A review of basic haematology

WHETHER you are already using an in-house laboratory, thinking of setting one up, or using a commercial laboratory, it is always worth reviewing the processes used in haematology to make sure you are getting the best from your samples.

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of Greendale Veterinary Diagnostics, review the processes used to ensure that you get the best results possible from your samples in the first part of this week's special feature on haematology

Blood collection

Correct blood collection and handling is critical: improper technique can result in inaccurate blood cell counts and morphological artifacts. Sample quality is *the* major contributor to analytical errors.

Where possible, it is advisable to use jugular puncture to minimise cell damage during sampling. 21 gauge needles should be used in dogs and 23 gauge in cats; remove needle first and avoid rapid aspiration and transfer of blood into the sample tube which can result in cell lysis.

For haematology you need liquid blood, so it is vital that blood should be drawn into tubes or syringes that contain anticoagulant-EDTA is the anti-coagulant of choice for most haematology, although it can cause platelet clumping in cats. Heparin can cause clumping and poor staining of leukocytes, so should not be used routinely for haematology.

Smears should be prepared as soon after collection as possible; prolonged exposure to EDTA produces artifacts in neutrophils and platelets.

If you are using a commercial laboratory it is advisable to submit a freshly-made, unstained smear. This is especially important for bloods obtained from birds and reptiles, as all cells are nucleated and deteriorate very quickly in transit. This will severely compromise interpretation of morphology.

Red cells show increased susceptibility to lysis after 24 hours in EDTA.

Sodium citrate is required for some platelet - and all coagulation - studies.

EDTA tubes should be filled to their capacity. Underfilled tubes have an excessive amount of EDTA relative to the blood volume and can cause shrinkage of RBCs, resulting in a reduction in the MCV.

Invert the tube several times after filling to ensure thorough mixing, but do not shake.

Note that blood from some exotic species haemolyse in EDTA - contact a specialist laboratory.

Handling samples

When handling samples, be aware of the following:

- label each tube clearly, even if testing in-house;
- blood should be processed as soon as possible after collection;
- blood films should be made immediately - if delay is anticipated before processing further, the blood should be refrigerated at 4°C (if kept at room temperature the red blood cells may swell, which raises the PCV and MCV and lowers MCHC);
- excessive time at room temperature can cause autolysis;
- platelet counts are most affected by delays in processing - platelets have short lifespans and tend to clump over time, even in the presence of anti-coagulants; and
- blood samples should be mixed again several times immediately before a portion is removed for testing - avoid excessive mixing to prevent physical trauma to cells (roll or use a mechanical blood mixing device.

Blood films

Well-prepared blood films are vital to allow accurate assessment of the haemogram.

If you suspect blood parasites, for example Mycoplasma spp, it is vital that a smear is made immediately after collection as the organisms quickly detach themselves from the cells (the gold standard in this case is PCR analysis).

Use only new, clean slides. Handle slides by the edges to avoid fingerprints.

Figure 1.

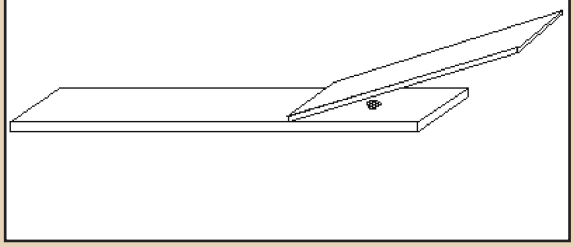


Figure 2.

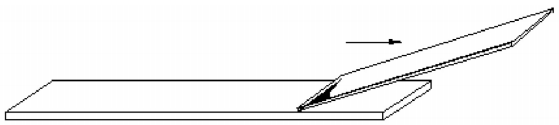
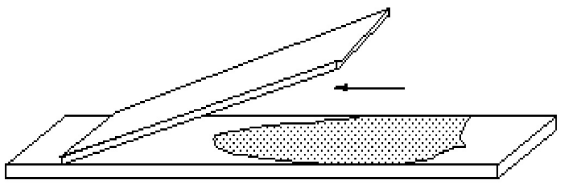


Figure 3.



Figures 1 to 3. Blood Smear preparation.

Blood smear preparation

The procedure for blood smear preparation is shown in Figures 1 to 3.

First, place one small drop of blood (approximately 3 µl) near the end of a clean glass slide. Place another slide at an angle of about 30-45~ to the first. Draw back until it touches the drop of blood in the acute angle between the slides (Figure 1).

Then, after the blood has spread (by capillary action) to within 2-3mm of the edge, push the second slide quickly and smoothly across the full-length of the first to produce a thin smear (Figure 2). For anaemic blood make the smear more quickly, for thick or polycythaemic blood make the smear more slowly.

Finally, make two smears from each sample. Label clearly and, when air-dried, submit in special slide containers if not evaluating them yourself. A well-formed smear has a flame shape (Figure 3). Bear in mind the following precautions:

- do not blot or wipe dry, as this introduces scratches;
- do not refrigerate, as the condensation that forms on cold slides can lyse cells;
- keep away from formalin; and
- do not fix until ready to stain, but keep covered.

