INTRODUCTION

These notes present some manual haematological techniques that may be used to evaluate common haematological disturbances in veterinary practice. Naturally, not all techniques will be used commonly, but they are all presented just in case they prove useful one day. For convenience, the techniques are categorized according to their usefulness in erythrocyte analysis, leukocyte analysis, bleeding (haemostasis/coagulation) analysis and in miscellaneous problem analysis. Some techniques, however, have general applicability to haematological analysis.

SOME ANTICOAGULANTS

Ideally, dry anticoagulants of known proportions should be used to minimize dilution, artefacts and alteration of staining properties. No one anticoagulant is entirely satisfactory for all haematological techniques.

- EDTA
  - disodium or dipotassium salt of ethylenediamine tetra-acetic acid
    (synonyms: sequestrene, versenate or versene)

EDTA acts by chelating calcium ions required in the clotting mechanism. At a concentration of 1 mg per ml of blood, it is the anti-coagulant of choice for routine blood counts and smears. Please note, excess EDTA, i.e. 2 mg per ml of blood, will cause shrinkage of the red blood cells and a significant decrease in PCV. So be careful to fill the tube to the graduated
level only.

With EDTA, thrombocyte (platelet) counts and cell counts may be performed up to several hours after the collection of blood. Refrigerated EDTA blood samples are suitable for cell counts, PCV and haemoglobin estimations up to 24 hours post-collection.

- **Heparin**

Heparin prevents coagulation by interfering with the conversion of prothrombin to thrombin and with the action of thrombin on fibrinogen. Heparin is naturally occurring and found abundantly in the liver. It does not alter erythrocyte cell size, even in excess; but it does interfere with the staining of leucocytes (the cell nuclei appear hazy or washed out). This is due to its strong affinity for basic dyes. Consequently, it is rarely used for haematological analysis.

Heparin is commonly used for biochemical analysis at a concentration of 10-50 international units per ml of blood.

- **Sodium Citrate**

Sodium citrate is used for some coagulation studies at the rate of one part of 3.8% aqueous solution to 9 parts of blood. Citrate removes the calcium which is essential for clotting. It is commonly used for coagulation studies.

**ERYTHROCYTE ANALYSIS**

**a) The Microhaematocrit value (PCV)**

This makes use of high speed centrifugation, using capillary tubes, which ensures complete packing of erythrocytes in a minimum time. The speed of microhaematocrit centrifuges range from 8000 to 16,000 r.p.m.

**Method:** After filling capillary tubes to 66-75% capacity by capillary attraction, the opposite end is sealed either by a small flame or by plasticine (preferred). The sealed tube is centrifuged for the requisite time - 2 minutes at 16,000 r.p.m. or 5 minutes at 8,000 r.p.m (follow the instructions on the centrifuge) - to give three distinct layers:

(i) the mass of erythrocytes at the bottom called the packed cell volume (PCV). This should really be called the volume of packed red cells (VPRC), as this was the first name used.

(ii) a mixed layer immediately above the erythrocytes, composed of leukocytes, platelets and other nucleated cells, called the buffy coat. The top white layer of the buffy coat is composed primarily of platelets. The leukocyte layer is immediately below the platelet layer and is usually an opaque dark red. This layer is difficult to visualize unless there are increased numbers of leukocytes.

(iii) the top layer composed of plasma. The microhaematocrit value is determined by use of a reader (see image at the end of the notes) supplied with the microhaematocrit centrifuge and is expressed as a fraction

\[
\text{microhaematocrit value (PCV)} = \frac{\text{red cell volume (Litres)}}{\text{total volume of blood (Litres)}}
\]
Notes:
The microhaematocrit reading is a good indication of the presence of anaemia or polycythaemia as long as the total plasma protein (TPP) concentration is determined - usually by refractometer. For instance, dehydration (TPP above normal units), by reducing plasma volume, may mask an anaemia or produce a spurious or relative polycythaemia (erythrocytosis).

The colour of the plasma layer provides useful information. For instance, it may depict the presence of jaundice (icterus), lipaemia or haemolysis.

b) Erythrocyte Count - (Unopette®)
This method uses a pre-measured diluent reservoir of 1.99 ml (normal saline with sodium azide as a growth inhibitor) and a disposable 10 μl micropipette. A haemocytometer is also required (see appendix 1). Note: Azide is dangerous. The protective shield for the pipette is used to puncture the diaphragm of the reservoir:

N.B. Discard shield after this step.

i) The pipette is filled with well mixed whole blood and the excess wiped off.
ii) The reservoir is squeezed and the pipette placed onto the reservoir and locked in.
iii) The index finger is placed over the top of the pipette and the pipette rinsed with diluent by releasing then squeezing the reservoir several times.
iv) The finger is removed and the apparatus is mixed by inversion.
v) The pipette is turned around and used as a dropper to fill the haemocytometer.

N.B. A few drops of diluent are discarded before filling the chamber. Five of the 25 intermediate squares in the central large square are counted (4 corners and centre). Both ruled areas are counted using 40 x objective and the mean is used in the calculation.

\[
\text{count} \\
\text{Calculation: } 100 \times 10^{12}/\text{Litre}
\]

(see haemocytometer notes for more detail)

Notes:
Haemoglobin estimation (method not given) and the erythrocyte count are usually not essential for establishing the presence of anaemia or polycythaemia but can provide supportive evidence. Also, they allow the determination of red cell indices which may be useful in categorizing an anaemia.

c) Erythrocyte Indices
These determine the relationships between the erythrocyte count, haemoglobin concentration, cell volume and cell thickness and are given for understanding rather than usage (most haematology machines determine these for you).

1. Mean corpuscular volume (MCV)
This is the average volume of a single cell expressed in femtoLitres (fL -- a femtoLitre is \(10^{-15}\) of a litre). It is obtained from the following formula:

\[
\text{MCV (fL)} = \frac{\text{PCV (L/L)} \times 1000 \text{ (expressed as a whole number)}}{\text{Erythrocyte count (x10}^{12}/\text{L})}
\]
2. **Mean corpuscular haemoglobin (MCH)**

This measures the mean or average haemoglobin content of a cell in picograms (pg -- a picogram is $10^{-12}$ of a gram). In some older and American texts it is referred to as the color index.

\[
MCH \text{ (pg)} = \frac{\text{Haemoglobin (g/L)}}{\text{Erythrocyte count (x } 10^{12}/\text{L)}} \text{ (expressed to one decimal place)}
\]

3. **Mean corpuscular haemoglobin concentration (MCHC)**

This measures the ratio of the weight of the haemoglobin to the volume of the erythrocyte, expressed in grams per litre. It takes into account the variation in size of erythrocytes, which is often present in anaemias. In some older and American texts it is referred to as the saturation index.

\[
MCHC \text{ (g/L)} = \frac{\text{Haemoglobin (g/L)}}{\text{PCV (L/L)}} \text{ (expressed as a whole number)}
\]

**Notes:**

On the basis of MCV, anaemias can be designated **macrocytic, normocytic or microcytic**. On the basis of MCHC, and to a lesser extent MCH, anaemias can be designated **hypochromic or normochromic**.

