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FELINE BLOOD SMEAR PREPARATION AND EVALUATION

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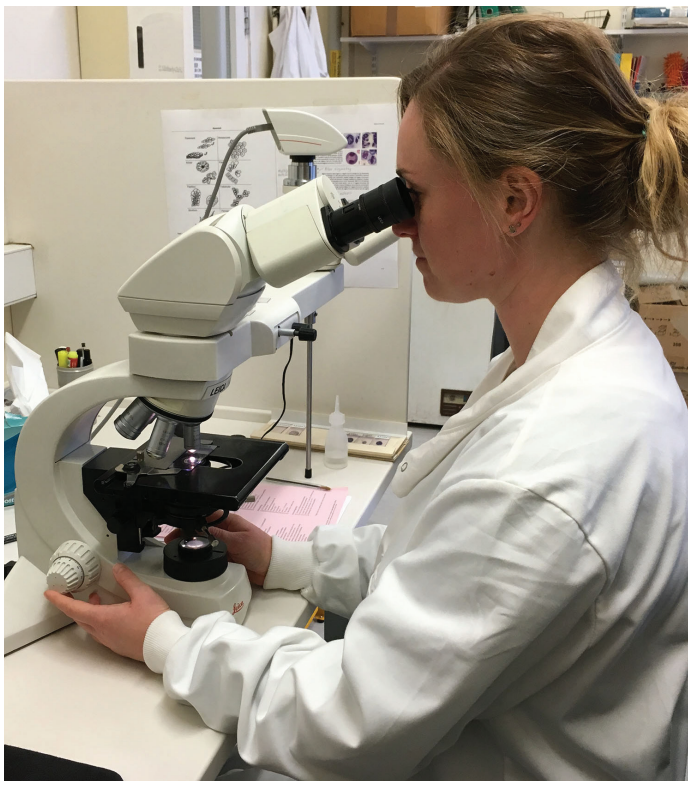
When carrying out a complete blood count, preparation and evaluation of a blood smear should always be performed alongside. A blood film review will help confirm numerical data provided by the analyser and perform a differential cell count.
It will also help identify morphological features that instruments cannot assess, such as left shift (increased immature neutrophils), toxic neutrophils, reactive lymphocytes, atypical white blood cells (WBC), abnormally shaped red blood cells (RBC)- poikilocytes, RBC inclusions, organisms and platelet abnormalities. Although we often rely on external laboratories with experienced personnel and established quality assurance and quality control to provide valuable and more accurate information, blood smear examination can also be performed in house. This can be particularly useful in situations where a rapid assessment is needed or when there are financial limitations. Evaluating a blood smear in-house and then comparing findings with a second smear sent to an experienced laboratory might also be used as a learning tool.

All blood smears in photographs are stained with modified Wright's stain, unless stated otherwise.

Effects of sampling and sample handling

Artefacts can be caused by traumatic blood collection, inappropriate filling of sampling tube or prolonged sample storage.

- Atraumatic venepuncture will prevent the activation of clotting mechanisms and minimize cellular trauma thus preventing erroneous results.
- Blood sampling tubes containing an anticoagulant should be filled as per manufacturer's instructions to avoid falsely decreased haematocrits and cell counts and to prevent RBC shrinkage. EDTA is the preferred anticoagulant for feline blood smears as it preserves cellular morphology better.



A high quality microscope (with eyepiece power of 4x, 10x, 40x and 100x magnification) is recommended in blood smear evaluation.

- Although EDTA preserves cellular morphology better, artefacts can be created by increased exposure to anticoagulants and cell deterioration and can be seen due to storage and shipment. Blood smears prepared shortly after blood collection (preferably within an hour) are always preferred over stored samples. Morphological artefacts due to storage include RBC crenation, changes in neutrophils that resemble toxic changes, such as cytoplasmic basophilia and cytoplasmic vacuolation, nuclear distortion in lymphocytes, and general WBC degeneration.

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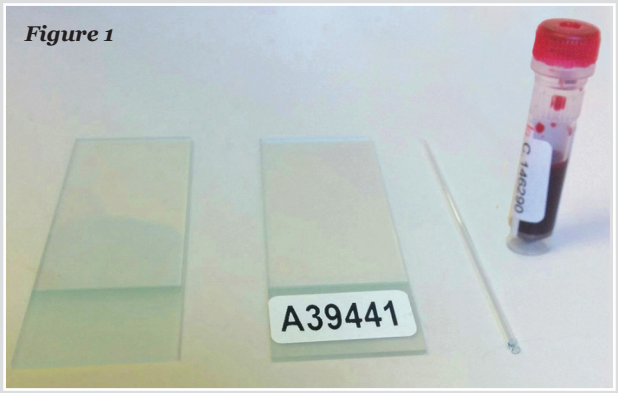

