis develops. If the anion that increases in the place of chloride is not bicarbonate, the anion gap (discussed below) increases and a titrational metabolic acidosis develops.

**Plasma Chloride Loss**

Net loss of chloride from the body can occur due to excessive loss of chloride containing fluids and/or excessive loss of chloride from the kidney without an adequate increase in chloride intake. Excessive loss of chloride can occur as a result of gastric loss (vomition) or sequestration (e.g., displaced abomasum, gastric torsion), sweat loss (e.g., endurance racing), salivary loss (e.g., esophageal obstruction in horses but not cattle), or renal loss. Excessive renal chloride loss is caused by increased plasma aldosterone concentration or an aldosterone agonist.
Paradoxical Aciduria

In order to maintain electroneutrality, a net loss of chloride must be accompanied by a net decrease in sodium and/or a net increase in other anions to replace the lost chloride anion. With conditions such as gastric vomition, chloride loss initially is accompanied by a decrease in sodium. However, only a slight decrease in sodium occurs before sodium receptors and baroreceptors react to the drop in sodium concentration and blood pressure which results from the sodium loss. As a result, aldosterone is released and stimulates avid renal sodium reabsorption. Initially, exchange of sodium for potassium is dominant and plasma bicarbonate concentration does not significantly change. However, when hypokalemia develops due to urinary potassium loss, renal tubular epithelial cells produce hydrogen and bicarbonate from carbon dioxide and water and renal tubular fluid sodium is exchanged across the epithelial cell luminal membrane for the hydrogen then transported into the blood with the bicarbonate, rather than chloride. Thus, exchange of tubular fluid sodium for hydrogen results in a disproportionate drop in plasma chloride concentration compared to sodium concentration with a simultaneous increase in plasma bicarbonate concentration. The increase in plasma bicarbonate concentration causes alkalemia (high blood pH) or exacerbates existing alkalemia while the passage of hydrogen into renal tubular fluid in exchange for sodium produces acid urine, i.e., paradoxical aciduria. Correction of hypokalemia usually is necessary to alleviate paradoxical aciduria.

Bicarbonate

Bicarbonate is the second most common anion of plasma. Regulation of its concentration is the means by which metabolic adaption to respiratory alkalosis and acidosis is achieved by the kidneys. Both bicarbonate and the hydrogen proton are generated from carbon dioxide and water. This reaction is accelerated by the carbonic anhydrase system. As long as sodium and chloride are plentiful, plasma bicarbonate and hydrogen proton concentration can be controlled by the kidneys and the lungs. When sodium or chloride are deficient, however, a conflict between the systems controlling blood pressure, plasma sodium concentration, and plasma effective osmolality and the systems controlling blood pH develops. Paradoxical aciduria, discussed above, is an example of this conflict.

Anion Gap

The anion gap is calculated by subtracting the milliequivalent concentration of the major anions of plasma (chloride and bicarbonate) from the milliequivalent concentration of the major cations of plasma (sodium and potassium). Physiologic adaptive processes change bicarbonate and chloride concentrations but do not affect the concentration of unmeasured anions. Therefore, changes in the anion gap indicate a pathologic process. Increases in the anion gap are usually due to titrational metabolic acidosis, but, occasionally, can result from increased negatively charged proteins (hyperalbuminemia and hyperbetaglobulinemia), decreased positively charged proteins (hypogammaglobulinemia), or decreased divalent plasma cations (hypocalcemia, hypomagnesemia). Decreases in the anion gap can result from decreased negative charges, e.g., hypoalbuminemia, or increased positive charges, e.g., hypercalcemia and hypergammaglobulinemia.

Buffer Systems

The major buffer systems of the blood are the bicarbonate, plasma protein, and phosphate buffer systems. Decreases in plasma proteins and/or hemoglobin can result in decreased buffering capacities of these systems and, hence, more pronounced changes in plasma pH. Although important in buffering changes in blood pH, the plasma protein and phosphate buffer systems are relatively stable and do not appreciably adapt (increase their buffering capacity) to changes in blood pH. The bicarbonate buffer system is highly reactive and is responsible for physiologic adaption to changes in blood pH.
Pathogenesis of Respiratory Acid/Base Imbalances