Regenerative anaemias (see explanation under reticulocyte count) are often macrocytic (due to the large numbers of reticulocytes) and, although the MCH is normal or increased, hypochromic due to lowered MCHC. However, these changes in red cell indices, if they do occur, are transient and return to normal on restoration of the red cell mass. For this reason, regenerative anaemias are often referred to as transitory or pseudomacrocytic anaemias.

True macrocytic anaemias may be found in non-regenerative anaemias associated with myeloproliferative disorders in the cat (due to elevated levels of nucleated red blood cells). Iron deficiency anaemia, a non-regenerative anaemia, is usually microcytic and hypochromic. However, the majority of non-regenerative anaemias are normocytic and normochromic.
d) Reticulocyte Count

**Reticulocyte stain:**
- Brilliant cresyl blue: 1.0 gm
- Sodium citrate: 0.4 gm
- Normal saline: 100.0 mls

Dissolve the stain in saline, add sodium citrate, mix and filter. The stain should always be filtered before use.

**Method:**
Equal quantities (ie 2 drops) of well mixed blood (in EDTA) and stain are mixed in a test tube and allowed to stain for approximately 20 minutes. If the animal is severely anaemic (e.g. PCV 0.10) then 3 quantities of blood to 1 quantity of stain is used. A blood film is prepared in the usual manner and can be counterstained with Giemsa/Diff Quik® although it is not advisable as the reticulocyte stain may be removed.

Select an area of the smear where there is a monolayer of cells and count the number of reticulocytes.

The mature erythrocytes are usually yellow-green while the reticulocytes contain dots or strings of blue staining material (residual cytoplasmic RNA).

The reticulocyte count is performed, under an oil immersion lens, by differentiating at least 1000 red blood cells (sometimes it may be possible to count only 500) and expressing the result as a percentage of the erythrocytes.

If the reticulocyte count is to be expressed as a percentage, ideally it should be corrected for the reduced PCV present in anaemia (fewer erythrocytes are present to dilute reticulocytes released from the bone marrow).

\[
\text{Corrected reticulocyte %} = \frac{\text{observed reticulocyte %}}{\text{patient's PCV}} \times \frac{\text{normal PCV}}{
\}

For this calculation the "average" PCV for a) Dogs is 0.45, b) Cats is 0.37.

The reticulocyte % can also be expressed as an absolute value:

\[
\text{Absolute reticulocyte count (x }10^9/L) = \frac{\text{observed reticulocyte %}}{\text{x erythrocyte cell count (x 1000)}}
\]

This is becoming the correction of choice for dogs and cats. The upper level of the reference interval for dogs is 75 (grey zone to 105) x 10^9/L and for cats 60 (grey zone to 100) x 10^9/L. If a dog has 4% reticulocytes and an erythrocyte count of 3.0 x 1012/L then the absolute reticulocyte value is 120 x 10^9/L. This suggests regeneration.

**Notes:**
In all domestic species, except the horse, the reticulocyte count is an indication of whether an anaemia is regenerative (i.e. an adequate bone marrow response to anaemia). In regenerative anaemia the marrow responds, from 3 days after the onset of anaemia, by elevating the numbers of circulating immature erythrocytes (primarily reticulocytes but some may be nucleated red blood cells). The dog has small numbers of circulating reticulocytes in health (0-1.5%). The cat has a reticulocyte percentage of 1.4 - 10.8% in health. The marked variation in reticulocyte numbers in the cat is partly due to the fact that 2 main types of reticulocytes exist. The *aggregate* type (strands or clumps of reticulum) is similar to other species and high values indicate current erythropoietic activity. The *punctate* type (dotted reticulum) may occur in large numbers in health (9-10%) and are increased in regenerative
anaemias. They remain increased at least 2 weeks after the aggregate count has returned to normal; therefore, they can indicate a bone marrow response as much as four weeks previously. *(In our laboratory we count only the aggregate type in cats which are 0-1.0% in health).*

The adult horse in health and in anaemia rarely has reticulocytes in the peripheral circulation (foals may develop reticulocytosis in response to anaemia); therefore, the response of bone marrow has to be assessed from changes in serial microhaematocrits and from, if required, bone marrow aspirate examination.

**LEUKOCYTE ANALYSIS**

*a) Leukocyte Count (Unopette®)*

The Unopette method employing a 20 μl capillary tube, haemocytometer counting chamber and Unopette reservoir containing 1.98 ml of diluent mixture (glacial acetic acid 3%, concentration made up in distilled water).

The protective shield for the pipette is used to puncture the diaphragm of the reservoir.

**N.B.** Discard shield after this step.

i) The pipette is filled with well mixed whole blood and the excess wiped off.

ii) The reservoir is squeezed and the pipette placed onto the reservoir and locked in.

iii) The index finger is placed over the top of the pipette and the pipette rinsed with diluent by releasing then squeezing the reservoir several times.

iv) Let the apparatus stand for 10 minutes, then mix thoroughly, convert the Unopette to the dropper assembly and fill the haemocytometer counting the chamber (ideally, both chambers should be filled and counted, and the average cell number per chamber used.)

Using 100 x magnification (i.e. 10 x objective) count the leukocytes in all nine large squares of the haemocytometer counting chamber (see Figure 1 at the end of the notes).

**Calculation:**

\[
\text{Count} + 10\% \times 10^9/\text{Litre}
\]

10

(see haemocytometer appendix for more detail)

**Notes:**

Total white blood cell count indicates the presence of leukocytosis or leukopenia. In the dog, cat and horse, leukopenia/Leukocytosis are usually due to changes in the number of neutrophils.

The total white blood cell count is essential for determining the absolute values of leucocyte types seen on the peripheral blood smear.