Figure 1

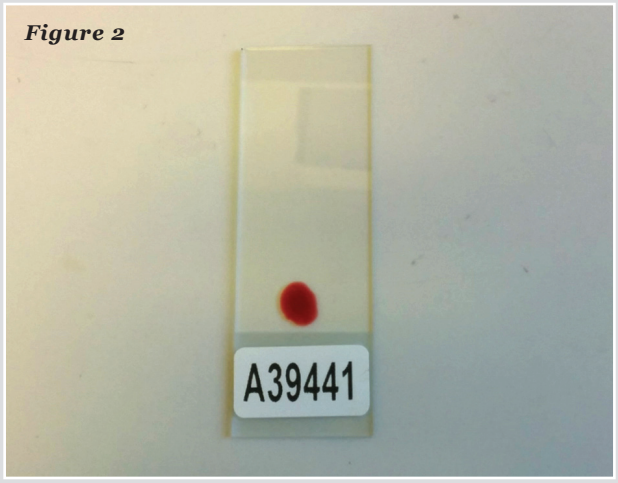

Figure 2

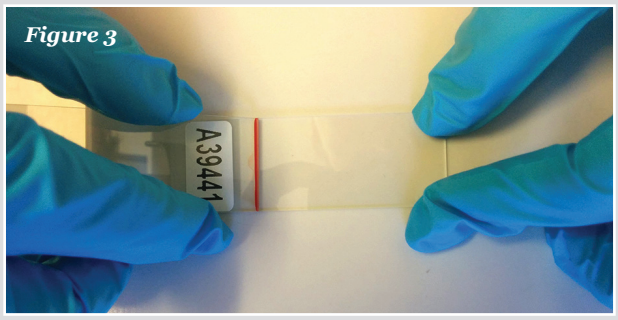

Figure 3

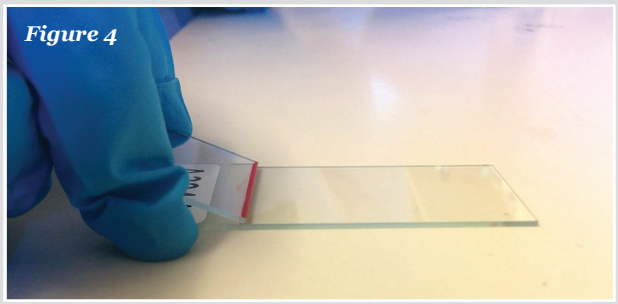
Figure 4

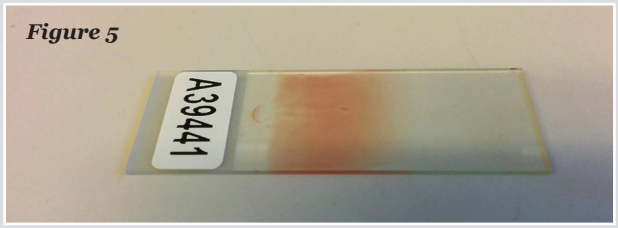

Figure 5

MAKING THE SMEAR

Figures 1-5: Making a blood smear

Materials needed: *Two clean microscope slides (one as a spreader and one for the smear that must be appropriately labelled) and a microhematocrit tube (figure 1). Care should be taken when handling the slide for the smear to prevent holes forming in the smear.*

1. Use a freshly collected blood sample and make sure there are no clots in the tube. Invert the tube gently several times and with a microhaematocrit tube remove a small amount of blood. Tilting the anticoagulant tube with the inserted microhaematocrit tube will help.
2. Place a small drop of the removed blood onto the edge of the slide by gently tapping the microhematocrit tube *(figure 2)*. If the drop is too large, the smear will be too long and thick whereas the opposite may happen if the drop is too small.
3. Take the second slide (the spreader) and place it at a 30-40° angle in front of the drop on the first slide *(figure 3)*. The length of the smear is partly determined by the angle. With a larger angle the smear is very short, with a lower angle, the smear may be too long. Maintain this angle through the duration of the spreading action. For blood samples from cats with a low heamatocrit, you may need to decrease the angle of the spreader; for samples from cats with high hematocrits, you may need to increase the angle of the spreader.
4. Maintaining the appropriate angle *(figure 4)*, slide the spreader towards the drop of blood and when it comes in contact with this wait until the blood spreads along the side of the spreader.
5. Still maintaining the same angle, steadily 'push' the spreader with a sliding movement away from the drop to create the blood smear *(figure 5)*. The speed at which the spreader is moved is also important. If this is done too quickly, the smear will be too short and all the cells will be at the feathered edge. If it is done too slowly, the smear will be too long and lack a feathered edge.
6. Allow the blood smear to air-dry completely before staining. Do not place a blood smear wet in a slide holder and do not store in the fridge as this will affect the morphology of the cells.