Normally, the lungs function to take up oxygen and to expel carbon dioxide. They do this by allowing blood oxygen and carbon dioxide concentrations to equilibrate with alveolar oxygen and carbon dioxide concentrations. Equilibration of blood carbon dioxide with alveolar carbon dioxide occurs much more rapidly (about 20 times more rapidly) than equilibration of blood oxygen with alveolar oxygen. To maintain normal blood oxygen and carbon dioxide concentrations of about 90 and 40 torr, respectively, at least three conditions must be present: (1) proper circulation and perfusion, i.e., shunting must not occur; (2) diffusion of oxygen and carbon dioxide must not be impeded; and (3) alveolar oxygen and carbon dioxide concentrations must be about 90 torr and 40 torr, respectively. Changes in any of these conditions can, and usually does, result in a respiratory acid/base imbalance.

Respiratory Alkalosis

Respiratory alkalosis develops when a primary respiratory problem results in too much carbon dioxide being blown off. Normally, alveolar carbon dioxide concentration is around 40 torr and is in equilibrium with pulmonary capillary blood carbon dioxide, giving arterial blood a normal carbon dioxide concentration of about 40 torr. If alveolar carbon dioxide concentration decreases, blood carbon dioxide concentration decreases, producing respiratory alkalosis. Except in artificial situations, such as gas anesthesia, decreased alveolar carbon dioxide concentration is always due to increased ventilation (hyperventilation). Hyperventilation and the resulting respiratory alkalosis can be caused by intrathoracic or extrathoracic problems.

Intrathoracic respiratory alkaloses are caused by increased ventilation in response to hypoxemia resulting from decreased pulmonary perfusion or impeded oxygen diffusion (ventilation/perfusion imbalance or mismatch) and can be further investigated by evaluation of the thoracic cavity (auscultation, radiographs, etc.).

Extrathoracic respiratory alkaloses are caused by increased ventilation either in response to tissue hypoxia resulting from conditions such as anemia or in response to central nervous system stimulation unrelated to hypoxemia, e.g., fear, pain, head trauma, etc. Extrathoracic respiratory alkaloses generally are further investigated by evaluation of the patient's central nervous system, emotional state, and/or hemoglobin concentration and quality.

Intrathoracic respiratory alkaloses can be differentiated from extrathoracic respiratory alkaloses by evaluation of arterial blood oxygen concentration. Intrathoracic respiratory alkaloses result from a decrease in blood oxygen concentration and, therefore, are always associated with low PaO2; whereas, extrathoracic respiratory alkaloses are associated with normal or increased PaO2. Anemia, which can cause extrathoracic respiratory alkalosis, causes normal to increased PaO2 rather than decreased PaO2 because PaO2 is measured in the plasma and thus represents only the PaO2 of the plasma not the actual blood oxygen content.

Intrathoracic Respiratory Alkalosis (Ventilation-Perfusion Imbalance or Mismatch)

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because normal alveolar oxygen concentration is very close to that of ambient air. As alveolar carbon dioxide concentration decreases and equilibration of blood and alveolar carbon dioxide concentration continues, \( \text{PaCO}_2 \) decreases and respiratory alkalosis develops.

Also, when perfusion of the lungs is impaired, intrathoracic respiratory alkalosis develops unless perfusion is severely impaired, in which case, respiratory acidosis develops. Decreased pulmonary perfusion results in decreased contact between pulmonary blood and the capillary/alveolar barrier and, hence, decreased carbon dioxide and oxygen equilibration. As a result, carbon dioxide (because of its more rapid equilibration) completely equilibrates, but oxygen does not fully equilibrate causing decreased \( \text{PaO}_2 \) and subsequently respiratory alkalosis, as described above for impeded oxygen and carbon dioxide perfusion.