*b) The Peripheral Blood Smear (Film)*

1. **Preparation of a peripheral blood smear.**

Using chemically clean slides, free from dust, films may be made in the following manner:
A small drop of blood (ideally the smear should be made directly from withdrawn blood rather than blood placed in anticoagulant) is placed on the slide 1 cm from the end. The spreading slide is placed at an angle of 30-45 degrees to the slide and then moved back to make contact with the drop. The drop should then spread out quickly along the line of contact of the spreader with the slide. The moment this occurs, the film should be spread by a smooth forward movement of the spreader. Films are then rapidly air dried.

It is essential that the spreader should have an absolutely smooth edge and should be narrower in breadth than the slide on which the film is to be made. If the edge is rough, films with ragged tails containing many leukocytes result.

The angle of the spreader determines the thickness of the film, i.e. the greater the angle, the thicker the film. The ideal thickness is such that there is some overlap of red cells throughout much of the smear's length with separation and lack of distortion towards the tail of the film.

2. Staining of a peripheral blood smear.

Flood the slide with blood Giemsa and leave to fix for 2 minutes. Dilute and mix the Giemsa with an equal quantity of water (pH7). Leave to stain for 7 minutes. Rinse the slide in tap water and allow to dry.

Blood Giemsa: Giemsa 4 gm
Methanol (A.R.) 1000 ml
Glycerine 15 ml

Blood Giemsa is placed in an incubator at 37°C for approximately 1 week before use. The solution is mixed daily while incubating.

Alternatively, stain with Diff Quik® or a similar rapid stain.

3. Staining the smear for Haemoplasma (originally called Haemobartonella) spp.

The blood smear is fixed for 4 minutes in methanol. It is then stained in a coplin jar in 6% stock Giemsa in tap water overnight. The coplin jar is flooded with water to remove the Giemsa. The slide is then dried, rinsed in methanol, rinsed in tap water, redried and examined.

Stock Giemsa: Giemsa 7.6 gm
Glycerine 250 ml
Methanol (AR) 750 ml

The solution is treated as for blood Giemsa

Notes:
1. A commercial stain ("Diff Quik" – Lab-Aids Pty Ltd) can be used for 2 and 3.
2. Mycoplasma (Haemobartonella) canis and Mycoplasma spp. in cats are usually found on the erythrocytes and may take one of several forms including chains of cocci, rods, bows and rings, or it may appear as a single coccus.

4. Examination of the Peripheral Blood Smear
Under low power note:

(i) if the leukocytes are well distributed. A bad film will show uneven distribution, with cells collecting at the margins or in the tail of the smear;
(ii) if there is a rouleaux formation;
(iii) if there is red cell agglutination;
(iv) if there are leukocyte clumps;
(v) if there are platelet or fibrin clots;
(vi) if there are any extracellular parasites present.

Under the oil objective (100x) note:

(i) Leukocyte Morphology

Note the presence of any primitive or immature leukocytes e.g. myelocytes, metamyelocytes. Note the presence of any atypical or abnormal leukocytes, e.g. atypical mononuclears, plasma cells. Note if the neutrophils show any toxic granulation, vacuolation and any inclusions. For the appearance of cell types please consult posters (supplied) and text books

In the differential leukocyte count, an attempt is made to determine the percentage distribution of the various leukocyte types in peripheral blood. The procedure is subject to considerable error, since an extremely small portion of the total number of leukocytes is observed. This error can be decreased by counting a large number of cells: at least 100 cells for every $10 \times 10^9$/L cells in the total white blood cell count.

From the results each leukocyte type is expressed as a percentage of the total count. However, the important value for each leukocyte type is the absolute number per litre of blood.

Absolute value for a leukocyte type = total leukocyte count x percentage of leukocyte type.

The absolute values determine the following: the presence of neutrophilia, neutropenia, eosinophilia, eosinopenia, lymphocytosis, lymphopenia and monocytosis.

However, the presence of a left shift can be determined from the percentage values for the segmented and immature neutrophils e.g. the dog has a normal ration of $> 16-18$ segmented neutrophils to 1 immature form; for a left shift to be definitely present, the ratio would have to be below that normal ratio. For instance, 60% segmented neutrophils and 5% band neutrophils. The ratio is 12:1, thus a left shift is presumed to be present. For dogs and cats, it is accepted that bands greater than $1.0 \times 10^9$/L indicate a left shift. The ratio of bands to segmenters is only used for values less than $1.0 \times 10^9$/L.

If there are any nucleated red cells present in the smear, they are usually recorded either as
numbers per 100 WBCs counted or as an absolute value derived from the initial leukocyte count. If there are over 5 nucleated red cells per 100 WBC’s present, the total leukocyte count should be corrected before determining the absolute values for leukocyte types.

Corrected total = \frac{\text{initial total leukocyte count} \times 100}{\text{leukocyte count} \left[ 100 + \text{nucleated RBCs (per 100 WBCs)} \right]}
Leukocyte morphology

This table is modified from one in *Outline of Veterinary Clinical Pathology* by Maxine M. Benjamin, 2nd Edition, The Iowa State University Press. 1964

