Essential haematology for companion animal vets in first opinion practice

Haematology provides a vital source of diagnostic information in veterinary medicine. Advancement in technology has improved the availability of in-house automated analysers in first opinion practices, allowing practitioners quick access to complete blood count results to aid day-to-day clinical decisions. Most in-house analysers use flow cytometry or electrical impedence technologies which provide fairly accurate complete blood count results. However, practitioners must exercise care when interpreting these results as sample factors such as lipaemia, haemolysis, and the presence of atypical white blood cells, may have an effect on the accuracy of the results. Interpreting complete blood count results alongside a good quality blood smear is important, as it serves as a quality control tool to assess the accuracy of the complete blood count results and also provides useful information on the cell morphology. Good sample handling and good smear making technique are important to produce a blood smear of diagnostic value.

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Haematology is an integral part of veterinary medicine, providing useful baseline information in situations such as routine screening tests or pre-anaesthetic blood tests, as well as diagnostic purposes. In-house haematology analysers are becoming more readily available for first opinion practices, allowing practitioners the benefit of rapid complete blood count results, which is particularly useful in cases of critical illness or other time-sensitive situations. However, practitioners must exercise care when interpreting these complete blood count results, because of there is a possibility of unreliable results arising from sample and patient factors, or from analyser factors, as in-house analysers can lack the vigorous quality control procedures that are performed by reference laboratories. A good quality blood smear examined alongside analyser results can help assess analyser accuracy and provide important information about cell morphology. Good sample handling and smear-making technique are important, as is a good understanding of smear interpretation. This article describes the important features of automated analysers, alongside the preparation and use of a good quality blood smear.

Automated haematology analysers

Types of in-house analysers

Understanding how analysers produce measurements allows prastitioners to use the results more efficiently. Currently, the majority of haematology analysers employ technologies such as electrical impedance and/or laser flow cytometry for measuring cell counts, cell sizes and white blood cell differentials (*Table 1*). Quantitative buffy coat-based analysers such as the IDEXX VetAutoread haematology analyser are beyond the scope of this article as they have been mostly superseded by flow cytometry and impedance analysers. More information on this type of analyser can be found in published literature (Stockham and Scott, 2008a; Villiers, 2016a).

Table 1. Examples of commonly used in-house haematology analysers (list not exhaustive).

Analyser	Laser flow cytometry	Electrical impedance
IDEXX LaserCyte	/	
IDEXX Procyte One/Dx	/	/
VETSCAN HM2/HM5		/
Woodley Insight V5	/	/

Red cell and platelet parameters

Both electrical impedance and flow cytometry analysers measure haemoglobin concentration, red blood cell count, mean corpus-

Table 2. Examples of commonly seen artefacts/errors in red cell parameters

Type of artefact/error	HGB	RBC	MCV	PCV (spun)	Hct (calculated)	MCHC	MCH
Heinz body	↑	—	—	—	—	↑	↑
Lipaemia	↑	—	—	—	—	↑	↑
In vitro haemolysis	—	↓	—	↓	↓	↑	↑
Sample ageing (cell swelling)	—	—	↑	↑	↑	↓	—
Autoagglutination	—	↓	↑	—	↑ / ↓	↑ / ↓	↑

↑ - value falsely elevated, ↓- value falsely reduced, — - no effect. HGB= haemoglobin concentration, RBC=red blood cell count, MCV= mean corpuscular volume, PCV= packed cell volume, HCT= haemocrit, MCHC=mean corpuscular haemoglobin concentration, MCH=mean corpuscular haemoglobin

cular volume (MCV), platelet concentration (PLT) and calculate other values such as haematocrit (HCT), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Practitioners need to bear in mind that errors in the measured values will cause susequent errors in the calculated values, with examples listed in *Table 2*.

Flow cytometry-based analysers measure the interruption of light and amount of light scatter when cells pass through a laser beam. This allows the analyser to determine cell counts, cell size and granularity/density, allowing accurate identification of the cells and generating red blood cell count, platelet concentration and mean corpuscular volume. Impedance-based analysers measure these values by detecting the changes in electrical resistance (frequency and magnitude) as cells pass through an aperture in an electrically conductive fluid.

Both types of analysers determine haemoglobin concentration by lysing red blood cells and measuring the free haemoglobin spectrophotometrically or colorimetrically.