**Extrathoracic Respiratory Alkaloses (Hyperventilation)**

Extrathoracic respiratory alkaloses occur as a result of hyperventilation caused by central nervous system lesions, emotional stress, or decreased oxygen carrying capacity of the blood and are associated with normal or slightly increased arterial blood oxygen concentrations. It is easy to understand how hyperventilation resulting in decreased arterial blood carbon dioxide concentration and normal or increased arterial blood oxygen concentration can result from central nervous system and emotional conditions. However, development of decreased arterial blood carbon dioxide concentration with normal or increased arterial blood oxygen concentration caused by decreased blood oxygen carrying capacity may, at first, be confusing. In blood most oxygen is carried bound to hemoglobin. Oxygen bound to hemoglobin is in equilibrium with oxygen in plasma and provides a reservoir of oxygen. As oxygen diffuses from plasma into tissues, plasma oxygen is replenished from the oxygen reservoir provided by hemoglobin bound oxygen. Decreased hemoglobin concentration (classic anemia) or decreased hemoglobin oxygen carrying capacity (cyanide toxicity, nitrate toxicity, carbon monoxide toxicity) decreases the hemoglobin bound oxygen reservoir. As a result plasma and functional hemoglobin are normally saturated, or slightly supersaturated, with oxygen when the blood leaves the lungs. However, plasma and hemoglobin are quickly depleted of oxygen (\( \text{PaO}_2 \) decreases) after blood enters the tissues because the hemoglobin bound oxygen reservoir is low. The decrease in \( \text{PaO}_2 \) stimulates oxygen sensitive receptors to increase respiratory rate and depth, increasing alveolar ventilation and inducing respiratory alkalosis. Because hyperventilation does not greatly increase the alveolar oxygen concentration, but does markedly decrease the alveolar carbon dioxide concentration, an extrathoracic respiratory alkalosis develops while increased oxygen delivery to tissues is minimal.

**Respiratory Acidoses**

Although respiratory acidoses can be caused by extrathoracic or intrathoracic problems, these two general categories cannot be differentiated by acid/base analysis. However, thorough physical examination is very helpful. Intrathoracic problems must be extremely severe to cause respiratory acidosis and thorough evaluation of the thoracic cavity by auscultation and percussion usually reveal abnormalities. Causes of intrathoracic respiratory acidoses include emphysema, tracheal obstructions including tracheal collapse, and the severe stage of conditions that cause intrathoracic respiratory alkalosis.

Extrathoracic causes of respiratory acidoses include central nervous system disease, myasthenia gravis, and extrathoracic airway obstruction (including tracheal collapse).

**Metabolic Acid/Base Imbalances**

Metabolic acid/base imbalances occur when a pathologic process decreases (metabolic acidosis) or increases (metabolic alkalosis) plasma bicarbonate. Determining the general cause of metabolic acid/base imbalances can help achieve a diagnosis and determine proper therapy.

**Metabolic Acidoses**

Metabolic acidoses are subclassified as secretional (hyperchloremic or normal anion gap) metabolic acidoses and titrational (normochloremic or increased anion gap) by evaluation of the anion gap. Secretional metabolic acidoses result from excessive bicarbonate loss; whereas, titrational metabolic acidoses result from the utilization of bicarbonate in
titrating (buffering) an excessive amount of acids entering the blood. Determining which of these processes is occurring or if both processes are occurring helps establish and refine the list of possible causes of the patient's illness.

**Secretional Metabolic Acidoses**

Secretional metabolic acidoses occur as a result of excessive bicarbonate loss from the body. Excessive amounts of bicarbonate can be lost in urine, gastrointestinal fluids (pancreatic juice), and saliva (ruminants only). The cellular processes which provide the bicarbonate for the fluid being lost also provide hydrogen protons to the blood. Normally, the bicarbonate in the fluid is reabsorbed from the fluid and there is no net increase in bicarbonate loss. However, during certain diseases, bicarbonate is not reabsorbed from the fluid, resulting in excessive bicarbonate loss and an increase in the hydrogen concentration in the blood. Hence, a secretional metabolic acidosis develops. In order to maintain electroneutrality, the decrease in plasma bicarbonate concentration must be accompanied by either a concurrent increase in the plasma concentration of another anion, or a concurrent decrease in plasma sodium concentration. Several physiologic systems, discussed earlier, protect the plasma sodium concentration and prevent it from decreasing dramatically concurrent with the decrease in plasma bicarbonate concentration. Therefore, in order to maintain electroneutrality, the plasma concentration of another anion must increase. Chloride is the only anion of significant concentration in plasma that can be physiologically controlled without causing further acid/base imbalances. At sites of bicarbonate formation and secretion, chloride is transported with H+ into blood while HCO₃⁻ is secreted into the fluid produced (e.g., saliva, pancreatic juice) with Na⁺ or K⁺. As a result, plasma chloride concentration increases concurrent with and equal to the decrease in plasma bicarbonate concentration. Secretional metabolic acidosis plasma chloride concentration increases to offset the decrease in bicarbonate concentration. In some cases of secretional metabolic acidosis plasma sodium concentration is low and plasma chloride concentration is not increased. As a result, depending on plasma/serum chloride concentration to determine the type of metabolic acidosis can cause misdiagnosis. Secretional metabolic acidosis have also been called normal anion gap acidoses because the anion gap does not change since the decrease in bicarbonate anions is accompanied by an equal increase in chloride anions. The term secretional metabolic acidosis more accurately reflects the pathophysiologic process which is occurring.