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Staining

Rapid stains *(figure 6)* are widely used for in-house staining of blood smear and fine needle aspirates.

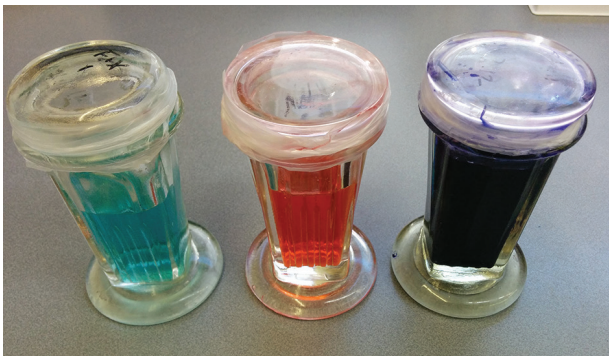

Figure 6: Rapid stain.

Romanowsky-type stains are widely used in veterinary medicine. Wright's stain is commonly used by veterinary laboratories while other rapid Romanowsky-type *(e.g Diff-Quik, Giemsa)* are utilised by veterinary practices.
For optimal results follow manufacturer's instructions and ensure the use of fresh stain as frequently as required. The pots containing the fixative and stains are commonly numbered to ensure the correct order for the staining process. Ideally have a second set of rapid stains for the "dirty" slides such as ear/skin cytology and aspiration of fatty lumps.

Examination: a general approach

A high quality microscope with the proper adjustments and maintenance is needed for blood smear examination. Most of these microscopes are equipped with four objectives (4x, 10x, 40x and 100x oil immersion) and a 10x eyepiece. During the examination the condenser needs to be raised to the top and the iris diaphragm opened.

Regular practice will enable you to review a blood smear within a few minutes. Follow a systematic approach aiming to always evaluate all three cell lines; platelets, erythrocytes and leukocytes.

1. Scan the whole smear at low power using 10x lens (100 magnification). Try to recognize the three areas of a blood smear; feathered edge *(figure 7)*, monolayer *(figure 8)* and body *(figure 9)*.
2. Check the entire feathered edge on a 10x lens for platelet clumps *(figure 10)*, large abnormal cells and parasites (e.g. filaria). If in any doubt, use the 100x oil lens to have a more detailed examination.

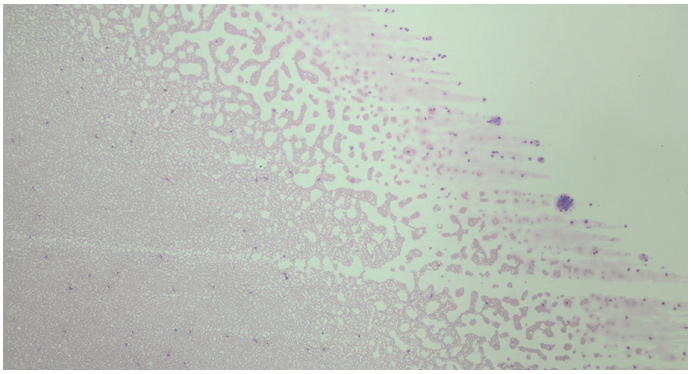

Figure 7: Feathered edge.

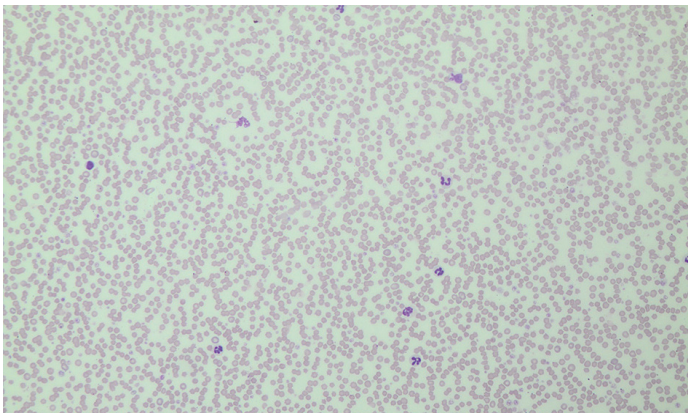

Figure 8: Monolayer.