The red cell indices, such as mean corpuscular volume and mean corpuscular haemoglobin concentration, are useful especially when an anaemia is present, allowing characterisation of the anaemia to assist with the diagnosis. It is also important to note that any elevation in the calculated mean corpuscular haemoglobin, or mean corpuscular haemoglobin concentration, is generally spurious which will prompt blood smear and plasma examination. Pre-analytical variables, such as sample lipaemia, sample haemolysis or inadequate mixing of the sample before analysis, can have a major impact on the red cell parameters (Braun et al, 2015). Occasionally, marked spherocytosis or marked eccentrocytosis can also result in false elevated mean corpuscular haemoglobin concentration (Villiers, 2016b).

Accuracy of platelet count can be variable for all haematology analysers (Stockham and Scott, 2008b). One of the most important, and common, errors is a falsely lowered platelet count. This is commonly a result of clumping (aggregation) of platelets in the sample. Platelet aggregates can be seen with platelet activation owing to suboptimal sampling techniques or handling (Stockham and Scott, 2008b; Flatland et al, 2013). As impedance-based analysers distinguish red cells and platelets based on their cell sizes, occasionally, larger platelets may be misclassified as small red cells

Table 3. Indication for blood smear examination

Indication	Examination
Elevated mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration	Examine red cell morphology for Heinz bodies and spherocytes
Low platelet concentration	Examine white cell morphology and perform manual differential if possible
Presence of leukocytosis or leukopenia	
Presence of anaemia	Check for the level of polychromasia and red blood cell morphology
When analysers raise flag on abnormalities	Examine white cell or red cell morphology as appropriate

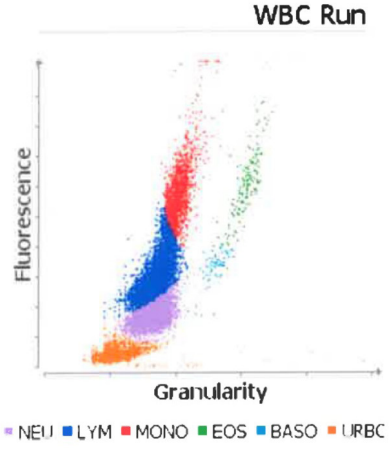

Figure 1. Dot plot from IDEXX Procyte analyser showing no distinction (overlapping) of white cell clusters.

