AUSTRALIAN SPECIALISED ANIMAL PATHOLOGY
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An Introduction to Haematology

and

Blood smear Preparation

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The blood is the major method of transportation within the body, providing:

a means of movement of substances, including vital nutrients to cells

assisting in removal of waste products,

transport of cells and protective proteins to help fight infection

transport of important messages e.g. via the use of hormones and cytokines.

Haematology encompasses a means of assessing and measuring the cellular components of the blood:

Red cells or erythrocytes are predominantly involved in oxygen transport and carbon dioxide removal

White blood cells or leukocytes are primarily involved in defense from invasion and infection

Platelets or thrombocytes responsible for initial protection from bleeding

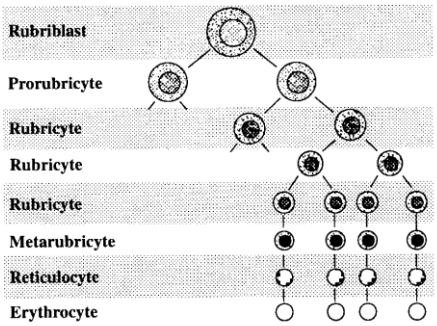
It is important to note measurement of haematological values only gives a small snap shot of the overall picture. Levels in the blood can be influenced by both increased demand for and decreased production of each cell line. Hence when interpreting any changes identified, need to take into consideration possible changes in both factors. e.g anaemia may result from both a reduction in bone marrow erythrocyte production, as well as an increase in erythrocyte loss or destruction (or in fact a combination of both).

Red Blood Cells (Erythrocytes)

The primary function of the red blood cell is to carry haemoglobin. Haemoglobin in turn functions as a carrier of oxygen (O_2) to tissue and in the elimination of carbon dioxide (CO_2).

Normal Canine RBCs 	• 7μm in diameter • biconcave disc with area of central pallor • uniform size
Normal Feline RBCs 	• 5.8μm in diameter • very slight or inapparent central pallor • slight anisocytosis

Red blood cell production occurs in the bone marrow in response to erythropoietin, produced by the kidney subsequent to inadequate oxygenation of tissue (hypoxia).



Sequence of Erythropoiesis

In a normal animal, the bone marrow will only release erythrocytes once the nuclei has been excluded towards the end of maturation. Once extruded, a fine net like structure of residual RNA and ribosomes (reticulum) remains. These intermediate cells containing reticulum are called reticulocytes (retics) and constitute the most immature cells released under normal circumstances.

A small number are present in the circulating blood of the normal animal (+/- 1%), although it should be noted, that horses do not have reticulocytes in the peripheral blood, even in the event of marrow stimulation e.g. blood loss.

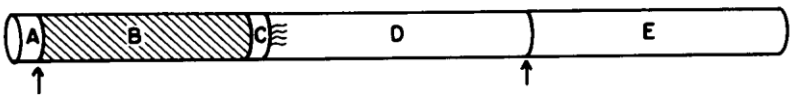
Means of Evaluating the Red Cell

The packed cell volume (PCV), red cell count (RCC) and Haemoglobin (HgB) are all measures of the red cell mass.

The red cell indices of Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Haemoglobin Concentration (MCHC) help to identify whether cells are normal, large or small in size (MCV) and whether they contain a normal concentration of HgB (MCH and MCHC).

The packed cell volume (PCV) or haematocrit (HCT) is the % of compacted red cells in a fixed amount of blood, and is measured by packing the red cells in an haematocrit tube in a centrifuge.

The PCV or HCT is one of the most accurate and reproducible methods of measuring red cells in the peripheral blood, falling in an anaemia and increased in polycythaemia.



MICROHEMATOCRIT TUBE

A - Clay seal
B - Packed erythrocytes
C - Packed leukocytes and platelets (buffy coat)
D - Plasma
E - Empty space at top of tube

Haemoglobin is the protein within the red cell which transports O_2 and CO_2 throughout the body. As a rule of thumb, the haemoglobin is usually about one third that of the HCT.

The red blood cell count (RCC) is measured most commonly with an automated process in impedance or laser instruments, indicating the number of red blood cells per litre.

The reticulocyte count is used to assess the number of immature erythrocytes in the blood. This then gives an indication of the degree of the bone marrow response to an anaemia and as such is a primary indicator of a regenerative versus a non regenerative anaemia. These may be automatically counted on some instruments, but may also be counted by examination of a blood film prepared from blood stained with new methylene blue.

White Blood Cells (Leukocytes)

The basic function of the white blood cell is to defend the body from infectious disease processes.

Evaluating the white blood cell count can help identify abnormalities that may suggest specific diseases such as a viral or bacterial infection or even a neoplastic process.

The leukocytes are comprised of five separate cell types, distinguished by:

- the nature of their cytoplasm
- the shape of their nucleus
- their staining characteristics

These are divided into

Granulocytes (3 types):

- Neutrophils comprise a majority of leukocytes in the peripheral blood of dogs and cats. These are primarily involved in fighting bacterial infections and cleaning up other causes of acute tissue damage

- Eosinophils are usually present in only small numbers, but may increase as a response to parasitic infections and allergic/hypersensitivity disease.