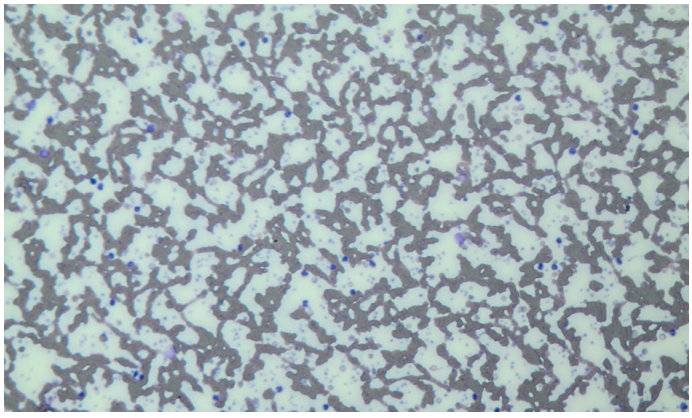

Figure 9: Body of the smear.

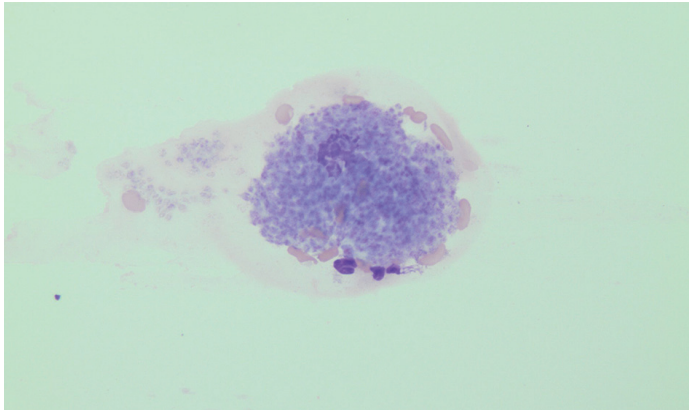

Figure 10: Platelet clump.

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3. Identify the monolayer and using the 40x lens estimate the nucleated cells count. Note any platelet clumps that were not seen with the 10x objective.
4. Using the 100x oil immersion lens systematically evaluate the platelets, erythrocytes and leukocytes.

> The monolayer or counting area is the zone where half of the erythrocytes are close to each other but not overlapping. Generally, from the feathered edge if you move two to three fields back to the body you are on the monolayer.

Platelet evaluation

Platelets are non-nucleated round to oval fragments of megakaryocytes, mostly pale staining; they may appear clear or containing purple cytoplasmic granules. Their diameter ranges from 2 µm up to 5 µm. Often larger platelets (macroplatelets or giant platelets) *(figure 11)*

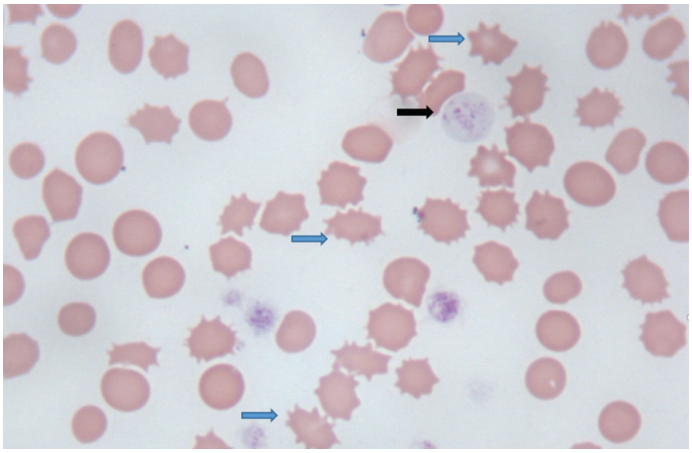

Figure 11: Acanthocytes (blue arrows), macrothrombocytes (black arrow). Microcytic red cells and anisocytosis are also demonstrated on this slide.

are seen in low numbers in normal cats. These can be miscounted as erythrocytes by the automated haematological analysers generating an inaccurate platelet count; this should not make a difference to the RBC count due to their presence in significantly higher numbers. Additionally platelet clumping occurs commonly in cats, thus a manual estimation of the platelet concentration in a feline smear is always essential.

Manual platelet count:

1. In the monolayer, count the number of platelets in at least 10 fields using the 100x oil immersion lens.
2. Calculate the average number.
3. Multiply the average platelet number by 20 = estimated platelet count (x 10^9/L). Around 200-700 x 10^9/L is normally expected.

Remember that the manual platelet count will be falsely decreased if platelet clumps are present.

> A minimum of 8 to 10 platelets per high power fields should be seen in order to be interpreted as adequate.

