A GUIDE TO INTERPRETING BLOOD PROFILES

Laboratory tests are just one part of the database from which clinical diagnoses can be made. Two diagnostic procedures must be completed before laboratory tests are used to pursue a possible diagnosis:

1. Obtain a full history
2. Perform a complete physical examination

Using the knowledge gained from these procedures you can then select and optimise diagnostic tests or further procedures (e.g. diagnostic imaging) to narrow down your differential list or clarify identified problems.

Laboratory tests should be interpreted with an understanding of:

- Knowledge of how disease processes change test results
- Reference Intervals
- Units used (SI or conventional)
- Potential for error (pre-analytical, analytical and post-analytical)

Importantly, the ***best possible diagnosis*** is made when the laboratory tests are evaluated in conjunction with the history, physical examination and any other ancillary diagnostic tests performed.

When evaluating cases:

1. Note any abnormalities in the laboratory data provided.

    - Remember that although this typically arises when results lie above or below an expected reference interval, you must also take into account the magnitude and significance of any variation found, and that occasionally an abnormal or unexpected result may also be one that actually lies *within* a reference interval.

2. Interpret the results, including any abnormalities found.

    - Interpretation involves listing the possible causes of any abnormalities (problems) identified. Include a brief discussion of the *most likely* cause/s for each abnormality and any possible associations between multiple abnormalities if present.
    - Grouping lab results by body systems can help identify associations and patterns.
    - Correct scientific terminology should be used.
    - Finally, can the abnormalities be drawn together into a conclusion and possible diagnosis? Note that this may not be possible in every case!

3. Determine whether further testing may be necessary, and what results you may expect.

4. Think about how you would manage the patient.

This guide is designed to assist with interpretation of the most common causes of abnormalities in biochemistry panels. For more comprehensive information and less common causes of biochemistry abnormalities please refer to one of the recommended textbooks at the end of this guide.

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HAEMATOLOGY

RBC Indices

RBC count – number of erythrocytes per litre

Haematocrit – expression of relative volume of blood that is composed of erythrocytes. Calculated by analyser from RBC count and MCV

Packed Cell Volume - expression of relative volume of blood that is composed of erythrocytes. Measured on haematocrit tube

Haemoglobin – amount of haemoglobin per litre

MCV – mean corpuscular volume = average volume of erythrocytes

MCH – mean corpuscular haemoglobin = average haemoglobin content of each erythrocyte

MCHC – mean corpuscular haemoglobin concentration = concentration of haemoglobin in the erythrocytes (takes into account erythrocyte volume)

RDW –red cell distribution width = measure of variation in cell size (anisocytosis)

Reticulocyte count – number of immature erythrocytes that contain increased RNA (seen on blood film as polychromatophils)

- Absolute Reticulocyte Count – number of reticulocytes per litre. Most accurate measure of marrow response to anaemia
- Automated reticulocyte count (absolute) – analyser detects erythrocytes with increased RNA
- Manual reticulocyte count – methylene blue smear is used to look for reticulocytes. Generates a reticulocyte % which can then be used to calculate the absolute reticulocyte count.

Erythrocytosis

- Increased RBC count/Haematocrit/Haemoglobin
    - Relative - haemoconcentration (dehydration), splenic contraction (adrenalin mediated)
- Absolute
    - appropriate - increased production in response to hypoxia
    - inappropriate – increased production in response to abnormal EPO secretion
    - inappropriate – increased production due to myeloproliferative disease (erythroid leukaemia)

Anaemia

- Decreased RBC count/Haematocrit/Haemoglobin
- Rarely due to over-hydration (fluid therapy) – will be mild

- Can reflect erythrocyte loss or destruction (haemolysis, haemorrhage, phagocytosis), or reduced production

Regenerative anaemia

- When anaemia reflects loss or production a regenerative response is expected by the marrow. This results in a reticulocytosis within 3-5 days of the erythrocyte loss.
- Characterised by macrocytosis, hypochromasia and polychromasia on the blood film
- May see increased MCV (macrocytic) and decreased MCHC (hypochromic)
- Causes include internal or external haemorrhage, haemolysis (e.g. IMHA, oxidative injury), and rarely haemophagocytic diseases (e.g. histiocytic sarcoma)
- Haemorrhage usually associated with concurrent decrease in protein (albumin and globulins)
- Haemolysis can be associated with haemolysed plasma, haemoglobinuria, hyperbilirubinaemia, bilirubinuria, normal or increased protein levels, agglutination (IMHA), spherocytes (IMHA), Heinz bodies or eccentrocytes (oxidative injury)