Staining

There are several rapid staining kits available for in-practice use. These consist of a three-stage staining procedure, based on a Wright stain, which can be varied in intensity according to requirements.

These stains contain both an acid stain (usually eosin) and a basic stain (such as methylene blue). Structures rich in basic compounds, such as eosinophil granules, bind the acidic dye and are stained red.

Acidic structures, such as DNA/RNA or basophil granules, are stained blue by the basic stain. Neutral structures stain purple. New methylene blue is used for reticulocyte counts and to stain Heinz bodies.

Reticulocyte counts are helpful in assessing the regenerative response of the bone marrow when the haemogram suggests anaemia. Heinz bodies are seen in a number of conditions and represent oxidative denaturation of haemoglobin; further evaluation of the patient and history would be required to establish the cause in each case.

Be aware that Heinz bodies can be seen routinely in low numbers in cat red blood cells. Other Romanovsky-type stains such as May-Grunwald/Giemsa are commonly used in diagnostic laboratories to accommodate the need to evaluate different species and specific disease problems.

Common artifacts/issues

- poor fixation;
- stain precipitate - a stain that is old, or has been left open over a longer period of time, may deposit precipitate on the slide that can be mistaken for haemoparasites (for example Mycoplasma spp), therefore, keep stain fresh and always covered when not in use and regularly filter or replace to minimise precipitate;
- overstaining - in overstained slides, all cells are deeply coloured with important cell details being lost, the red cells appear to be more dense and more basophilic (blue) than normal.
- understaining - in understained slides all the cells are pale, cellular details of the leukocytes are barely distinguishable and red cells are very faint, however, this should not be confused with hypochromia. Practice with staining procedures to avoid overstaining or understaining.

Figure 4 shows some examples of poorly-prepared slides.

Sample evaluation

Blood cell counts alone are not sufficient to adequately evaluate the haemogram.

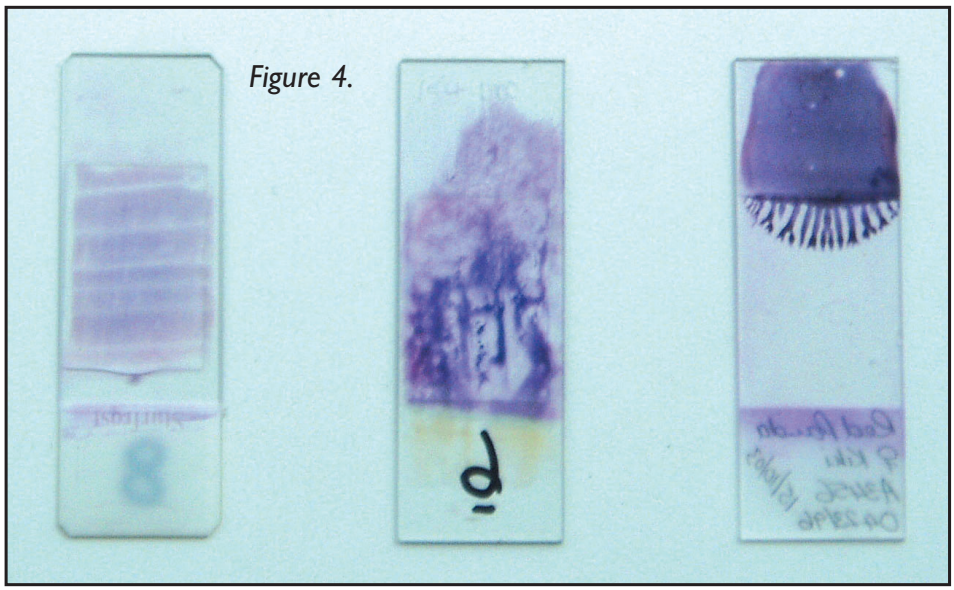


Figure 4.

Figure 4 Examples of poorly-prepared slides.

• Initial low-power examination: Microscopic examination of a blood smear is an essential part of any haematological evaluation, regardless of the methodology to be used for cell counting. The usual site for examination of the stained smear is the small area of monolayered cells between the thick body of the smear and the feathered edge. Leukocytes in this area are flattened and not overlapping or touching and their morphology can be easily recognised.

A systematic approach to evaluation of the blood smear is essential to obtain accurate and complete results. A common error is to begin to identify and count the white cells immediately at high magnification. Failing to observe the characteristics of the leukocytes erythrocytes and platelets. Always precede any cell counting by scanning at low magnification (10x) for rouleaux formation and for RBC. WBC or platelet aggregation. which can cause errors in cell counts in most automated counters. This will allow you to find the most suitable area for the cell count.

The size nature and condition of cells and platelets provide vital information when investigating disease.

Some diseases - blood parasites and certain neoplasms – can be diagnosed directly from examination of the blood film.

Estimate the total number of leukocytes and develop a mental image of the appearance of typical leukocytes of each cell line (neutrophil, eosinophil, lymphocyte, Monocyte, basophil).

Evaluate the red cells for evidence of polychromasia, anisocytosis, Hypochromasia, poikilocytosis, etc.

Note any unusual findings such as atypical cells or parasites.