Erythrocyte (RBC) evaluation

Erythrocytes are non-nucleated, round biconcave shaped, with very limited central pallor. Their diameter is approximately 6 µm but a slight anisocytosis (variation is size) is commonly found. Rouleaux formation (red cells arranged in stacks, 'like coins') *(figure 12)* is often seen in feline smears and should not

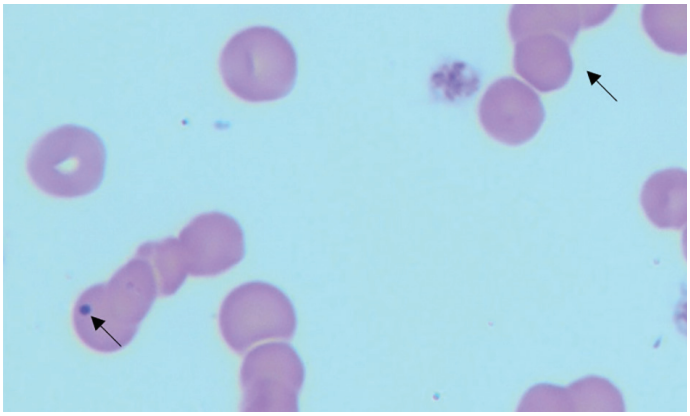

Figure 12: Howell-Jolly bodies (left arrow), Rouleaux formation (right arrow).

be associated with agglutination. If however seen in high numbers in the monolayer, they may be suggestive of increased globulin concentration (e.g. in inflammatory disorders or B cell neoplasms). The following are some of the most important changes we should always try to identify if present:

- *Polychromatophils:* Immature RBCs that stain slightly basophilic and are larger than normal *(figure 13, 14)*. Low numbers of polychromatophils can be seen in blood smear from healthy cats. These cells are the best tool to judge regeneration on an in-house blood smear. Marked polychromasia is suggestive of a regenerative response. Anaemias caused by haemolysis and blood loss tend to exhibit marked regeneration, following an initial period of 3 to 5 days that the bone marrow needs to respond.
- *Nucleated RBCs:* Commonly seen in high numbers due to regenerative response of the bone marrow to anaemia *(figure 14)*. Other causes include bone marrow injury, infiltrative marrow disease, FeLV associated erythroid neoplasia and splenic dysfunction.
- Howell-Jolly bodies: Remnants of the nucleus that appear a dark blue round inclusion *(figure 12)*.

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Low numbers can be present in healthy cats. Increased numbers can be seen in regenerative anaemia, splenic dysfunction and erythroid dysplasia.

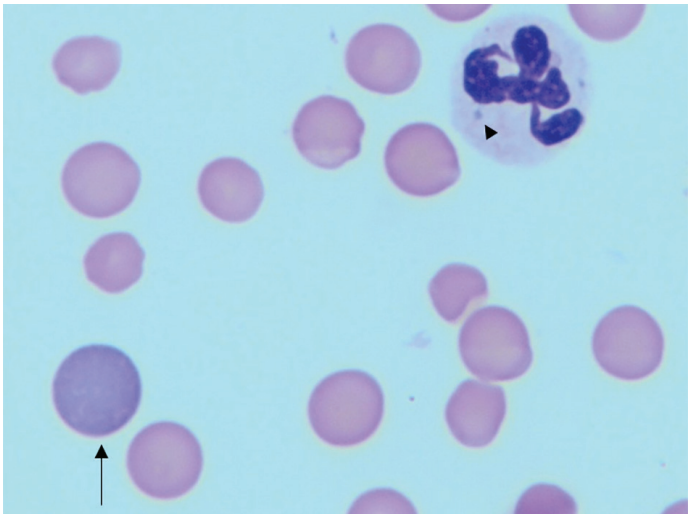

Figure 13: Polychromatophils (left arrow), Neutrophil with Döhle bodies (arrowhead). Anisocytosis is also demonstrated on this slide.

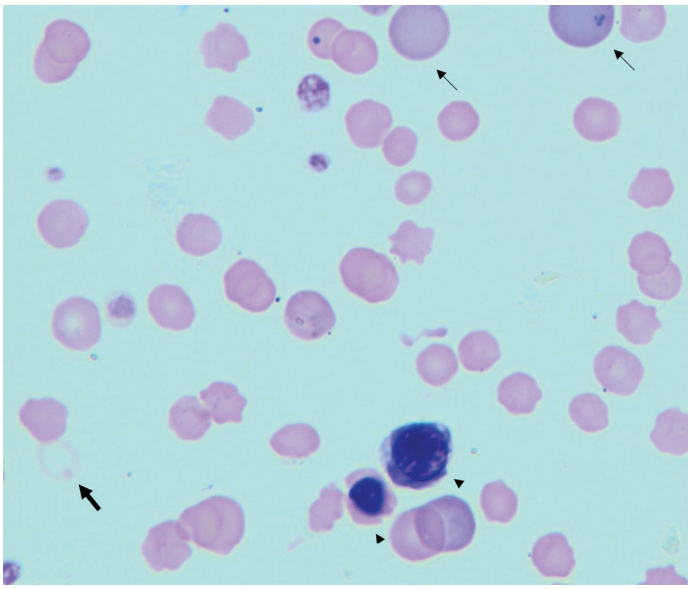

Figure 14: Feline ghost cell (left arrow), nucleated RBCs (arrowheads), polychromatophils (right arrows).