Administration of chloride containing acid (HCL) or administration of large amounts of sodium chloride (dilutional acidosis) can cause hyperchloremic metabolic acidosis that is not of secretional origin. However, these iatrogenic metabolic acidoses should be easily recognized.

**Titrational Metabolic Acidoses**

Titrational metabolic acidosis occur as a result of bicarbonate utilization by titration (buffering) of excessive amounts of acids entering the blood. An acid is an anion which is weakly associated with a hydrogen proton. When an acid enters the blood, the sodium from sodium bicarbonate associates with the anion and the bicarbonate associates with the hydrogen proton. This results in the sodium concentration remaining stable, but an increase of one mEq of unmeasured anion (hence, an increase of one in the anion gap) for every mEq of bicarbonate utilized in buffering the acid. Therefore, titrational metabolic acidosis increase the anion gap, but tend not to change the plasma chloride concentration. As a result, titrational metabolic acidosis have also been called "euchloremic or normochloremic metabolic acidoses" and "increased anion gap metabolic acidoses". The term "titrational metabolic acidosis" more accurately reflects the pathophysiologic process which is occurring.

The source of the acid causing titrational metabolic acidosis may be endogenous or exogenous. Conditions that cause endogenous production of acids and result in a titrational metabolic acidosis include shock (lactic acid, pyruvic acid, and others), extreme anaerobic metabolism (lactic acid, pyruvic acid, and others), grain overload in ruminants (lactic acid and others) (arguably, this could be considered exogenous production), ketoacidotic stage of diabetes mellitus (keto acids), starvation (keto acids), and late renal failure (phosphates, sulfates, and others).

Historical information, physical examination, and, when necessary, serum lactic acid analysis can help determine if titrational metabolic acidosis is due to shock, extreme anaerobic metabolism, or grain overload. Patients in the ketoacidotic stage of diabetes mellitus should have marked hyperglycemia and ketonuria; whereas, Patients with ketonemia due to starvation should have a normal or low blood glucose with ketonuria.
Early renal disease causes excess bicarbonate loss, producing secretional metabolic acidosis; whereas, late-stage renal disease (when BUN, creatinine, phosphorus, etc. concentrations are increased) causes titrational metabolic acidosis in addition to the secretional metabolic acidosis (mixed metabolic acidosis). Therefore, if titrational metabolic acidosis is due to renal disease, the BUN, creatinine, and phosphorus concentrations should also be increased. Decrease in plasma HCO$_3^-$ concentration is in excess of the increase in anion gap, indicates concurrent secretional metabolic acidosis.

If a likely cause of endogenous acid production is not found, an exogenous source of acid should be suspected. There are many exogenous sources of acids that can cause titrational metabolic acidosis. Ethylene glycol toxicity and cantharidin (blister beetle) poisoning are renowned as causes of titrational metabolic acidosis. If a patient does not have a titrational metabolic acidosis, these diseases can be ruled out.

**Metabolic Alkaloses**

Metabolic alkaloses can be subclassified as chloride responsive or chloride resistant by evaluating urine chloride concentration. Chloride responsive metabolic alkaloses result from excess chloride loss in gastric fluid, sweat, or saliva (in the horse) and results in an extremely low urine chloride concentration (less than 10 mEq/L). Chloride resistant metabolic alkaloses result from excess plasma concentrations of aldosterone or aldosterone agonists, which cause excess renal chloride excretion producing high urine chloride concentration (greater than 20 mEq/L). Treatment of chloride responsive metabolic alkalosis is aimed at finding the source of chloride loss and providing an adequate source of chloride; whereas, treatment of chloride resistant metabolic alkalosis is aimed at finding the source of excess aldosterone or the aldosterone agonist.

