

ANTICOAGULANTS & THEIR APPLICATION FOR BLOOD SAMPLING

TYPE OF TUBE COLOUR OF TOP FUNCTION

PLAIN (*clotted*) **BROWN/CLEAR** General biochemistry

Bile acids
Serology
Endocrinology

HEPARIN (*plasma*) **ORANGE** Biochemistry including enzymes and potassium
ANP 31-67

HEPARIN (*whole blood*) **ORANGE** Lead
Transketolase
G S H-PX
Reptilian/avian haematology

EDTA (*whole blood*) **LAVENDER/RED** Haematology

YELLOW

OXALATE FLUORIDE **YELLOW OR GREY** Glucose
(*whole blood or plasma*)

SODIUM CITRATE **GREEN** Clotting times (*OSPT/AAPTT*)
(*whole blood or plasma*) von Willebrand factor antigen

N.B. Tubes should be filled exactly to the fill line and then mixed well. Over or under filling of tubes can significantly affect results. This is particularly true when assessing clotting times.

MODERN VETERINARY HAEMATOLOGY

There are few specialist areas in veterinary diagnostic medicine which have been more severely devalued by “*technological advances*” than veterinary haematology. The morphological assessment of red blood cells, white blood cells and platelets is a critical part of the haematological investigation, without which the diagnostic value of the haematological profile is seriously flawed. Profiles offered by Axiom Veterinary Laboratories include a full haematological assessment which includes cells counts by a sophisticated haematology analyser and manual film examination by a qualified haematologist. A further blood film review by a Veterinary Clinical Pathologist is performed if the findings fulfil specific abnormal criteria e.g. presence of atypical lymphocytes, spherocytes etc. We strongly recommend that samples of blood in EDTA and fresh blood smears are submitted from all cases requiring a haematology profile. Inclusion of blood film examination without cell counts in our price list is a concession to clinicians who use their own cell counters, but in general the information obtained from such cases is inferior to that from a full haematology profile. Bone marrow examination is underused in veterinary practice largely because of the perceived difficulties with sample collection. We have special expertise in the evaluation of bone marrow aspirates and in order to encourage the use of this invaluable diagnostic technique we have invested in a range of bone marrow needles which are available at low cost and can be sent out on the request date.

THE HAEMATOLOGICAL EXAMINATION

A full haematological examination should always contain the following parameters:

- Red cell indices - RBC count, PCV/HCT, Hb, MCV, MCHC
- White blood cell count with manual differential
- Platelet count
- Morphology - RBC, WBC and platelets
- For interpretative purposes cell counts reflect the kinetics of entry and loss of cells from circulation, and cell morphology reflects the status of individual cells which is a direct reflection of the health of the bone marrow, the circulation and the tissues.

Clinically relevant haematological artefacts

Anticoagulated blood can be kept refrigerated for 24 hours before significant morphological artefacts occur. High ambient temperature accelerates morphological artefacts.

Sample ageing causes:

- Swelling and/or crenation of red blood cells
- Nuclear degeneration, lobulation, rupture
- Cytoplasmic vacuolation
- Formation of smear cells, cytoplasmic rupture
- Karyolysis, pyknosis

Anticoagulants are also associated with some specific artefacts

- WBC shrinkage and abnormal staining characteristics (*heparin*)
- Red cell cremation (*EDTA*)
- Platelet aggregation, cats particularly (*EDTA*)

All haematology analysers produce artefactual cell counts on occasions for differing reasons which depend upon the underlying principle of cell counting (*e.g. coulter vs QBC*). The best approach to these problems is to check each blood film visually and ensure that estimated cell counts approximate to those measured by the analyser.

HAEMATOLOGY PATTERNS

Haematological diseases often produce patterns of well defined abnormalities on haematological profiles. Recognition of the common patterns is relatively easy but interpretation of the more complex variations requires extensive experience and a good understanding of the underlying pathophysiology in order to arrive at the correct diagnosis.