- *Basophilic stippling:* aggregation of ribosomes appearing as small basophilic granules. It can be seen in regenerative anaemia, mainly within polychromatophils. When not associated with severe anaemia, it may indicate lead toxicity.
- *Heinz bodies:* Appear as paler or of the same colour, small round structures inside or protruding from the RBCs *(figure 15, 16)*. These structures are a result of oxidative damage that denatured haemoglobin. If they are present in high numbers (more than 20%) then oxidative damage is present (toxins, drugs). Small Heinz bodies are normally present in low numbers without causing anaemia. Cats with hyperthyroidism, lymphoma or diabetes mellitus (especially with ketoacidosis) can have increased numbers of Heinz bodies without anaemia (still the lifespan of RBCs is reduced).

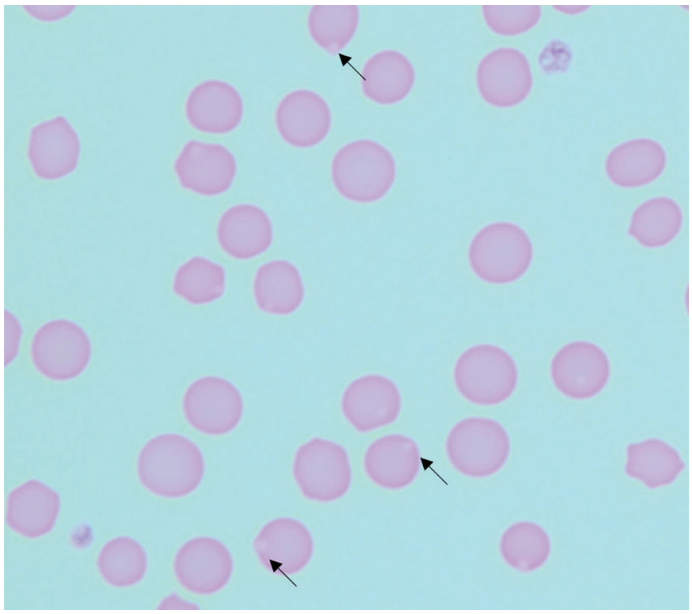

Figure 15: Heinz bodies (black arrows).

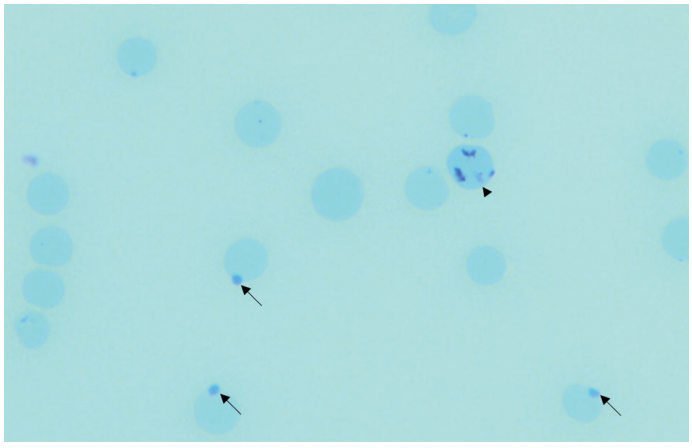

Figure 16: Heinz bodies stained with new methylene blue (black arrows), reticulocyte (arrowheads).

- *Poikilocytosis:* Variation in the morphology of the erythrocytes. Low numbers of poikilocytes can be seen in healthy animals or may not be of diagnostic relevance in some ill animals. If found, these should be interpreted in view of other haematological findings, other laboratory findings and clinical signs. Amongst the most significant are:

  Echinocytes: Have numerous small projections, which are evenly spaced *(figure 11)*.

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These are mostly artefactual (crenation) but can be caused by electrolyte imbalances and renal disease.

Acanthocytes: Have few large projections unevenly distributed *(figure 17)*. Often a result of liver disease, such as hepatic lipidosis (due to changes in the lipid content of cell membrane) or fragmentation injury (e.g. disseminating intravascular coagulation(DIC)).