Paradoxical aciduria may develop with chloride responsive metabolic alkalosis. Often, when paradoxical aciduria develops, the potassium deficiency must be corrected for complete correction of the acid/base imbalance.

**Mixed Acid/Base Imbalances**

Sometimes acid/base problems occur in the respiratory and metabolic systems simultaneously, metabolic acidotic processes occur simultaneous with metabolic alkalotic processes, or secretory and titrational metabolic acidotic processes occur simultaneously. These conditions are called mixed or complex acid/base imbalances. They are identified by recognizing that appropriate adaption to an acid/base imbalance has not occurred (concurrent metabolic and respiratory problems), the anion gap is increased concurrent with an increase in the plasma bicarbonate concentration (titrational metabolic acidosis with concurrent metabolic alkalosis or respiratory acidosis), or the decrease in plasma bicarbonate concentration is in excess of the increase in anion gap (concurrent titrational and secretional metabolic acidosis or concurrent titrational metabolic acidosis with respiratory alkalosis and metabolic adaption).

**Physiologic Adaption**

Physiologic adaption is classified as respiratory or metabolic (nonrespiratory). The lungs, of course, are the site of respiratory adaption and act by increasing (retaining) or decreasing (blowing off) blood carbon dioxide. The kidneys are the major site of metabolic adaption and act by increasing or decreasing blood bicarbonate concentration.

**Respiratory Adaption**

When metabolic acidosis (decreased blood bicarbonate concentration) occurs, receptors in the brainstem increase the rate and depth of respiration. As a result, more carbon dioxide is blown off and blood carbon dioxide concentration decreases, dampening the decrease in blood pH. Generally respiratory adaption occurs as fast, or faster, than metabolic acidosis can develop. Therefore, respiratory adaption to metabolic acidosis is considered immediate. Table 1 gives formulas for predicting respiratory and metabolic adaption.

When metabolic alkalosis causes an increase in blood bicarbonate concentration, receptors in the brainstem are stimulated to decrease respiratory rate and depth. As a result, carbon dioxide is retained and blood carbon dioxide concentration increases, dampening the increase in blood pH. The decrease in respiratory rate and depth also decreases blood oxygen concentration. When the blood oxygen concentration decreases to about 60 torr, hypoxemia stimulates brainstem oxygen
receptors to increase the respiratory rate and depth. The conflict between brainstem receptors responding to increased blood pH and those responding to hypoxemia results in the respiratory adaption to a metabolic alkalosis being less effective and less predictable than the respiratory adaption to a metabolic acidosis.

**Metabolic Adaption**

When respiratory acidosis (increased blood carbon dioxide concentration) occurs, the kidneys increase their production and reabsorption of bicarbonate and excretion of hydrogen. As a result, plasma bicarbonate concentration increases, dampening the decrease in blood pH. When respiratory alkalosis (decreased blood carbon dioxide concentration) occurs, the kidneys increase their rate of hydrogen production and reabsorption and bicarbonate excretion, decreasing plasma bicarbonate concentration and dampening the increase in blood pH. This is the major mechanism of metabolic adaption to respiratory acidosis/alkalosis. Complete metabolic adaption may require up to 72 hours. Therefore, separate formulas are used to predict appropriate metabolic adaption to acute and chronic (2 days or greater) respiratory acidoses/alkalosis. Clinical judgment must be used in determining which formula is appropriate. Table 1 gives formulas for predicting respiratory and metabolic adaption.

**Interpretation of Acid/Base, and Blood Gas Results**

Reliable interpretation of acid/base and blood gas results begins with evaluation of the pH. If the patient's blood pH is decreased, the patient is acidemic and its acid/base and electrolyte analysis should be interpreted as indicated in the "acidemia" section. If the blood pH is within the normal range, the patient's acid/base and electrolyte analysis should be interpreted as indicated in the "normal pH" section. And, if the pH is increased, the patient is alkalemic and its acid/base and electrolyte analysis should be interpreted as indicated in the "alkalemia" section.