Abnormalities in red blood cell morphology

- **Hypochromic RBCs** flatten more than normochromic cells in a film and have a thin rim of haemoglobin around an increased pale central concavity. They are deficient in haemoglobin and therefore undergo an extra division during maturation becoming smaller cells (*microcytes*). In iron deficiency the red cells become microcytic before becoming hypochromic.
- **Stomatocytes and target cells** are RBCs with increased membrane in comparison to content and therefore adopt different folded conformations, appearing mouth-like or like targets when viewed in 2 dimensions. All reticulocytes have excessive surface membrane and can appear like polychromatic stomatocytes or target cells and this finding is irrelevant. When mature RBCs adopt these shapes it usually indicates that the membrane lipids have altered in response to some underlying disease process *e.g.* chronic inflammation or liver disease.
- **Reticulocytes** are large, folded polychromatic cells. These are the primary indicators of RBC regeneration in cats and dogs.
- **Echinocytes or Burr cells** are crenated cells with uniform circumferential spiculation. They occur in renal disease but the most common cause is RBC dehydration/EDTA artefact in an aged blood sample.
- **Acanthocytes** are shrunken RBCs with uneven, assymetrical spiculation. This is a special form of RBC fragmentation Acanthocytes are important early markers of vascular anomalies such as haemangiosarcoma or microvascular angiopathies. They also accompany chronic liver disease.
- **Spherocytes** are RBCs which have had pieces of membrane removed by macrophages. The normal biconcave shape is lost and the cells become small dark spheres and lose their central pallor. Spherocytes are the hallmark of immune-mediated damage to RBCs. They are a more powerful diagnostic criterion than a positive Coombs' test. Incomplete sphere formation occurs with partial loss of the biconcave shape. Such cells have reduced central pallor when viewed in 2 dimensions.
- **Keratocytes** are cells which develop a membranous blister on one side which ruptures leaving claw-like spikes. This is a form of fragmentation in response to chemical and physical injury.
- **Eccentricocytes** are cells which have undergone oxidative damage causing adhesion of opposing membranes. This squeezes out the haemoglobin from one side of the cell. Such cells appear clear at one end and dark at the other when viewed in 2 dimensions. Their presence is a good marker for oxidative red cell damage in dogs. Whereas cats tend to form Heinz bodies, dogs form eccentricocytes (*although Heinz bodies can often be seen on the surface of eccentricocytes*). The most common cause of eccentricocyte formation in dogs and cats is onion poisoning and paracetamol toxicity respectively.

- **Heinz bodies** are non-staining (*with Romanowsky stains*) particles of denatured haemoglobin. They stain basophilic with new methylene blue. They are common in cats, because feline haemoglobin is particularly susceptible to oxidative damage.
- **Howell Jolly bodies** are nuclear remnants which result from incomplete extrusion of the nucleus from maturing reticulocytes. They are present in normal blood films but increased numbers tend to be seen in splenectomised patients and in any regenerative anaemia.
- **Nucleated RBCs** Normoblasts frequently appear in regenerative anaemias. The presence of nucleated RBCs in the absence of polychromasia is a strong indicator of bone marrow dysfunction. It may also occur in patients with splenic diseases or following splenectomy. Normal dogs sometimes have occasional late normoblasts in the circulation with minimal evidence of polychromasia. The presence of megaloblastic RBCs or red cells with other nuclear or cytoplasmic maturation abnormalities in the peripheral circulation is a very useful indicator of bone marrow dysfunction/dysplasia. This is of particular relevance in cats with FELV. The presence of nucleated red cells +/- basophilic stippling without polychromasia can be a feature of lead poisoning.
- **Proerythroblasts** and early normoblasts can be markers of myelodysplasia or myeloproliferative disease e.g. erythremic myelosis.
- **Basophilic stippling** is quite a common finding in regenerative anaemias in cats and ruminants and can be interpreted as a feature of RBC regeneration in these species.
- **Red cell parasites** The most common red cell parasite in the UK is mycoplasma felis which is a very difficult microscopic diagnosis. Microscopy is an insensitive way of detecting the parasite because the organisms may detach from the RBCs in anticoagulated blood.
- **Inclusion bodies** Distemper inclusions can sometimes be seen in early distemper cases.

Abnormalities in leukocyte morphology

Any of the bone marrow stages of leukocyte development can appear in the peripheral circulation in different disease processes. Acute leukaemias are characterised by the presence of poorly differentiated cells in the circulation which in some cases cannot be assigned to any particular cell line.

NEUTROPHIL TOXICITY

- This is a group of morphological abnormalities which, when present, help to discriminate between inflammatory-infectious and neoplastic processes. In severe toxæmic states, granulopoiesis becomes suppressed and neutrophil morphology is altered by maturation defects. These are termed toxic changes. The different types of toxic change are as follows:
- Doehle bodies are remnants of rough endoplasmic reticulum resulting from defective cytoplasmic maturation. These can be seen as angular, poorly defined basophilic bodies in the outer regions of the cytoplasm. A small numbers of neutrophils containing Doehle bodies are normal especially in cats.
- Foaminess of the cytoplasm is due to the intracytoplasmic release of lysosomal enzymes and restricted cytolysis.
- Increased cytoplasmic basophilia due to increased cytoplasmic ribosomal RNA.
- Cytoplasmic vacuolation due to lysosomal cytolysis (*note that this is a common sample ageing change in normal neutrophils*).
- Inappropriate appearance of intracytoplasmic azurophilic granules (*'toxic' granules*). These are primary granules which are retained instead of being lost during maturation.

Other maturation defects may occur for example the nucleus may undergo maturation without cell division resulting in the formation of giant bands, mature neutrophils with bizarre twisted/ribbon-like nuclei, or neutrophils with ringed or doughnut nuclei.