### MORPHOLOGY OF BLOOD CELLS USING ROMANONSKY TYPE STAINS

<table>
<thead>
<tr>
<th>CELL</th>
<th>Size (microns)</th>
<th>NUCLEUS</th>
<th>Staining Quality</th>
<th>Chromatin</th>
<th>Nucleoli</th>
<th>CYTOPLASM</th>
<th>Colour</th>
<th>Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shape</td>
<td></td>
<td>Quality</td>
<td></td>
<td>Relative Amount</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Granulocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Myeloblast</td>
<td>15-20</td>
<td>Round or oval</td>
<td>Light purple</td>
<td>Finely reticulated</td>
<td>2 - 5 Pale Blue</td>
<td>Small</td>
<td>Deep blue</td>
<td>--</td>
</tr>
<tr>
<td>b. Progranulocyte</td>
<td>14-20</td>
<td>Round or oval</td>
<td>Purple</td>
<td>Slightly coarse</td>
<td>1-3 Small</td>
<td>Blue</td>
<td>Few azurophilic</td>
<td></td>
</tr>
<tr>
<td>c. Myelocyte</td>
<td>10-18</td>
<td>Round or oval</td>
<td>Purple</td>
<td>Fairly coarse</td>
<td>--</td>
<td>Moderate</td>
<td>Bluish pink</td>
<td></td>
</tr>
<tr>
<td>d. Metamyelocyte (Juvenile)</td>
<td>10-18</td>
<td>Indented oval, resembling kidney or bean</td>
<td>Deep purple</td>
<td>Coarse</td>
<td>--</td>
<td>Large</td>
<td>Pink</td>
<td></td>
</tr>
<tr>
<td>e. Band cell (Stab)</td>
<td>10-15</td>
<td>Curved with parallel sides</td>
<td>Purplish blue</td>
<td>Coarse</td>
<td>--</td>
<td>Large</td>
<td>Pink</td>
<td></td>
</tr>
<tr>
<td>f. Segmented - Neutrophil</td>
<td>10-15</td>
<td>Ragged or divided into lobes 2-3 lobes</td>
<td>Purplish blue</td>
<td>Coarse</td>
<td>--</td>
<td>Large</td>
<td>Faint pink</td>
<td></td>
</tr>
<tr>
<td>- Eosinophil</td>
<td>10-15</td>
<td>Oval or sl. indented</td>
<td>Deep reddish blue</td>
<td>Fine to sl Coarse.</td>
<td>Occasional</td>
<td>Large</td>
<td>Dark to med. blue</td>
<td></td>
</tr>
<tr>
<td>- Basophil</td>
<td>10-15</td>
<td>Outline covered with granules</td>
<td>Pale blue</td>
<td>Indistinct</td>
<td>--</td>
<td>Moderate</td>
<td>Bluish pink</td>
<td></td>
</tr>
<tr>
<td>2. Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Lymphoblast</td>
<td>10-18</td>
<td>Round or oval</td>
<td>Lt. reddish purple</td>
<td>Fine</td>
<td>1-2 Pale blue</td>
<td>Small</td>
<td>Deep blue</td>
<td>--</td>
</tr>
<tr>
<td>b. Prolymphocyte</td>
<td>10-18</td>
<td>Oval or sl. indented</td>
<td>Deep reddish blue</td>
<td>Fine to sl Coarse.</td>
<td>Occasional</td>
<td>Large</td>
<td>Dark to med. blue</td>
<td></td>
</tr>
<tr>
<td>c. Lymphocyte</td>
<td>6-18</td>
<td>Round, oval or sl. indented</td>
<td>Deep purplish blue</td>
<td>Large coarse clumps</td>
<td>--</td>
<td>Small to moderate</td>
<td>Sky blue</td>
<td>Few azurophilic</td>
</tr>
<tr>
<td>3. Monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Monoblast</td>
<td>14-18</td>
<td>Round or oval</td>
<td>Deep purple</td>
<td>Fine retic.</td>
<td>1-2</td>
<td>Moderate</td>
<td>Deep blue</td>
<td></td>
</tr>
<tr>
<td>b. Promonocyte</td>
<td>14-18</td>
<td>Irregular</td>
<td>Purple</td>
<td>Fine retic.</td>
<td>0-1</td>
<td>Moderate</td>
<td>Gray blue</td>
<td></td>
</tr>
<tr>
<td>c. Monocyte</td>
<td>12-18</td>
<td>Round, indented, band or lobed</td>
<td>Pale purple</td>
<td>Fine strands</td>
<td>--</td>
<td>Large</td>
<td>Gray or gray blue</td>
<td></td>
</tr>
<tr>
<td>4. Erythrocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Rubriblast</td>
<td>14-19</td>
<td>Round</td>
<td>Purple</td>
<td>Fine stippled</td>
<td>Usually 1-2</td>
<td>Small</td>
<td>Deep blue to gray blue</td>
<td>--</td>
</tr>
<tr>
<td>b. Prorubricyte</td>
<td>10-15</td>
<td>Round</td>
<td>Purple</td>
<td>Coarse</td>
<td>Usually 1</td>
<td>Moderate</td>
<td>Blue to pink</td>
<td>--</td>
</tr>
<tr>
<td>c. Rubricyte</td>
<td>8-12</td>
<td>Round</td>
<td>Deep purple</td>
<td>Dense purple</td>
<td>Definite</td>
<td>--</td>
<td>Moderate</td>
<td>Pink</td>
</tr>
<tr>
<td>d. Metarubricyte (Late Normoblast)</td>
<td>7-10</td>
<td>Pyknotic, fragmented, or partially extruded</td>
<td>Dense purple</td>
<td>Solid</td>
<td>--</td>
<td>Moderate</td>
<td>Pink</td>
<td></td>
</tr>
<tr>
<td>e. Erythrocyte</td>
<td>4-8</td>
<td>Total</td>
<td>Pink</td>
<td>--</td>
<td>Total</td>
<td>Pale blue</td>
<td>Azurophilic</td>
<td></td>
</tr>
<tr>
<td>5. Thrombocytes</td>
<td>2-4</td>
<td>Total</td>
<td>Pale blue</td>
<td>--</td>
<td>Total</td>
<td>Azurophilic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Platelets)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>


Erythrocyte Morphology

Note any variation in size (anisocytosis) and the presence of microcytes, macrocytes. Note any variation in shape (poikilocytosis) and the presence of target cells, burr cells (acanthocytes) and spherocytes. Note any variation in colour (polychromasia) and the presence of hypochromatic or polychromatic (polychromatophilic) cells. Note the presence of erythrocyte inclusions e.g. Howell Jolly bodies, punctate basophilia (basophilic stippling) and parasites.

N.B. E.R. bodies (Heinz bodies) can be stained with 0.5% brilliant cresyl blue in normal saline.

Reticulocytes, in the Giemsa stained peripheral blood smear, will appear blue and will be larger than the mature erythrocytes. Accordingly, a blood smear from a regenerative anaemia will show anisocytosis and polychromasia. The presence of nucleated red cells in the blood smear may also occur in regenerative anaemias. However, if in an anaemia, nucleated red cells appear in the blood unaccompanied by anisocytosis and polychromasia, a non-regenerative anaemia associated with abnormal marrow should be suspected.