**Acidemia**

If the pH is decreased (acidemia), there must be a primary derangement causing an acidosis in the metabolic system, the respiratory system, or both. To establish which system the primary derangement is in or if the patient has a primary derangement in both systems, the bicarbonate concentration and PCO₂ are evaluated to determine which is shifted in the acid direction.

**Decreased pH and Decreased Bicarbonate**

These results indicate primary metabolic acidosis. To determine if the patient has a concurrent derangement in the respiratory system, the PCO₂ is evaluated next.

**Decreased pH, Decreased Bicarbonate, and Increased PCO₂**

These results indicate concurrent primary metabolic and respiratory acids.

**Decreased pH, Decreased Bicarbonate with the PCO₂ in the Normal Reference Range**

These results usually indicate metabolic acidosis with concurrent respiratory acidosis. The formula (Table 1) for predicting respiratory adaption to metabolic acidosis can be used to ascertain that the patient has a respiratory acidosis. If the measured PCO₂ matches the PCO₂ predicted by the formula, the respiratory system has appropriately adapted and the patient has only a metabolic acidosis. On the other hand, if the measured PCO₂ is greater than the PCO₂ predicted by the formula, the patient has a primary derangement in the respiratory system which is causing a respiratory acidosis concurrent with the metabolic acidosis.

**Decreased pH, Decreased Bicarbonate, and Decreased PCO₂**

These results indicate metabolic acidosis. To determine whether the respiratory system has appropriately adapted or whether the patient has a concurrent derangement in the respiratory system causing either a respiratory acidosis or a respiratory alkalosis, the formula in Table 1 for respiratory adaption to metabolic acidosis should be applied. If the
measured PCO₂ matches the PCO₂ predicted by the formula, the respiratory system has appropriately adapted and the patient has only a metabolic acidosis. However, if the measured PCO₂ is greater than the PCO₂ predicted by the formula, the patient has a primary acid/base derangement in the respiratory system causing respiratory acidosis concurrent with the metabolic acidosis. On the other hand, if the measured PCO₂ is less than the PCO₂ predicted by the formula, the patient has a primary acid/base derangement in the respiratory system causing respiratory alkalosis concurrent with the metabolic acidosis.

**Decreased pH and Bicarbonate Not Decreased**

If the bicarbonate concentration is not decreased, the PCO₂ must be increased for the patient to be acidemic. In this situation, the patient must have a primary acid/base derangement in the respiratory system causing a respiratory acidosis. To determine if the metabolic system has appropriately adapted or if the patient has a concurrent acid/base derangement in the metabolic system causing either a metabolic acidosis or a metabolic alkalosis, the bicarbonate must be evaluated.

**Decreased pH, Normal Bicarbonate, and Increased PCO₂**

These results generally indicate respiratory acidosis with a concurrent derangement in the metabolic system causing metabolic acidosis. To ascertain that the patient has a concurrent metabolic acidosis and not appropriate metabolic adaption, the formula in Table 1 for metabolic adaption to respiratory acidosis can be applied. If the measured bicarbonate concentration matches the bicarbonate concentration predicted by the formula, the metabolic system has appropriately adapted to the respiratory acidosis. However, if the measured bicarbonate concentration is less than the bicarbonate concentration predicted by the formula, the patient has a primary acid/base derangement in the metabolic system causing metabolic acidosis concurrent with the respiratory acidosis.

**Decreased pH, Increased Bicarbonate, and Increased PCO₂**

These results indicate respiratory acidosis. To determine if the metabolic system has appropriately adapted or if the patient has a concurrent acid/base imbalance in the metabolic system causing either a metabolic acidosis or a metabolic alkalosis, the formula in Table 1 for metabolic adaption to respiratory acidosis should be applied. If the measured bicarbonate concentration matches the bicarbonate concentration predicted by the formula, the patient does not have an acid/base imbalance in the metabolic system, i.e., the patient has a respiratory acidosis with appropriate metabolic adaption. However, if the measured bicarbonate concentration is greater than the bicarbonate concentration predicted by the formula, the patient has a primary acid/base imbalance in the metabolic system causing a metabolic alkalosis concurrent with the respiratory acidosis. On the other hand, if the measured bicarbonate concentration is less than the bicarbonate concentration predicted by the formula, the patient has a primary acid/base derangement in the metabolic system causing metabolic acidosis concurrent with the respiratory acidosis.