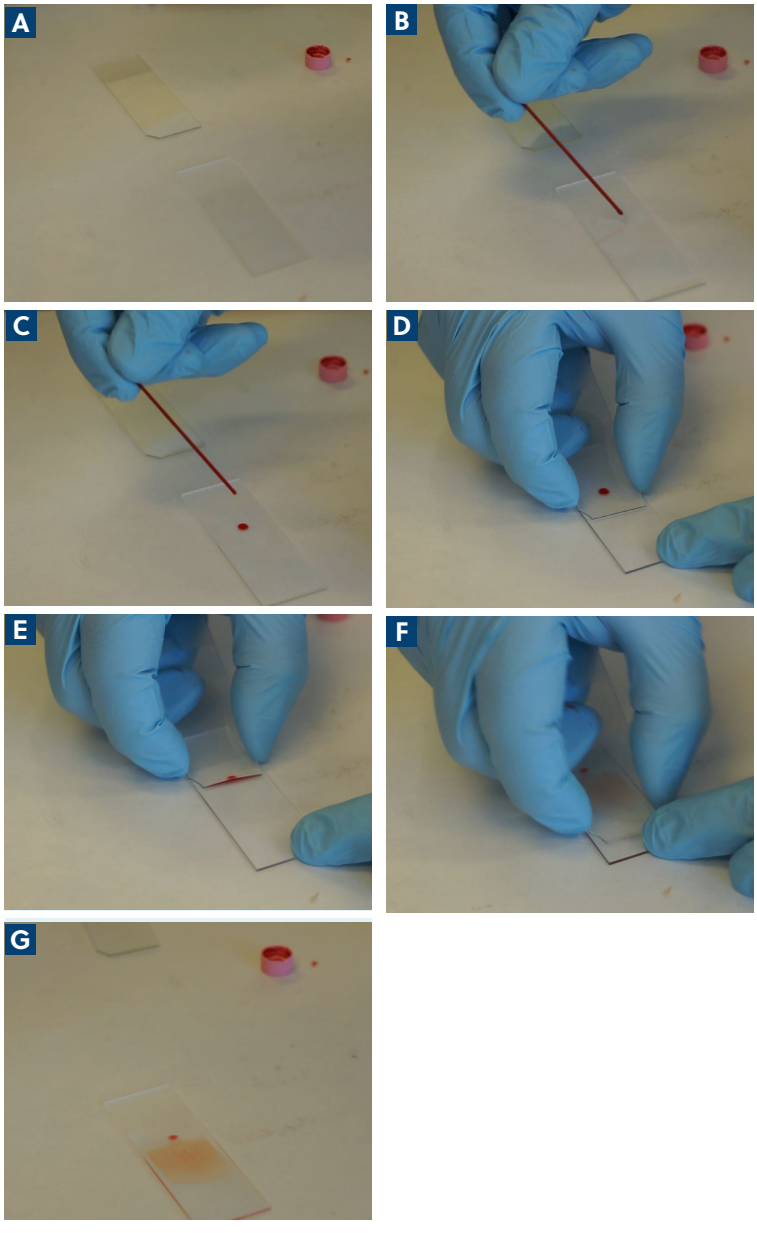

Figure 2. a) A spreader slide has been prepared by removing one corner and is ready to use. b) A small amount of blood is drawn up into a a capilliary tube. c) A small amount of blood is placed on the slide. d) Hold the spreader slide at 30° and draw it back toward the drop of blood. e) When the spreader slide touches the drop of blood, allow the blood to spread along the edge of the spreader slide. f) Push the spreader slide forward to create a blood smear with a feathered edge. g) A finished blood smear.

(especially in cats), resulting in an artifactually lowered platelet concentration and/or increased red blood cell count (Stockham and Scott, 2008b). Microcytic red cells may also be mistaken for platelets, resulting in a falsely increased platelet concentration. It is prudent to examine the blood smear for any platelet aggregates or changes to platelet morphology when thrombocytopenia is present on the automated analyser.

White blood cell parameters

Both flow cytometry-based and impedance-based analysers can generate white blood cell count and white blood cell differentials. Flow cytometry-based analysers measure white blood cell count and generate differentials in a similar way to red blood cells and platelets, identifying the white blood cells by differences in cell size, internal structures and granularity. To generate total and differential white blood cell counts, impedance-based analysers lyse the cells and measure the changes in electrical resistance caused by the naked nuclei. Impedance analysers may have a relatively poorer ability to differentiate types of white cells compared to flow cytometry (Villiers, 2016a).

White blood cell count and white blood cell differentials are generally fairly accurate in a fresh, normal sample. However, morphological changes such as left shift, toxic change and atypical cells may render the differential less reliable. Some analysers such as IDEXX Procyte and Woodleys Insight V5 appear to be able to pick up some of these morphological abnormalities, such as band neutrophils, and raise flags to alert practitioners.

Nucleated red blood cells are usually included in white blood cell counts and may occasionally be misclassified as lymphocytes. Nucleated red blood cells usually represent a very small proportion of the total nucleated cell count, hence in a healthy individual, the effect on white blood cell count is negligible. However, in an event of normoblastosis, white blood cell count can be falsely elevated and adjustments may be needed to obtain a corrected WBC.

Use of graphical plots

Most analysers generate graphical visuals of the results presented as scatter plots/dot plots or histograms. The manufacturer of these analysers usually provides guides to help with the interpretation of these graphs (Zoetis, 2022; Idexx, 2022). They can allow quick visual assessment of the white blood cells population and can also be used to verify the results generated. For example, with scatter plots, if 'overlapping' of the clusters is identified (and there is no clear distinction between the clusters of different white cells) as shown in *Figure 1*, then the differential is less likely to be accurate.

Quality control

Correct operation and maintenance of the analysers is important to reduce analytical errors, giving practitioners more confidence to rely on the results generated for clinical decision-making. Appropriate training of staff involved with operation and maintenance is essential to optimise performance of the analysers. It is also advisable to perform maintenance and cleaning as per the manufacturer's recommendations/guidance to ensure reliability of the analysers and reagents.