Assessing regeneration

- A reticulocytosis confirms an anaemia is regenerative
    - Elevated absolute reticulocyte count (i.e. above the reference range)
    - Or elevated corrected reticulocyte % (i.e. above the reference range)
- Reticulocyte counts can be determined by some haematology analysers or we can do a manual count with methylene blue stained blood
- Increased polychromasia on the blood film is expected with a regenerative anaemia
    - Most but not all reticulocytes are polychromatophilic (reflects our inability to perceive a slight colour difference)
    - All polychromatophils are reticulocytes
- A regenerative anaemia is often macrocytic and hypochromic….but not always

Non regenerative anaemia

Consider the following causes;

- Anaemia of chronic disease or inflammation – common cause of a mild normocytic normochromic non-regenerative anaemia
- Renal disease
- Bone marrow disease
- Iron deficiency – in the early stages can still be regenerative with a variable MCV and MCHC, but once iron stores are severely depleted the anaemia will become microcytic hypochromic and non-regenerative

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LEUKOGRAMS

Stress Leukogram

- Hallmark is Lymphopenia
- May also have a mature Neutrophilia, Eosinopenia, and in dogs Monocytosis
- There should NOT be a left shift or toxic change unless there is concurrent inflammation

Inflammatory Leukogram

- Neutrophilia +/- Monocytosis
- May see left shift and/or toxic change (but not always)
- Neutropenia and/or degenerative left shift (where immature neutrophils outnumber segmented neutrophils) can be seen with severe inflammation e.g. sepsis

Beware of analyser errors with the differential

- Analysers CAN NOT assess morphology and hence will not identify leukaemia cells, toxic change, bands etc.
- Leukaemia cells can be misclassified as lymphocytes or monocytes
- NRBCs can be misclassified as lymphocytes
- The blood smear should be examined for EVERY sick patient

PLATELETS

Thrombocytopenia is a common analyser artefact due to

- Sample clotting
- Platelet clumping invitro

We must always confirm thrombocytopenia by checking the sample for clots and checking the blood film for platelet clumping, particularly in cats.

PLASMA

Lipaemia can lead to false elevations in Hb, MCH, MCHC and refractometer protein (total solids)

Lipaemia and haemolysis also interfere with accurate biochemistry testing so be wary when interpreting results from a lipaemic or haemolysed patient samples. Fasting the patient reduces the risk of lipaemia.

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KIDNEY

Urea (BUN)

Protein metabolism waste product filtered and excreted by kidneys. Increased levels are called AZOTAEMIA and this can reflect the following causes;

1. Prerenal – e.g. dehydration, high protein meal, GIT haemorrhage or protein loss
2. Renal disease – decreased glomerular filtration rate
3. Post renal – e.g. urinary obstruction or uroperitoneum

Creatinine

By product of muscle metabolism filtered and excreted by kidneys. Increased levels are called AZOTAEMIA and this can reflect the following causes;

1. Prerenal – e.g. dehydration, high muscle mass
2. Renal disease – decreased glomerular filtration rate
3. Post renal – e.g. urinary obstruction or uroperitoneum

Azotaemia needs to be interpreted in light of hydration status, serum electrolyte levels, urine specific gravity (USG) and urine output. If an animal is dehydrated then it should maximally concentrate its urine to conserve water. Poorly concentrated urine in a dehydrated animal supports renal insufficiency (primary renal disease) or extra renal disease causing impaired renal concentrating ability e.g. hyperadrenocorticism.

	Usual range USG	**Hyposthenuric USG**	**Isosthenuric USG**	**Mildly to Moderately concentrated USG**	**Highly Concentrated USG**
Cat	1.035-1.060	<1.008	1.008-1.012	1.013-1.034	≥1.035 (1.040)
Dog	1.015-1.045	<1.008	1.008-1.012	1.013-1.029	≥1.030
Large Animals	1.015-1.030	<1.008	1.008-1.012	1.013-1.024	≥1.025

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LIVER, PANCREAS AND GIT

Alanine aminotransferase (ALT)

Cytoplasmic enzyme found within hepatocytes (liver cells) and to a lesser degree in muscle. Increases in blood levels usually reflect liver damage but levels can also mildly increase with muscle damage. Useful for screening for liver disease in dogs and cats.