**Normal pH**

If the pH is normal and the bicarbonate concentration and PCO₂ are normal, there is no acid/base imbalance. However, if the pH is normal but the bicarbonate concentration and PCO₂ are abnormal, the patient has an acid/base imbalance even if the pH is normal. Normal physiologic adaption does not return the pH to normal. Therefore, if the pH is normal, but bicarbonate concentration and PCO₂ are markedly abnormal, there are primary derangements, acting in opposite directions, in both systems.

If the pH is normal and the bicarbonate concentration and PCO₂ are only slightly abnormal, the patient may have an acid/base imbalance in both systems or only in one system. When the bicarbonate concentration and PCO₂ are only slightly abnormal, acid/base analysis does not reliably establish whether only one system or both systems are involved or which system is involved.
Alkaelemia

After establishing that the patient's blood pH is increased, next evaluate the plasma bicarbonate concentration and PCO2 to determine which is shifted in the alkalosis direction, i.e., increased bicarbonate concentration or decreased PCO2.

**Increased pH and Increased Bicarbonate**

If the pH is increased and the bicarbonate concentration is increased, the patient has at least an alkalosis in the metabolic system. To determine if there is a concurrent acid/base derangement in the respiratory system the PCO2 is evaluated.

**Increased pH, Increased Bicarbonate, and Decreased PCO2**

These results indicate a mixed respiratory/metabolic alkalosis. That is, a primary derangement in the respiratory system causing an alkalosis and a primary derangement in the metabolic system causing an alkalosis.

**Increased pH, Increased Bicarbonate with PCO2 in Normal Reference Range**

If the bicarbonate concentration is increased and the PCO2 is in the reference range for normal animals, the patient has a mixed metabolic/respiratory alkalosis. The respiratory system, if normal, will adapt to the metabolic alkalosis by increasing PCO2 and dampening the increase in pH. Therefore, failure of the PCO2 to increase indicates that the PCO2 is lower than it should be for appropriate adaption and, hence, the patient has a respiratory alkalosis concurrent with a metabolic alkalosis.

**Increased pH, Increased Bicarbonate, and Increased PCO2**

If the bicarbonate concentration and the PCO2 are increased, the patient has at least a metabolic alkalosis. A reliable formula for evaluating respiratory adaption to metabolic alkalosis is not available. Therefore, if the plasma PCO2 of an alkaelemic patient with a metabolic alkalosis is increased, the respiratory system is assumed to have adapted appropriately unless there is evidence to the contrary.

**Increased pH and BicarbonateNot Increased**

If bicarbonate concentration is not increased, the PCO2 concentration must be decreased in order for the patient to be alkaelemic. In this situation, the decrease in PCO2 establishes that the patient has a respiratory alkalosis. The respiratory alkalosis should be further differentiated into intrathoracic or extrathoracic respiratory alkalosis as indicated in the respiratory alkalosis section.

Knowing that the patient has a respiratory alkalosis does not establish whether the metabolic system has appropriately adapted or if the patient has a concurrent metabolic alkalosis or acidosis. To determine if there is a metabolic abnormality, the bicarbonate concentration must again be reviewed.

**Increased pH, Decreased PCO2 with Bicarbonate Concentration in the Normal Reference Range**

These results indicate respiratory alkalosis and concurrent metabolic alkalosis. To insure that a concurrent metabolic alkalosis is present, the formula for respiratory alkalosis in Table 1 should be applied. If the measured bicarbonate concentration matches the bicarbonate concentration predicted by the formula, the metabolic system has appropriately adapted, i.e., the patient has a respiratory alkalosis with appropriate metabolic adaption. However, if the measured bicarbonate concentration is greater than the bicarbonate concentration predicted using the formula, the patient has a primary derangement in the respiratory system causing a respiratory alkalosis and a primary derangement in the metabolic system causing a metabolic alkalosis, i.e., the patient has a respiratory alkalosis with concurrent metabolic alkalosis. The metabolic alkalosis should be further evaluated as discussed in the metabolic alkalosis section below.
Increased pH, Decreased Bicarbonate, and Decreased PCO₂

During alkalemia, if the PCO₂ is decreased and the bicarbonate concentration is also decreased, the patient has a respiratory alkalosis. To determine if the patient's metabolic system has adapted appropriately or if the patient has a concurrent primary derangement in its metabolic system causing either a metabolic alkalosis or acidosis, the formula for respiratory alkalosis can be applied.