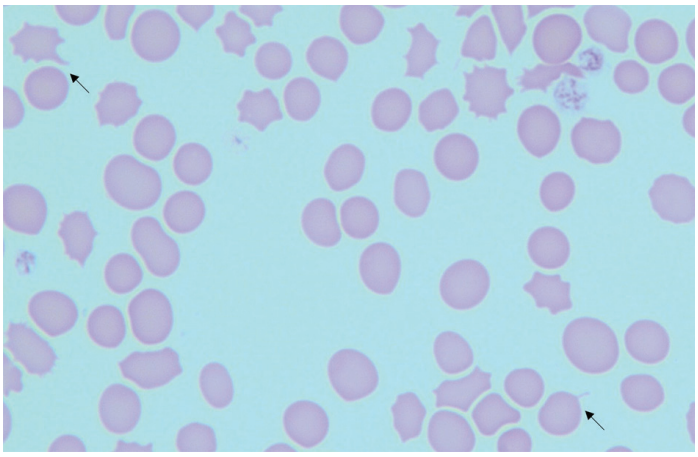

Figure 17: Acanthocytes

Schistocytes: Fragments of RBCs, seen less commonly in cats than in dogs. Result of fragmentation injury (e.g. DIC, microangiopathies, abnormal blood flow, severe iron deficiency) and are often found along acanthocytes.

Eccentrocytes: Appear condensed on one side and clear on the other, with the edge of the membrane barely visible; they are a result of oxidative injury on the cell membrane of the RBC.

Keratocytes: Have a single or two projections and give a "bite-shape" appearance *(figure 18)*. Low numbers can be seen in cats without a clinical significance. When they are present in high numbers or with other poikilocytes they can indicate fragmentation injury or liver disease.

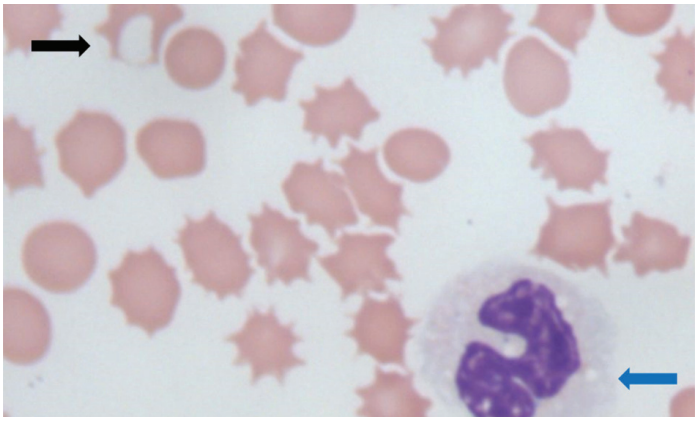

Figure 18: Karatocytes (black arrow), cytoplasmic vacuolation (blue arrow)

- *Agglutination:* Grape shaped aggregates of RBCs. They suggest immune-mediated haemolytic anaemia. It is important not to confuse agglutination with rouleaux formation *(figure 12:* where RBCs stack like coins) which is often see in healthy cats. Washing the RBCs with physiologic saline will eliminate rouleaux formation.
- *Hypochromasia:* Erythrocytes that appear pale with central pallor due to lower haemoglobin concentration. When seen with microcytosis (smaller RBCs), iron deficiency should be strongly suspected.
- Ghost cells: Appear as pale-staining RBCs with minimal haemoglobin and a faint cell membrane *(figure 14)*. Can be formed during intravascular haemolysis or in vitro.

Leukocytes

A rough estimate of the leukocyte count can be performed by counting the white blood cells in 10 different fields on the monolayer using the 40x lens. Then you multiply the average by 1.6 and that will give you an estimate of your WBCs x 10^9/L. Uneven distribution of the white blood cells, especially when they accumulate at the feathered edge, can generate an inaccurate estimate.

Neutrophils:

- Normal: The predominant leukocyte in healthy cats. Normal neutrophils have a well-segmented nucleus *(figure 19)*. Neutrophils with more than five

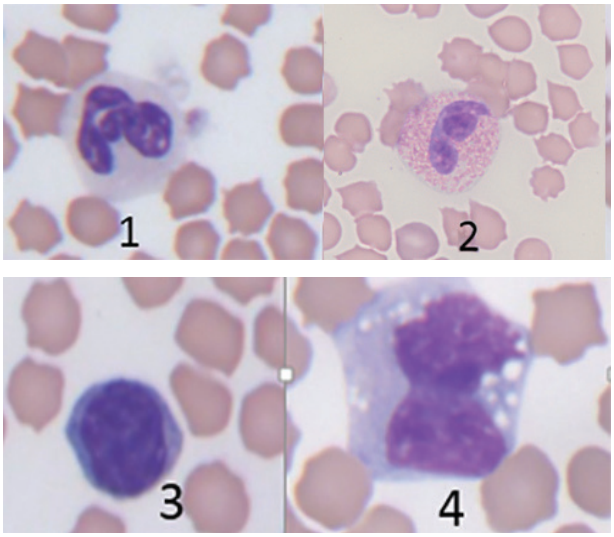

Figure 19: (1) Neutrophil, (2) Eosinophil, (3) Lymphocyte, (4) Monocyte

segments within the nucleus (hypersegmented, often referred to as a "right shift") may be seen secondary to corticosteroid treatment due to longer lifespan of neutrophils in circulation (although this is not very commonly seen). They have a clear or very pale eosinophilic cytoplasm on most commonly used rapid stains. With some stains, small and very pale eosinophilic cytoplasmic granules may be seen.