Aspartate transaminase (AST)

Cytoplasmic and mitochondrial enzyme found within hepatocytes, muscle and erythrocytes. Increases in blood levels can reflect liver damage, muscle damage or sample haemolysis. Less sensitive than ALT for detecting liver damage in dogs and cats, but more sensitive and specific than ALT for hepatocellular damage in horses and cattle.

Glutamate dehydrogenase (GLDH)

Mitochondrial enzyme found within hepatocytes, heart muscle and kidney. Increases in blood levels usually reflect hepatocellular damage. Primarily used for detection of liver damage in horses, cattle and birds.

Sorbitol dehydrogenase (SDH)

Liver specific enzyme very useful for screening for liver damage in horses and ruminants, however it is very labile requiring immediate testing which limits its usefulness in practice.

Lactate dehydrogenase (LDH)

Enzyme found within hepatocytes, muscle and erythrocytes. Increases in blood levels can reflect liver damage, muscle damage or sample haemolysis.

Alkaline phosphatase (ALP)

Enzyme found in liver (membrane of hepatocytes and biliary epithelial cells), bone, intestine, and placenta. Increased blood levels can reflect cholestasis, hepatic lipidosis in cats, induction by drugs (particularly the corticosteroid isoform in dogs), bone remodelling, hyperthyroidism in cats, colic in horses, and late pregnancy.

Gamma glutamyl transferase (GGT)

GGT found in liver (membrane of hepatocytes and biliary epithelial cells), kidney and mammary gland. Increased blood levels can reflect cholestasis, induction by corticosteroids, passive transfer in neonates (dog, sheep and cattle). More sensitive than ALP for screening for cholestasis in cats, horses, cattle and birds.

Bilirubin (TBIL)

Elevated total bilirubin (hyperbilirubinaemia/icterus) can be pre-hepatic (haemolysis), hepatic (cholestasis), or post hepatic (bile duct obstruction), or anorexia in horses. Evaluation of PCV/haematocrit and liver enzymes can help differentiated between these causes. Some

laboratories also offer measurement of conjugated (direct) bilirubin which can assist with differentiating the causes of icterus. Birds form biliverdin rather than bilirubin.

Bile acids

Synthesized in the liver from cholesterol and secreted in bile. Feeding induces release of a bolus of bile into the intestine from the gall bladder. Increased serum bile acids can occur post prandially, with cholestasis, liver disease and portosytemic shunts. Bile acid stimulation test (where we take a 0hr and 2hr post prandial sample) is more sensitive in screening for hepatic dysfunction in dogs and cats than fasted or random serum bile acids. In horses that lack of a gall bladder means we don't see significant increases post prandially and thus we just do a single bile acid level. Serum bile acids rise with cholestasis and thus measurement of bile acids in an icteric patient does not help us evaluate hepatic function.

Ammonia

Produced in GIT as a result of breakdown of urea and amino acids by bacteria, and then transferred to the liver via portal circulation for conversion to urea. Elevated levels can indicate hepatic dysfunction, portosystemic shunt, excess urea supplementation in ruminants, or severe intestinal disease in horses. Very labile and special handling is required.

Glucose (G)

Elevated G levels (hyperglycaemia) can reflect stress (particularly in cats), diabetes mellitus, hyperadrenocorticism, equine metabolic syndrome, fluid therapy, sepsis. Hypoglycaemia can reflect anorexia (very young animals), liver disease, hypoadrenocorticism, neoplasia (e.g. insulinoma), insulin excess or oral hypoglycaemic drugs, sepsis, or laboratory error.

Amylase

Amylase is an enzyme found in pancreatic secretions, gastrointestinal tract, and saliva (varies with species), and undergoes renal excretion. Elevated amylase levels (hyperamylasaemia) can reflect pancreatitis, pancreatic neoplasia, or renal insufficiency. Low amylase is not considered clinically significant. Monitoring of amylase is most useful in the dog as a screening test for pancreatitis.