Erythrocyte Morphology - number of cells per oil field of 200 - 250 erythrocytes

<table>
<thead>
<tr>
<th>ABNORMALITY</th>
<th>SLIGHT</th>
<th>MODERATE</th>
<th>MARKED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anisocytosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>7-10</td>
<td>11-20</td>
<td>&gt;21</td>
</tr>
<tr>
<td>Cat</td>
<td>5-8</td>
<td>9-20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Horse</td>
<td>1-3</td>
<td>4-10</td>
<td>&gt;10</td>
</tr>
<tr>
<td><strong>Polychromasia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>1-2</td>
<td>3-10</td>
<td>&gt;11</td>
</tr>
<tr>
<td>Cat</td>
<td>1-2</td>
<td>3-15</td>
<td>&gt;15</td>
</tr>
<tr>
<td>Horse</td>
<td>rarely observed</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Poikilocytosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td>8-25</td>
<td>26-150</td>
<td>&gt;150</td>
</tr>
<tr>
<td><strong>Hypochromasia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td>1-2</td>
<td>3-10</td>
<td>&gt;10</td>
</tr>
<tr>
<td><strong>Codocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs only</td>
<td>1-2</td>
<td>3-6</td>
<td>&gt;6</td>
</tr>
<tr>
<td><strong>Spherocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species*</td>
<td>1-2</td>
<td>3-10</td>
<td>&gt;10</td>
</tr>
<tr>
<td><strong>Echinocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td>1-2</td>
<td>3-10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Acanthocytes, schizocytes, stomatocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All species</td>
<td>1-2</td>
<td>3-5</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

* spherocytes are not easily identified in species with small erythrocytes (eg cats, ruminants)

(iii) Platelets

Note their size and numbers. See Haemostasis section g (ii) for indirect count methods.
LABORATORY EVALUATION OF HAEMOSTASIS (BLEEDING DISORDERS)

Haemostasis is dependent on vascular integrity, platelet function and numbers, and clotting factors. Consequently, laboratory evaluation of haemostasis involves the determination of these components. Although there are many tests available, these notes concentrate on the simpler and more commonly used coagulation tests. It is advisable to provide samples from control (normal) animals along with the test sample.

a) Bleeding Time

The interval between the occurrence of injury to the blood vessel and the cessation of bleeding is called the bleeding time. The test may be performed on the skin of the ear, the nose, the pad of the foot, or the inside of the lip.

Method:
A thin coat of petroleum jelly is spread over the area, and a moderately deep puncture is made with a number II Bard Parker blade so that the blood flows freely. Do not use pressure. Drops of blood are absorbed onto a filter paper at 30 second intervals, and the time at which bleeding stops is noted.

Normal bleeding time for most animals is 1-5 minutes.

Notes:
Bleeding time is an estimation of vascular integrity and platelet function and numbers, and is not dependent on clotting factors. Therefore, prolonged bleeding times occur in thrombocytopenia, platelet function defects and vessel wall defects. Von Willebrand’s factor deficiency (allows platelet adhesion) will also cause an extended bleeding time. A normal bleeding time occurs in health and in deficiencies of clotting factors.

b) Clotting Time

This test measures the time required for fibrin clot formation in non-anticoagulated blood in vitro.

Method:
A 5 ml syringe is rinsed with normal saline solution immediately before use. 3 ml of blood is withdrawn from a vein. The blood is divided into 1 ml lots and is transferred to 3 clean glass test tubes (8 mm bore). The tubes are maintained in a water bath at 25-37°C. After 3 minutes, the tubes are tilted at 30 second intervals, starting with tube 1; when this clots, i.e. when the tube can be inverted without blood flowing out, the time is recorded. Clotting times are noted for tubes 2 and 3. The average time for coagulation in all 3 tubes is then determined.

Normal values:
3-13 minutes for the dog
8 minutes for the cat
4-15 minutes for the horse
Notes:
Clotting (coagulation) time is not dependent on vascular integrity or platelet function and numbers (as long as glass tubes are used). Clotting in vitro is primarily due to activation of the intrinsic pathway of clotting factors by direct contact with glass. However, in siliconized or plastic tubes, platelet factors are important in activating the intrinsic pathway of clotting factors. Also, clotting time is not affected by the extrinsic system of clotting factors as this system is activated only by the release of tissue thromboplastin from injured vascular endothelium.

Prolonged clotting times occur in deficiencies or inhibition of intrinsic or common pathways of clotting factors. Normal clotting times occur in health, thrombocytopenia, platelet function defects and vessel wall defects.

*the deficiency must be less than 5% of the normal level before clotting time is extended.

c) Clot Retraction

Clot retraction in vitro is primarily a function of platelets, although the fibrinogen content of the blood does have an influence.

Method:
5 ml of blood is placed into a clean, dry glass test tube (with no added anticoagulant) and incubated at 37°C. A normal clot will retract markedly within 2-4 hours and by the end of 24 hours will be a compact mass.

Notes:
The above is purely a screening test and more quantitative methods are available. Clot retraction is impaired primarily by platelet function and number defects. It may be impaired in afibrinogenaemia and anaemia. When fibrinolysis is very active, the fibrin clot may be dissolved as quickly as it is formed, and clot retraction will obviously be impaired.

d) Fibrinogen Estimation

Method: A
2 microhaematocrit tubes are filled with blood containing EDTA and centrifuged for 5 minutes as would routinely be done for determining packed cell volume. The plasma protein level is determined from a tube by using the refractometer. The second tube is placed in a water bath at 56°C for 3 minutes. This will precipitate fibrinogen, which is removed from the plasma by further centrifugation for 5 minutes. The total plasma protein of the sample in the heated tube is determined with the refractometer. Fibrinogen concentration is calculated by subtracting the protein value of the plasma in the heated tube from that of the unheated tube.

Method B (Millar Method - suggested method))
A microhaematocrit tube is filled with blood containing EDTA and centrifuged for 5 minutes as would routinely be done for determining packed cell volume. The tube is placed in a water bath at 56°C for 3 minutes. Making sure that all the fluid is immersed. This will precipitate fibrinogen, which is packed on top of the buffy coat by further centrifugation for 5 minutes. The microhaematocrit tube is then attached to a slide so that measurements can be made of the different interfaces in the tube using the microscope's scale and its vernier. If the microscope does not have a scale, an ocular micrometer eyepiece can be used. For this calculation the interface between the buffy coat and the precipitated fibrinogen is called A. The interface between the precipitated fibrinogen and the serum is called B. The base of the meniscus of the plasma (now serum) is called C. The length of the column of the precipitated fibrinogen (AB) is measured in relation to the original length of the column of plasma (AC).
Fibrinogen concentration (g/L) is calculated from the formula:

\[(B - A) \times \frac{100}{(C - A)}\]

Normal fibrinogen (Factor I) levels:
- 2-4 g/L for the dog
- 1-3 g/L for the cat
- 2-4 g/L for the horse

Notes:
(These methods are limited in their ability to detect reduced levels. Other more sensitive methods are available to detect reduced fibrinogen.)