In-house quality control can be performed by comparing the packed cell volume value (spun haematocrit) to the haematocrit value generated by the automated analyser. Generally, these numerical values should be comparable with the maximum difference expected within 3% (Stockham and Scott, 2008c; Flatland et al, 2013).

The plasma in the microhaematocrit tube can also be assessed for lipaemia or haemolysis. This can be especially useful when elevated mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are observed in the automated complete blood count results. Another useful tip for cross-checking

the automated results is that the numerical value for measured Haemoglobin concentration should be approximately one-third of the numerical value for calculated haematocrit (Stockham and Scott, 2008c; Flatland et al, 2013). If the values do not correlate, investigation should be performed to identify the source of error.

Although there has been improvement in the accuracy of white blood cell differentials, analysers still have limitations when encountering morphologically abnormal white cells or red cells. They are unable to identify changes such as toxic neutrophils, Heinz bodies in red cells and haemoparasites, and may only be able to raise flags which require a blood smear to confirm or identify the abnormality (Flatland et al, 2013). Blood smear examination should ideally be performed for all complete blood cell counts analysed in-house, as this can serve as a quality control and as a diagnostic procedure. However, this may not always be achievable in first opinion practice. Nevertheless, blood smears should at least be examined in clinically ill patients or in the situations listed in *Table 3*.

Making and reading blood smears

Sample handling

In most cases ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant used to preserve blood samples for making smears, although heparin may also be used. If using heparin, bear in mind that leukocytes may stain suboptimally and platelets may aggregate more than when using EDTA (Harvey, 2012). Whenever possible, blood should be drawn in one smooth motion, as slow or traumatic sampling with multiple needle sticks can contribute to clotting. Blood should be placed in an anticoagulant tube immediately after sampling to prevent clotting and gently inverted 8–10 times to mix the blood with the anticoagulant. Samples should not be shaken, as this will damage the cells. Tubes should be filled to the line indicated, as underfilling may result in crenation of cells and echinocyte formation owing to water leaving the cells, and overfilling can lead to clotting.

Smears should be made as soon as possible after sampling so that optimal cell morphology is preserved. Blood samples can be refrigerated if it is not possible to make smears immediately, but should not be frozen. If using chilled samples, the blood should be warmed to room temperature and mixed by gently inverting 8-10 times to ensure good cell distribution before making the smear.

Blood smear technique

Ensure that the workspace and slides used are clean. Using slides with twin frosted edges makes labelling easier. Ideally pencil should be used for labelling because ink may dissolve during staining. A spreader slide can be prepared by scoring and removing one corner with a blade or diamond pen (*Figure 2a*). The edge of the spreader slide should be smooth, as this will help prevent cell damage. This can be checked by running a fingernail along the spreading edge. If necessary, a diamond pen can be used to smooth the edge of the slide. The spreader slide can be cleaned with tap water and kept for future use. If the spreading edge becomes rough, the spreader should be replaced. The standard technique for making blood smears is shown in *Figure 2*.

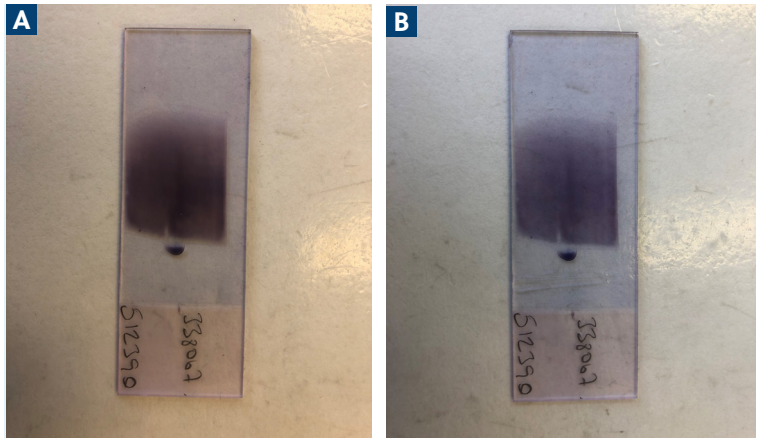

Figure 3. a) Stained blood smear before application of oil. b) Slide after application of oil.