- Basophils are the least populous with only occasional cells identified, but when increased may also be associated with parasitic and allergic/hypersensitivity disease.

and

Non-granular leukocytes (2 types):

- Lymphocytes are generally the second most common leukocyte in the peripheral blood, primarily involved in maintenance of immune function, the body's defence against infectious agents (viral, bacterial and parasitic agents) and some forms of neoplasia.

- Monocytes are fewer in number, involved in destruction of foreign organisms and miscellaneous debris throughout the body by phagocytosis, and may be considered the garbage collectors of the body.

Note further subdivision of neutrophils into immature (band) and mature neutrophils will also add valuable information.

In normal animals, very few immature cells are present in the peripheral blood.

The bone marrow will however release progressively immature cells when appropriately stimulated, usually an increase in demand, most commonly a consequence of an underlying inflammatory process.

Means of Evaluating the White Cells

Determination of the white blood cell count (WCC) will give an appreciation of the total number of leukocytes. This can then be subdivided into the proportion (%) and absolute count for each of the varying types of leukocytes.

The total number and proportion of leukocytes that comprise the white blood cell count will then aid in identifying a possible underlying disease process.

It is important to note that although automated processes may give some definition of total numbers of the various leukocyte types, blood smear examination remains a vital step in confirmation and identification.

The white blood cell count (WCC) is a measurement of the number of leukocytes in a litre of blood.

White cell differential is the proportion of each cell type expressed as a percentage. This can be obtained from automated analysers, or obtained from examination of a blood film (still strongly recommended, even with sophisticated analysers). This will include the proportion of

Absolute counts for each cell line may be directly counted, but also derived from the proportion (%) of the total white cell count.

The pattern of change in the white cell differential and cell counts for each cell can provide valuable information concerning a possible underlying process, summarized in the table below:

General Patterns of Leukocyte Responses

	WCC	Seg	Band	Lymph	Mono	Eos
Acute Inflammation	Increased	Increased	Increased	Decreased or no change	Variable	Variable
Chronic Inflammation	Increased or no change	Increased or no change	Increased or no change	Increased or no change	Increased	Variable
Overwhelming Inflammation	Decreased or no change	Decreased or no change	Increased	Decreased or no change	Variable	Variable
Hypersensitivity or Allergic reaction	Increased or no change	Increased or no change	No change	Decreased or no change	Variable	Increased
Excitement leukocytosis	Increased	Increased in dogs; increased or no change in cats	No change	No change in dogs; increased in cats	No change	No change
Stress leukogram	Increased	Increased	No change	Decreased	Increased or no change	Decreased or no change

Platelets (Thrombocytes)

Platelets are cytoplasmic fragments of megakaryocytes, produced in the bone marrow and responsible for forming the initial plug to prevent bleeding following vascular injury.

Low platelet numbers can be seen with decreased production (primary marrow disease), increased destruction, sequestration or utilization

A platelet can be counted directly or numbers can be estimated from the blood film.

A platelet count $<100 \times 10^9$/L is significant and can be classified as thrombocytopenia, however bleeding doesn't occur unless platelets are $<10\text{-}20 \times 10^9$/L.

Blood smear preparation

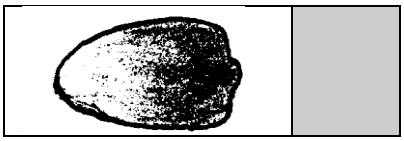
The aim of preparing smears is to spread out the cells for identification with a minimum of damage. This requires the use of good quality, oil-free slides with an even edge and surface

- Rest a frosted glass slide on firm support and place a small drop of blood just in from one end of the slide.
- Place the spreader at approximately 30 -40° in front of the drop and move the spreader back until it just touches the drop. The drop will immediately run along the edge of the spreader.
- With the same angle of the spreader, move it smoothly along until the smear is approximately 3 cm in length.
- Thick viscous blood may be spread rapidly with the spreader at a lower angle.
- Thin anaemic blood should be spread slowly with the spreader held at a greater angle (up to 70° - 80°).

The finished smear should have a nicely rounded tail. The red cells should lie side by side with minimal distortion and the white cells should be well spread.

- Allow the smear to dry in air at room temperature. Waving the smear in the air or holding over a gas flame or hair dryer can be used to hasten the process. Direct drying of the smear with blotting paper or filter paper is not recommended because it damages the cells.

Label the smear with the owner's name, patient name and date of preparation.



If smears are poor, consider the following:

a. the drop of blood is too large

b. the smear is made too rapidly

c. spreader edge is dirty or not even

d. spreader moved in jerky manner

e. entire edge of spreader not kept on slide while making smear

f. angle of spreader not constant

g. the patient has a high plasma protein concentration

h. there is a very high white cell count

i. the patient is grossly anaemic

j. the smear has extended to the edges of the slide