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- Left Shift: The presence of immature neutrophils in circulation in numbers exceeding the established reference interval for that species. This is triggered by increased demand, such as in severe inflammatory conditions. Band neutrophils are the most commonly seen (with an unsegmented nucleus with two parallel bands or "S" shaped) *(figure 20)*; these are one stage less mature than mature neutrophils. Metamyelocytes (one step before the band, with a plumper, similarly shaped, unsegmented nucleus) can be seen in more severe cases. Earlier precursors (myelocytes- with a kidney shaped nucleus) are less commonly seen; these indicate severe demand.
- Toxic change: morphologic abnormalities caused by an accelerated maturation in the bone marrow secondary to increased demand. This occurs due to cytokine stimulation, which is usually in response to inflammation. Toxic changes are commonly seen with a left shift, as the stimulus is the same, however these can be seen individually.

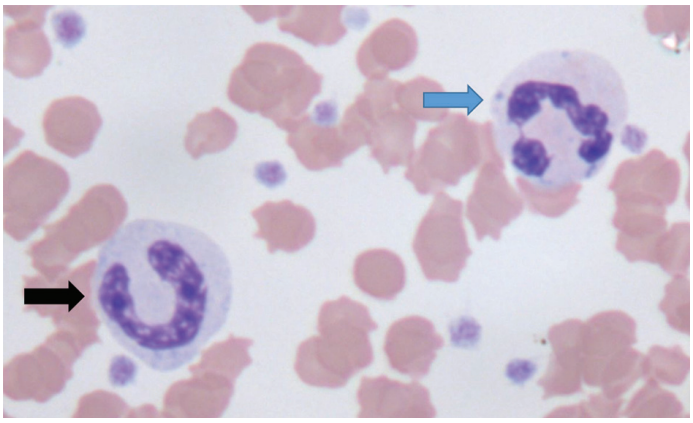

Figure 20: Band nuetrohphil (black arrow), Döhle bodies (blue arrow).

1. Cytoplasmic basophilia: A diffuse blue appearance to the cytoplasm *(figure 20)*.
2. Döhle bodies: Pale blue aggregates in the cytoplasm *(figure 13, 20)*. However, some healthy cats have low numbers of small Döhle bodies, so these alone do not always indicate toxic change- look for other toxic changes.
3. Cytoplasmic foaminess: Indistinct vacuoles in the cytoplasm, creating a foamy appearance *(figure 18)*. Note that clear punctate vacuoles often develop in neutrophils as a storage-related artefact.
4. Toxic granulation: These are distinct magenta staining granules in the cytoplasm due to the primary granules taking up stain.
5. Ring form or 'doughnut' shaped nuclei. Look for a perfect ring with no indentation.

Lymphocytes

Most of the lymphocytes that circulate in healthy cats are small (mature) cells; they have a high nuclear to cytoplasmic ratio, a round nucleus with smudged, dense chromatin and a small amount of pale blue cytoplasm *(figure 19)*. Normal lymphocytes need to be differentiated from nucleated red blood cells, reactive lymphocytes and neoplastic cells. Reactive lymphocytes usually have deeper blue cytoplasm and their nuclear to cytoplasmic ratio is often smaller than in normal lymphocytes; these are associated with antigenic stimulation. Granular lymphocytes, containing small magenta staining granules within their cytoplasm, can be seen occasionally (cytotoxic T cells or natural killer cells).

Eosinophils

These are larger than neutrophils. Their nucleus is less segmented than that of neutrophils and has a dense chromatin pattern. Their cytoplasm contains small rod-shaped pink-orange granules *(figure 19)*.

Basophils

These are a rare finding in healthy cats. They are similar in size to eosinophils. Their nucleus is often described as "ribbon-like" because it is long and folded and has a smooth/lacy chromatin pattern. Their cytoplasm stains gray to pale purple/blue and contains pale purple/blue granules.

Monocytes

These are larger than neutrophils and can be fairly variable in size and appearance. Their nucleus varies in shape and is often complex with a lacy to slightly clumped chromatin. Their cytoplasm is moderate to abundant, usually stains blue-grey and often contains a few variably sized vacuoles *(figure 19)*.

Blood smears are a useful and inexpensive diagnostic tool. With practice they can be reviewed in house for rapid assessment of red and white blood cells.

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