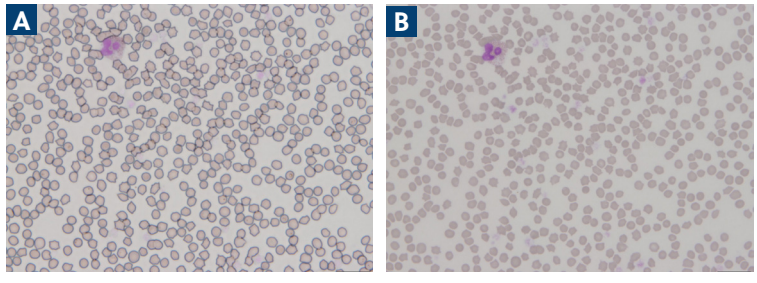

Figure 4. a) Cat blood smear without oil, 40x objective. b) Cat blood smear with oil, 40x objective.

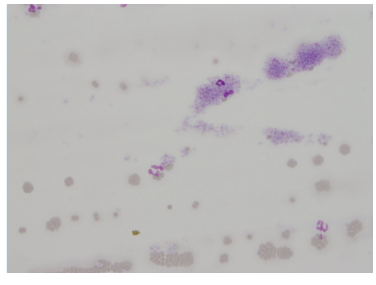

Figure 5. Platelet aggregates at the feathered edge of a cat blood smear.

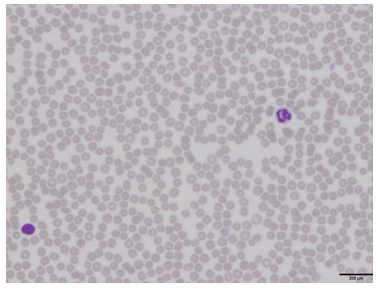

Figure 7. Normal cat erythrocytes 40x objective. A lymphocyte (left) and neutrophil (right) are also visible.

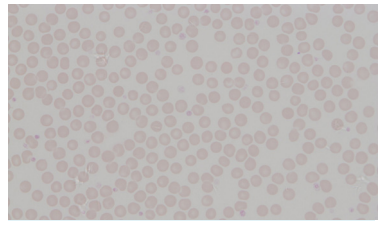

Figure 6. Normal dog erythrocytes 40x objective.

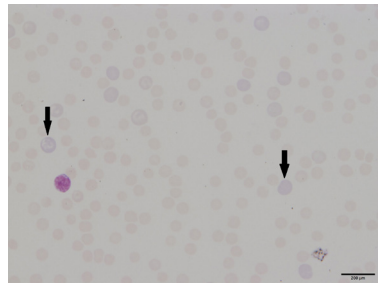

Figure 8. Dog blood smear showing increased polychromasia, 40x objective. Reticulocytes are visible as larger, lightly basophilic erythrocytes (arrows). A lymphocyte is also visible on the left side of the image.

Staining

Romanowsky stains containing eosin and methylene blue are used for most haematology samples. Rapid Romanowsky stains such as

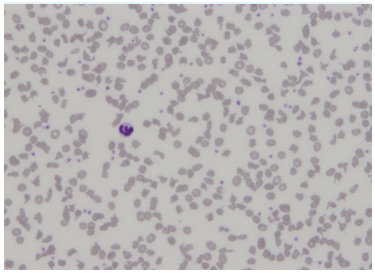

Figure 9. Cat blood smears showing marked poikilocytosis, 40x objective. Schistocytes and acantocytes are among the shapes seen, a neutrophil is also seen.

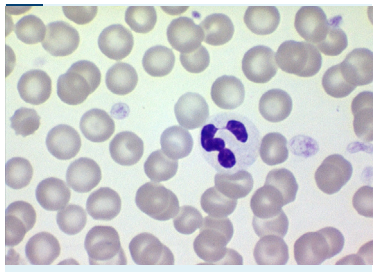

Figure 10. Normal dog neutrophil, 100x objective.

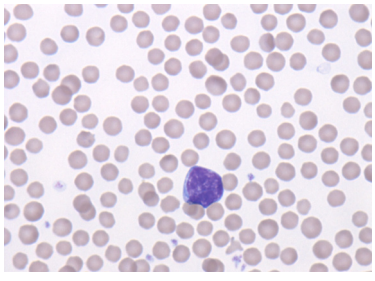

Figure 11. Normal cat lymphocyte, 100x objective.