Lipase

Lipase is an enzyme found in pancreatic secretions, gastrointestinal tract, and liver, and undergoes renal excretion. Elevated total lipase can reflect pancreatitis, pancreatic or hepatic neoplasia, renal insufficiency or hypercortisolaemia. Low lipase is not considered clinically significant. Monitoring of lipase is most useful in the dog as a screening test for pancreatitis.

Pancreatic lipase (spec-CPL)

This assay measures lipase produced in the pancreas only and is a more specific test for pancreatitis in dogs and cats than amylase and total lipase.

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MUSCLE

Creatine Kinase (CK)

CK is a cytosolic enzyme found in skeletal muscle, cardiac muscle and brain. Elevated serum CK indicates muscle damage but mild increases are also seen with anorexia in cats. Haemolysis can lead to falsely high results due to assay interference.

AST and LDH are also useful in screening for muscle damage, but are less specific as levels are also affected by hepatic disease. Mild elevations in ALT can also occur with muscle damage.

ELECTROLYTES

Calcium

This ion is critical to the function of muscle and many enzyme systems and thus blood levels are tightly regulated by the parathyroid gland, kidneys and intestine. Total calcium levels reflect the sum of protein bound calcium (albumin), calcium complexes (e.g. phosphate, sulphate), and ionized calcium, and thus we need to interpret total calcium levels in light of the serum albumin level.

↑ total calcium can reflect neoplasia, renal disease, hypoadrenocorticism, vitamin D toxicity, granulomatous inflammation, hyperparathyroidism, hyperalbuminaemia (haemoconcentration), or bone lysis.

↓ total calcium can reflect renal disease, hypoparathyroidism, hypoalbuminaemia, GIT loss.

Phosphate

Phosphate is present in many tissues including bone, muscle and erythrocytes, and is excreted by the kidneys and thus levels are often elevated with diseases that cause reduced glomerular filtration rates e.g. renal disease.

↑Phosphate can reflect bone activity (young animals), reduced renal function, diet, tissue damage or cellular release of Phosphate e.g. sample haemolysis or myonecrosis.

↓Phosphate can reflect hyperparathyroidism, neoplasia, prolonged anorexia or diuresis.

Sodium (Na) and Chloride (Cl)

Most of the time Na and Cl changes are in parallel and tend to follow the movement of water.

↑ Na and Cl usually reflects dehydration (look for hyperalbuminaemia or clinical history to support this).

↓Na and Cl can reflect loss of electrolyte rich fluid through vomiting and/or diarrhoea, loss into a body cavity (third space loss), renal loss or exudation (e.g. burns). Less commonly this reflects water gain e.g. with congestive heart failure or excessive fluid therapy (which would also give us slight hypoalbuminaemia – however this could also occur with protein loss).

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Occasionally Na and Cl changes are not in parallel. This can occur with selective Cl loss e.g. the loss of HCl with vomiting or sequestration of HCl in the stomach or abomasums leads loss of Cl that exceeds the loss of Na. This process often leads to a metabolic alkalosis. A secretory metabolic acidosis (e.g. renal or GIT loss of HCO3) or selective Na loss (which is rare) can lead to a selective Cl gain. We can evaluate this by looking at either the Corrected Cl or the Na-Cl difference (they achieve the same thing and it's just a matter of personal preference as to which method you use).

a) **Corrected Cl** = serum Cl level x (midpoint reference Na level/ serum Na)

Low corrected Cl = selective Cl loss (met alkalosis)

High corrected Cl = loss HCO3 (secretory met acidosis)

b) **Na-Cl difference** = serum Na – serum Cl mmol/L

Normal Na-Cl in mmol/L are:

Dog & Cat 29-42	Horse 34-43
Cow 35-45	Goat 33-43

↑Na-Cl diff = selective Cl loss (met alkalosis)

↓ Na-Cl diff = loss HCO3 (secretory met acidosis)

Here is an example: Both Na and Cl are low, but subjectively Cl appears lower than expected for the decrease in Na, so we need to evaluate this further……..

Canine results	Patient result	Reference Interval
Sodium mmol/L	140	144-160
Chloride mmol/L	87	109-122

Corrected Cl = 87 x (152/140) = 94 (i.e. lower than reference interval supporting selective Cl loss)

Na-Cl difference = 140-87 = 53 (i.e. high supporting selective Cl loss)

Potassium (K)

K is an important electrolyte for normal nerve and muscle function and thus the K level is tightly controlled by the kidneys.