Fibrinogen (Factor I) levels are increased in a wide variety of inflammatory and tissue destructive conditions. They can be decreased in certain chronic liver diseases, D.I.C. and genetic disorders. Fibrinogen levels can have an effect on the results of certain coagulation tests.

e) Prothrombin Time (ProT, PT or OSPT)

Measures the time required for fibrin clot formation in recalcified, fresh, citrated plasma after addition of tissue thromboplastin (which should be absent if the sample was taken correctly) in vitro i.e. it measures the extrinsic (tissue) and common pathways' clotting factors. Sometimes referred to as one stage prothrombin time (OSPT)

Notes:
The PT is prolonged only when the deficient factor is less than 30% of the normal level. A prolonged PT occurs in deficiencies or inhibitions of extrinsic and common system clotting factors, and in heparin inhibition (if heparinized blood had been given by transfusion). A normal PT occurs in deficiencies or inhibitions of intrinsic system clotting factors and in health.

f) Partial Thromboplastin Time (PTT)

Measures the time required for fibrin clot formation in recalcified, fresh, citrated plasma after addition of a contact activator in vitro (which activates the intrinsic [intravascular] pathways of clotting factors).

The PTT yields the same, but more precise, information as clotting time, i.e. it measures the intrinsic and common pathways of clotting factors.

Note: The PTT is prolonged only when the deficient factor is less than 30% of the normal level. The PTT is very low in birds and reptiles.

The PT and PTT methods are the laboratory methods of choice and are now automated on a machine available for veterinary practice
g) Platelet (Thrombocyte) Count

Direct methods are available, but this section will only provide the indirect method, as that can be done simply in veterinary practice.

Indirect Methods:
To cross check or estimate platelet numbers, the indirect methods can be employed. These methods have clinical application only when more sophisticated methods are not available.

A. From the stained blood film, under oil immersion, the number of platelets per 100 leukocytes can be estimated.

**Calculation:** \[ \text{platelets (x } 10^9\text{) per Litre} = \text{number of platelets per 100 leukocytes} \times \text{total leukocyte count} \]

B. When using the Olympus microscope with the 100X oil immersion lens, an estimate of the number of platelets, in the peripheral blood smear, can be made by taking an average of 5 scattered fields and referring to the table below. *This mainly works for the dog*, and when there is not a severe anaemia present (upsets the relativity). This is an estimate only because the count does vary on the type of microscope, objective size and quality, and the ocular size and quality.

<table>
<thead>
<tr>
<th>Number</th>
<th>Estimate X 10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>103</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>15</td>
<td>298</td>
</tr>
<tr>
<td>20</td>
<td>396</td>
</tr>
<tr>
<td>25</td>
<td>494</td>
</tr>
<tr>
<td>30</td>
<td>592</td>
</tr>
<tr>
<td>35</td>
<td>690</td>
</tr>
<tr>
<td>40</td>
<td>780</td>
</tr>
</tbody>
</table>

**Notes:**
The platelet count assesses the presence of thrombocytopenia e.g. less than 200 x 10^9 per litre for the dog. However, spontaneous haemorrhages (thrombocytopenia purpura) will not usually occur until the count is much lower than the minimum e.g. below 50-70 x 10^9 per litre for the dog, or there is a concomitant platelet defect.

Thrombocytopenia may occur due to marrow damage, splenic disorders (stores a third of the platelets at any one time), accelerated use e.g. D.I.C. (disseminated intravascular coagulation), and immune-mediated destruction e.g. idiopathic thrombocytopenia purpura. Thrombocytopenia due to marrow damage is accompanied by decreased numbers of megakaryocytes, thrombocytopenia due to other causes usually has normal to increased numbers of megakaryocytes.
MISCELLANEOUS PROCEDURES

a) Bone Marrow Examination

Bone marrow samples can be collected from the iliac crest, femur, rib and sternum. The site will depend on the species and size of the animal.

(i) Making the smear

Methods of aspiration are available in most texts. As bone marrow rapidly clots, a sample is usually withdrawn in a syringe washed out with EDTA (make an aqueous solution of 2 ml from a vial with dried EDTA normally used for blood collection). Only a small amount of bone marrow should be aspirated (stop when it enters the main barrel of the syringe) and transferred to a watchglass. The bone marrow particles (white flecks) are carefully aspirated with a pasteur pipette so as to leave behind as much of the peripheral blood as possible. They are transferred to a cleaned glass slide, gently pushed into a mound and then squashed between two slides ("T" or "L" method). The pressure of squashing will vary between individual but it is usually mild to moderate. A correctly made bone marrow smear should be elliptical and have any peripheral blood present around the periphery of the squashed bone marrow particles. The slide is rapidly air dried, fixed in methanol and stained routinely with Giemsa but may be stained with prussian blue (for iron) and peroxidase (for the granulocytes).

(ii) Examination of the Stained Smear

The degree of cellularity can be assessed under low power.

Under oil immersion, a differential cell count can be performed. The most reliable results are obtained in the tail area of the smear, where 500-1000 cells are counted.

From the differential cell count: the myeloid to erythroid (M:E) ratio (expresses the proportion of total granulocytes to nucleated erythroid cells), the proportions of myeloid and erythroid cells in the proliferating pools (promyelocytes and myelocytes for myeloid, usually less than 20%; pronormoblasts and early normoblasts for erythroid, usually less than 10%), and the proportion of megakaryocytes and other cell types can be determined.

M:E ratios:  
1.2:1 for the dog  
1.5:1 for the cat  
1.1-10.20:1 for the horse

Notes:  
Bone marrow examination is useful for:

1. Investigating non-regenerative anaemias in most species.  
   In the horse it can be useful for investigating all anaemias.  
2. Establishing or confirming the presence of a myeloproliferative disorder.  
3. Investigating a platelet disorder.  
4. Investigating a myelophthisic disorder.

b) Cross-matching Blood for Transfusion

Saline Cross-Match (dog)  
This is the most simple method available. Although it is not as sensitive as the antiglobulin test, reagents are readily available whereas for the antiglobulin method they are hard to obtain
and very expensive.

a) Samples
The sample of choice is clotted blood. From this sample both serum and cells may be obtained. After the sample has clotted it should be centrifuged to separate both serum and the cells. The serum is stored at 4°C. The clotted blood should not be refrigerated until the serum and cells have been removed. The serum is only stable for up to 24 hours due to loss of complement. It is best to use fresh serum, but plasma must not be used as anticoagulants are anti-complement. Red cells are teased from the clot and used within 24 hours. If there is any difficulty in obtaining cells from the clot then cells from the EDTA sample may be used. Cells obtained in such a manner should be washed three times in buffered normal saline before use. If there is no alternative, cells collected in ACD (Acid Citrate Dextrose) may be used and these are stable for several days at 4°C.

b) Technique
The clotted blood sample is centrifuged and the serum removed. The clotted samples need to be completely clotted - if the request is urgent, then the clotting process may be speeded up by placing the samples in the water bath at 37°C. After the serum is removed a little normal saline is added to the clot and the clot broken up with a glass rod to free the cells. Some of the freed red cells are placed into a test tube and centrifuged at 200g for one minute. Take care that there is no clots in the cells. The saline is replaced and the process repeated twice more. The saline on the last wash is removed and a 3% suspension of red cells is made in LISS (1 drop of cells and 32 drops of LISS). If LISS is not available saline may be used. Both donor and recipient samples are treated in the same manner.