If the measured bicarbonate concentration matches the bicarbonate concentration predicted by the formula, the patient has only a primary derangement in the respiratory system causing a respiratory alkalosis. The patient does not have a primary derangement in the metabolic system; instead, the patient's metabolic system has appropriately adapted to the respiratory alkalosis.

On the other hand, if the measured bicarbonate concentration does not match the bicarbonate concentration predicted by the formula for respiratory alkalosis, the patient has a primary derangement in the respiratory system causing a respiratory alkalosis and a primary derangement in the metabolic system. If the measured bicarbonate concentration is higher than the predicted bicarbonate concentration, the patient has a respiratory alkalosis with concurrent metabolic alkalosis; whereas, if the measured bicarbonate concentration is lower than the predicted bicarbonate concentration, the patient has a respiratory alkalosis with a concurrent metabolic acidosis.

When respiratory alkaloses, metabolic alkaloses, and/or metabolic acidoses are recognized they should be further evaluated as discussed below.

Evaluation of Metabolic Acidoses

The anion gap can be used to differentiate titrational metabolic acidoses from secretional metabolic acidoses. An increase in the anion gap indicates titrational metabolic acidosis regardless of the blood pH, the PCO₂, or the bicarbonate concentration. If the anion gap is not increased, the metabolic acidosis is secretional. Administration of chloride containing acid (HCL) or administration of large amounts of sodium chloride (dilutional acidosis) can cause hyperchloremic metabolic acidosis that is not of secretional origin. However, these iatrogenic metabolic acidoses should be easily recognized.

In some conditions, secretional metabolic acidosis and titrational metabolic acidosis occur concurrently. In this situation, the anion gap will be increased but not to the extent that the bicarbonate concentration is decreased.

Evaluation of Metabolic Alkaloses

The urinary chloride concentration is used to differentiate chloride responsive metabolic alkaloses from chloride resistant metabolic alkaloses. Chloride responsive metabolic alkaloses are caused by conditions which result in excessive loss of chloride from the body in gastric fluid, sweat, and saliva (horses). As a result, chloride responsive metabolic alkaloses are associated with low urinary chloride (less than 10 mEq/L). Whereas, chloride resistant metabolic alkaloses result from excessive concentrations of aldosterone or aldosterone antagonists and subsequent excessive loss of chloride by the kidneys. Therefore, chloride resistant metabolic alkaloses are associated with high urinary chloride (greater than 20 mEq/L).

Evaluation of Respiratory Alkaloses

Arterial blood PO₂ can be used to differentiate intrathoracic respiratory alkaloses from extrathoracic respiratory alkaloses. Intrathoracic respiratory alkaloses are associated with decreased PO₂; whereas, extrathoracic respiratory alkaloses are associated with a normal or increased PO₂.
Formulas for calculating normal physiologic adaption

A. **Acute Respiratory Acidosis**

A 10 torr increase in PCO₂ causes an increase of 1 mEq/L HCO⁻₃

B. **Acute Respiratory Alkalosis**

A 10 torr decrease in PCO₂ causes a decrease of 2 mEq/L HCO⁻₃

C. **Chronic Respiratory Acidosis**

A 10 torr increase in PCO₂ causes an increase of 4 mEq/L HCO⁻₃

D. **Chronic Respiratory Alkalosis**

A 10 torr decrease in PCO₂ causes a decrease of 5 to 6 mEq/L HCO⁻₃

E. **Metabolic Acidosis**

\[ \text{PCO}_2 = 1.54 \times (\text{HCO}_3) + 8.4 \pm 1.1 \]

F. **Metabolic Alkalosis**

Gives a variable response. No reliable formula is available.

**CONCLUDING THOUGHTS**

I hope that these notes will provide a reference for ongoing study and a document which you will continue to study and use in developing your skills in equine clinical pathology and interpretation. Please feel free to e-mail me should you have cases or aspects that you would like to discuss. I’d appreciate any feedback regarding sections of these notes that are of particular benefit to you or which you feel need to be improved.

Sincerely, Kathy Freeman (kathy-freeman@talktalk.net)

January, 2010