↑ K can reflect failure of urinary excretion with acute renal insufficiency, urinary obstruction, or hypoadrenocorticism, or K retention with a metabolic acidosis, or cellular release of K e.g. myonecrosis or haemolysis in species with high erythrocyte K such as horses and ruminants

↓K can reflect excess loss through renal loss (e.g. renal insufficiency, frusemide Rx), GIT disease, or reduced intake through prolonged anorexia.

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Na:K ratio

A low Na:K ratio is useful to assess for the likelihood of hypoadrenocorticism in dogs where we see hyperkalaemia and hyponatraemia. If Na:K is <27:1 then hypoadrenocorticism should be considered (but also consider pseudoaddisons e.g. whipworm, Salmonellosis), and if Na:K is <25:1 it's very highly likely due to hypoadrenocorticism.

PROTEINS

Total Protein (TP)

TP levels reflect the sum of albumin and globulins. Elevated protein levels (hyperproteinaemia) can reflect dehydration, neoplasia, or inflammation (hyperglobulinaemia). Low TP levels (hypoproteinaemia) can reflect reduced protein synthesis (hepatic disease), malabsorption/maldigestion, or protein loss through kidney, GIT, exudation, or blood loss.

Albumin

Synthesized by the liver and functions to maintain colloidal osmotic pressure and also has anti-thrombotic properties. Elevated albumin levels (hyperalbuminaemia) usually reflect dehydration. Decreased albumin levels (hypoalbuminaemia) can reflect reduced liver synthesis due to inflammation (negative acute phase protein) or liver disease, or can reflect albumin loss through kidney, GIT or exudation. Selective hypoalbuminaemia usually reflects renal loss e.g. glomerulopathy.

Globulins

Calculated by TP-albumin. Can be more accurately measured by serum protein electrophoresis. Increased levels can be seen with inflammation or neoplasia (e.g. multiple myeloma, B cell lymphoma). Decreased levels are seen with failure of passive transfer, immunodeficiencies (rare) or protein loss (e.g. GIT loss).

What causes spurious results and how do we identify them?

Sampling error – clots in the sample can cause the probe to partially block leading to short sampling. This can result in falsely low results.

Haemolysis or **Icterus**– many biochemistry tests are chromogenic assays, relying on machine detection of a colour change to evaluate the level of an enzyme or substrate. Thus discolouration of the sample by haemolysis or icterus can lead to false results, which depending on the assay can be falsely increased or decreased.

Lipaemia – lipaemia can interfere with chromogenic assays as well as resulting in short sampling of serum/plasma. Lipaemia causes falsely high total protein results with refractometers and can also affect electrolyte levels.

EDTA contamination of the serum sample – this leads to spurious hypocalcaemia, hyperkalaemia and/or hypernatraemia.

Prolonged sample storage – can lead to decreases in some tests and increased haemolysis.

High ambient temperatures – the room temperature will affect analyser performance and excessive heat can lead to falsely high or low results

LIPIDS

Cholesterol (CHOL)

Elevations in cholesterol (hypercholesterolaemia) can reflect recent food intake (post prandial), cholestasis, endocrine disease (hypothyroidism, diabetes mellitus, hyperadrenocorticism), protein losing nephropathy, or can be hereditary (e.g. hyperlipidaemia of miniature schnauzers).

Triglycerides (TG/TAG)

Elevations in triglyceride (hypertriglyceridaemia) can reflect recent food intake (post prandial), cholestasis, endocrine disease (hypothyroidism, diabetes mellitus, hyperadrenocorticism or can be hereditary (e.g. hyperlipidaemia of miniature schnauzers).

RECOMMENDED TEXTBOOKS FOR MORE INFORMATION

Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology 5th ed. Kenneth S. Latimer

electronic book through UOM library:
http://ebookcentral.proquest.com/lib/unimelb/detail.action?docID=821970

Fundamentals of Veterinary Clinical Pathology 2nd ed. Steven L. Stockham, Michael A. Scott

Veterinary Hematology and Clinical Chemistry 2nd ed. Mary Anna Thrall, Glade Weiser, Robin Allison, Terry W. Campbell

electronic book through UOM library:
http://ebookcentral.proquest.com/lib/unimelb/detail.action?docID=918265