• **Oil immersion magnification:** Once the counting area has been selected examine erythrocytes and confirm observations made at low magnification (size, shape, colour, abnormalities and any inclusions). This will aid in interpretation of any anaemia and some specific disease processes.

Examine platelet morphology and distribution and estimate relative number. A minimum of six to 10 platelets per high power field is classed as "adequate" (average microscope with wide held of view). If platelets appear to be low, check for clumping (this occurs frequently in cats): look in the tail of the smear using low power magnification. Large platelets are called macroplatelets and can suggest accelerated regeneration in dogs.

Examine leukocyte morphology (abnormalities and inclusions).

Perform differential leukocyte count if not using automated equipment. A minimum of 100 consecutively encountered cells are counted in a systematic manner using the customary "battlement" pattern within the counting area. These are classified into their individual categories, with a category for "other" if required. The total leukocyte count is then multiplied by the percentage of each fraction to give the absolute count.

If using automated equipment it is helpful to estimate the differential count on the smear and compare to that reported by the instrument.

Note that it is possible for automated cell counters to give erroneous results on occasions: if any doubt about the result it is advisable to check manually or submit to a commercial laboratory.

• Packed cell volume (microhaematocrit): The packed cell volume is used to estimate erythroid mass. It is measured after high speed centrifugation of a column of blood (in a microhaematocrit tube) and subsequently a tube reading device. Three distinct layers are produced: the plasma column. A small white band known as the buffy coat (consisting of nucleated cells and platelets) and the packed erythrocytes (Figure 5).

The appearance of plasma in haematocrit tubes can also provide

important information: icterus haemolysis and lipaemia may all be detected.

Micro-haematocrit are accurate and repeatable. Instrumentation and supplies are inexpensive and suitable for all practices.

Do not try to save time by not allowing the sample to spin for long enough. This produces an overestimate of the PCV: as the plasma and cells are not fully separated. Haematocrits may also be computed and reported by in-clinic automated analysers. However these are calculated and may not reflect the true picture in all cases.

Commercial laboratories

Many commercial laboratories offer haematology services at reasonable prices to veterinarians. Leading laboratories have state-of-the-art equipment as they are able to spread the cost over a large number of samples **(Figure 6).**

Counts are performed on automated equipment, supplemented by microscopic examination by trained personnel.

In order to get the best from a commercial laboratory it is always advisable to give full details of clinical history and medication when submitting the samples.

The advantages of using a commercial laboratory are:

- well-run laboratories are enrolled in quality control programmes for example RIQUAS, VLA to ensure accuracy;
- films are evaluated by personnel who process hundreds of smears daily and are therefore more likely to notice and diagnose rare and unusual abnormalities;
- identification of bizarre or neoplastic cells is a job for an expert;
- red cell parasites and inclusions can also be difficult to differentiate and are best submitted for expert confirmation;
- they can provide additional commentary on unusual findings and consultation with veterinary clinical pathologists;
- they often have several trained personnel to discuss or interpret results;
- veterinary advice is offered on further tests and investigations that may assist in a particular case; and
- trained personnel are more likely to pick up faults with the equipment and often have back-up analysers or manual facilities to compensate for problems.

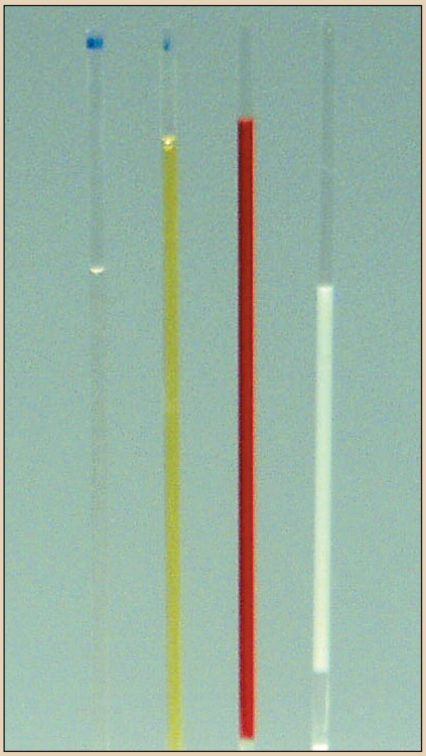


Figure 5. Serum Samples

Figure 6. A centrifuge

The limitations associated with employing a commercial laboratory are:

- time - results are not usually available for several hours (or the next day) which can be an issue for time-sensitive cases or pre-anaesthetic screening;
- fresh blood smears should be prepared in-clinic and sent with EDTA samples - check with your reference laboratory and confirm that blood smears are at least scanned by trained professionals;
- automated haematology equipment must be re-calibrated for each species or it will produce inaccurate results;
- persons evaluating blood smears must be familiar with species differences or they may misclassify white blood cell types and may misinterpret normal variation as a disease condition;
- the largest laboratories have many technicians each of whom evaluates blood films differently meaning a veterinarian cannot assume that all slides are evaluated in the same way; and
- not all laboratories are familiar with evaluating veterinary samples and have veterinary

pathologists on staff you need to find this out or use a veterinary reference laboratory.