Duplicates of each of the following tubes are prepared.

<table>
<thead>
<tr>
<th></th>
<th>1 drop</th>
<th>2 drops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control</td>
<td>Recipient Cells</td>
<td>saline (LISS)</td>
</tr>
<tr>
<td>Auto-Control</td>
<td>Recipient Cells</td>
<td>Recipient Serum</td>
</tr>
<tr>
<td>Donor Test</td>
<td>Donor Cells</td>
<td>Recipient Serum</td>
</tr>
<tr>
<td>Donor Control</td>
<td>Recipient Cells</td>
<td>Donor Serum</td>
</tr>
</tbody>
</table>

Tubes 3 and 4 are repeated for each additional donor.

One set of tubes is placed in a 37°C waterbath and the other left at room temperature for 30 minutes. The room temperature should be in the range of 20-24°C, preferably closer to 24°C as below 20°C the test is affected by cold agglutinins. It is best to run at both temperatures as the literature is in conflict as to the ideal temperature and until this is determined it is best to cover all possibilities.

At the end of 30 minutes the tubes are centrifuged for 1 minute at 750 G (slow speed) and then examined for haemolysis and/or macro-agglutination. Negative tubes should then be examined for micro-agglutination. If any doubt exists with the result then the blood is declared incompatible.

Notes:
Care must be taken not to confuse rouleaux for agglutination. The donor dogs are commonly checked for microfilaria prior to use. All animals having a second transfusion must be cross-matched. Breeding bitches that have already had litters should be cross-matched using the antiglobulin test as well as the saline cross-match.

The horse is cross-matched using the antiglobulin test or the haemolysin test.
The cat is commonly blood typed for transfusions since the saline cross-match often produces inadequate results.

c) Blood typing for cats and dogs

Blood typing for dogs and cats is now available for the veterinary practitioner and has, in many cases, replaced the need for cross matching. It is recommended that all cats undergoing a transfusion be typed as there may be high levels of circulating isoantibodies to incompatible blood groups. Dogs undergoing a transfusion for the first time usually do not have significant levels of isoantibodies to incompatible blood groups. These usually develop after the initial transfusion. Consequently, typing of dog erythrocytes is optional for first transfusions but essential for second and subsequent transfusions.

**RapidVet-H (Feline)** (Kansas State University and dms/agrolabo products ag, Neuhausen am Rheinfali, Switzerland): is intended for use to classify cats as blood group Type A, Type B or Type AB. This is one blood group system in the cat, with the majority of cats possessing the A antigen. One third of the cats possessing A antigen have naturally occurring, low-titred, anti-B antibody. All Type B cats have naturally occurring, highly titred anti-A antibody. Type AB cats are rare and do not have or develop anti-A or anti-B antibodies. Cats with B erythrocytes will develop a marked anaphylactic and haemolytic response to Type A blood because of their natural, high-titred anti-A antibodies. Cats with A erythrocytes and natural, low-titred anti-B antibodies will exhibit only a mild reaction when transfused with Type B blood for the first time (but significant haemolysis can occur over a period of time). Cats with AB erythrocytes do not usually develop transfusion reactions.

The kit assay is based on the agglutination reaction that occurs when an erythrocyte which contains either a Type A, Type B or a Type AB antigen on its surface membrane interacts with lyophilized specific antisera present on the test card. Whole blood collected in EDTA and test diluent are added to the Auto-agglutination Saline Screen well, the Patient Type A well and the Patient Type B well and mixed. The test card is rocked until agglutination occurs. The Patient Type A well agglutinates if the cat is Type A, the Patient Type B well agglutinates if the cat is Type B. Both wells agglutinate if the cat is AB. There are limitations to the test which are discussed in the information sheet.

**RapidVet-H (Canine DEA 1.1)** (Kansas State University and dms/agrolabo products ag, Neuhausen am Rheinfali, Switzerland): is intended for use to classify dogs as DEA 1.1. Eight specific antigens have been identified on the surface of canine erythrocytes. The internationally accepted canine blood group system, the DEA (Dog Erythrocyte Antigen), is based on these antigens. The antigens are DEA 1.1-1.8. DEA 1.1 and 1.2 are the most significant. Both are highly antigenic but DEA 1.1 is the primary lytic factor in canine transfusions. Dog erythrocytes with DEA 1.1 have the greatest potential to stimulate formation of isoantibodies. Thus most reactions resulting from the transfusion of incompatible cells occur when DEA 1.1 positive blood is given to DEA 1.1 negative recipients. DEA 1.2 and 1.7 may produce minor transfusion reactions. Greyhounds are particularly low in DEA 1.1, 1.2 and 1.7 antigens and, therefore, are useful donors. About 40% of dogs are DEA 1.1 positive. A DEA 1.1 positive dog can receive both DEA 1.1 positive and negative blood. A dog that is DEA 1.1 negative should not receive DEA 1.1 positive blood.

The kit assay is based on the agglutination reaction that occurs when an erythrocyte which contains a DEA 1.1 antigen on its surface membrane interacts with a murine monoclonal antibody proven specific to DEA 1.1 which is lyophilized on the test card. Whole blood collected in EDTA and test diluent are added to the Auto-agglutination Saline Screen well. Positive Control fluid is added to the DEA 1.1 Positive Control well. Whole blood collected
in EDTA is added to the Patient Test well and mixed. The test card is rocked until agglutination occurs. The Positive Control should agglutinate. If the Patient Test agglutinates then the animal is DEA 1.1 positive. There are limitations to the test which are discussed in the information sheet.

d) The Blood/Erythrocyte Sedimentation Rate (BSR:ESR)

When blood containing an anti-coagulant is allowed to stand in a tube in a vertical position, the red cells will gradually settle, leaving the plasma as a clear supernatant. The rate of sedimentation over a specified time is known as the sedimentation rate.

Method:
The Wintrobe method entails the use of a tube, in which 10 cm are graduated from the base in mm. The bore is 2.5 mm to 3 mm and the volume approximately 0.7 ml. The tube is filled to the 10 cm mark with blood and allowed to stand vertically for one hour, for the dog and cat, at which time a reading is taken of the sedimentation of the cells (for the horse, readings are taken at 10, 20, and 30 minutes).

The test should be set up within 2 hours of collection of the sample (in EDTA). As variations in temperature affect the sedimentation rate, care should be taken to ensure that the test is performed under standard conditions (usually 22-27°C). The rate of sedimentation is obtained by reading from the top of the blood plasma to the top of the layer of sedimented erythrocytes in mm.

Ideally, the result should be corrected for PCV as the ESR varies inversely with PCV.

Significant ESR difference = observed ESR - anticipated ESR at that PCV (read from a table)

Notes:
The usefulness of the ESR has been questioned. The ESR is a non-specific reaction which does not aid differential diagnosis. Even a normal ESR does not exclude the possibility that a disease process exists. Today, it is only utilised by a few equine veterinarians. Elevated ESR may be found in infections, skin alterations, tissue injury or destruction and pregnancy. A depressed ESR may occur when there are significant numbers of abnormal or immature red cells, and when there is a low total plasma protein e.g. chronic liver disease, poor nutrition.
## REFERENCE VALUES FOR HAEMATOLOGY

**VETERINARY PATHOLOGY DIAGNOSTIC SERVICES**  
**FACULTY OF VETERINARY SCIENCE**  
**UNIVERSITY OF SYDNEY**

<table>
<thead>
<tr>
<th></th>
<th>DOG</th>
<th>CAT</th>
<th>HORSE (adult)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV L/L</td>
<td>0.37-0.55</td>
<td>0.30-0.45</td>
<td>0.32-0.52</td>
</tr>
<tr>
<td>Plasma protein g/L</td>
<td>55-75</td>
<td>59-78</td>
<td>58-84</td>
</tr>
<tr>
<td>Haemoglobin g/L</td>
<td>100-150</td>
<td>80-140</td>
<td>110-160</td>
</tr>
<tr>
<td>Erythrocytes x10^{12}/L</td>
<td>5-7</td>
<td>6-10</td>
<td>8-11</td>
</tr>
<tr>
<td>MCV fl</td>
<td>60-75</td>
<td>40-45</td>
<td>41-49</td>
</tr>
<tr>
<td>MCHC g/L</td>
<td>300-350</td>
<td>310-350</td>
<td>300-360</td>
</tr>
<tr>
<td>MCH pg</td>
<td>20-25</td>
<td>13-17</td>
<td>13-16</td>
</tr>
<tr>
<td>Fibrinogen g/L</td>
<td>2-4</td>
<td>1-3</td>
<td>2-4</td>
</tr>
<tr>
<td>Leukocytes x10^9/L</td>
<td>7-12</td>
<td>8-14</td>
<td>6.0-13.0</td>
</tr>
<tr>
<td>Neutrophils seg cells/10^9/L</td>
<td>4.06-9.36</td>
<td>3.76-10.08</td>
<td>2.47-6.96</td>
</tr>
<tr>
<td>Lymphocytes band cells/10^9/L</td>
<td>0-0.24</td>
<td>0-0.42</td>
<td>0-0.24</td>
</tr>
<tr>
<td>Monocytes cells/10^9/L</td>
<td>0.91-3.6</td>
<td>1.6-7.0</td>
<td>1.6-5.4</td>
</tr>
<tr>
<td>Eosinophils cells/10^9/L</td>
<td>0.21-0.96</td>
<td>0.08-0.56</td>
<td>0.0-0.72</td>
</tr>
<tr>
<td>Basophils cells/10^9/L</td>
<td>0.14-1.2</td>
<td>0.16-1.4</td>
<td>0.16-0.96</td>
</tr>
<tr>
<td>Platelets x10^9/L</td>
<td>200-900</td>
<td>300-700</td>
<td>100-300</td>
</tr>
<tr>
<td>Reticulocytes %</td>
<td>0-1.5</td>
<td>0-1.0</td>
<td>0</td>
</tr>
</tbody>
</table>

*There may be marked variations in normal values due to age, sex, breed and use. These are crude approximations only. They are not necessarily applicable to results from other laboratories.*
THE HAEMOCYTOMETER

The improved Neubauer haemocytometer is used here (see Figure 1). A special optical plane coverslip must be used which is thicker than an ordinary coverslip.

The depth between the coverslip and the ruled area is 0.1 mm. The ruled area is 9 mm² and is divided into nine large squares. Each of the four large corner squares is divided into 25 smaller squares. The central large square is divided into 400 tiny squares arranged in 25 groups of 16 by triple boundary lines.

There are two ruled areas per haemocytometer separated by a moat. When filling a haemocytometer the clean coverslip is placed on the clean haemocytometer and the diluted sample run in each side. The chamber must be filled quickly (i.e. reduce error in distribution) and must fill the raised area completely without the sample flowing into the moat.

When counting, those cells touching or lying on the upper or left boundaries of the squares are included while those on the right and lower boundaries disregarded (or vice versa). For cell counts it is the usual routine for both chambers to be counted and the mean count used for the calculation.

The total volume of the ruled area is 0.9 μl (mm³) with each large square 0.1 μl. From these values and the dilution factor it is a simple matter to calculate the cells per litre of blood.

For Leukocytes (when using Unopettes) this is: count \( \times 10^\frac{9}{9} \times \text{D.F.} \times 10^6/\text{Litre} \)

\[ \text{D.F. (Dilution factor)} = 100 \]

\[ 10^6 \text{ brings the total volume to one litre} \]

A simple calculation although not totally correct is:

\[ \frac{\text{count} \times 10^\% \times 10^9/\text{Litre}}{10} \]

For Erythrocytes the volume counted is 5/25 of 0.1 μl (i.e. 1/ 50μl).

The calculation is thus:

\[ \text{count} \times 50 \times \text{D.F.} \times 10^6/\text{Litre} \]

if D.F. = 200 then

\[ \text{count} \times 50 \times 200 \times 10^6 \]

Put simply

\[ \frac{\text{count} \times 10^{12}/\text{Litre}}{100} \]

The same approach may be used for all other cells counts using the haemocytometer.
All 9 squares are counted for white cell analysis (x10 objective).
5 squares in the central square are counted for red cell analysis (x40 objective).
MICRO HAEMATOCCRIT SCALE