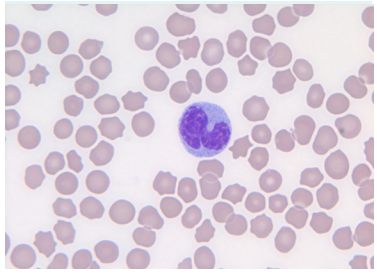

Figure 12. Cat monocyte, 100x objective.

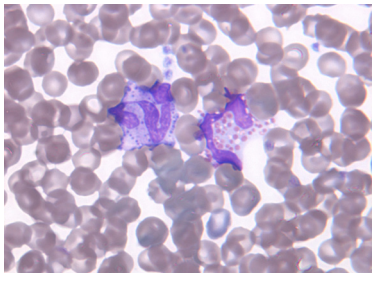

Figure 13. Dog basophil (left) and eosinophil (right), 100x objective.

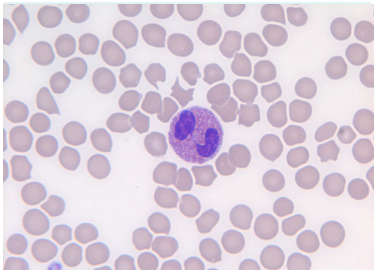

Figure 14: Cat eosinophil, 100x objective.

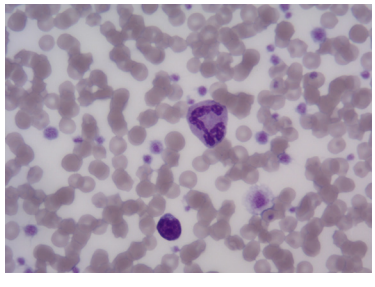

Figure 15. Cat basophil (100x objective). Note the lacy nuclear chromatin pattern and fine granules.

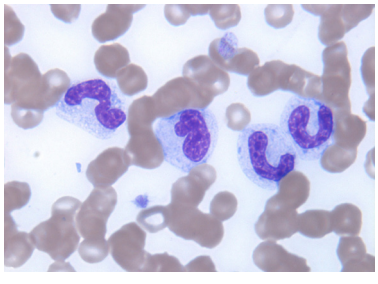

Figure 16. Cat neutrophils showing toxic change, 100x objective. Note the basophilic cytoplasm. Dohle bodies may also be seen on close examination.

Diff-Quik are quick and easy to use and are suitable for in-house laboratories. Blood smears should be processed separately from types of cytology samples which can lead to contamination, particularly those from external areas such as skin or ears.

Ensure all samples are adequately fixed. Thicker samples may require a slightly longer fixation time than specified in the instructions. Stains and fixatives should be replaced regularly, especially if being used for these types of cytologies as debris and bacteria can affect subsequent samples. From the author's experience, depending on the frequency of use, the stains and fixatives should be replaced every 2–4 weeks; sooner if more stain precipitate is observed or if depth of staining is not adequate. Stain and fixative containers should be covered when not in use to prevent evaporation. Ensure no water gets into the fixative, as this can lead to artefact.

How to examine a blood smear

Once stained and dry, slides can be examined immediately by applying a thin film of immersion oil to the entire slide. Immersion oil has a similar refractive index as a cover slip. This eliminates the need for a cover slip and allows good appreciation of cellular detail. A drop of oil can be spread evenly across the slide using a capillary tube. A blood smear before and after application of oil is shown in *Figures 3a* and *3b*. *Figures 4a* and *4b* illustrate the microscopic appearance before and after applying oil. Cellular detail is easier to appreciate in in *Figure 4b* with oil than in *Figure 4a* without oil.

First, examine the whole smear with a low power objective. Look for any large platelet aggregates in the feathered edge, around the edges of the smear, or around the blood drop at the base of the smear (*Figure 5*). Also look at the feathered edge as larger cells or parasites are often located here. However, bear in mind that cellular detail can become distorted in the feathered edge, so morphology of erythrocytes and leukocytes should be examined in the monolayer. Platelets should be visibly scattered among the cells in the monolayer.

Next, examine the monolayer. Red blood cells should be in a single layer with minimal overlap and evenly spaced here. Normal canine red blood cells often have a small degree of central pallor (*Figure 6*), while feline erythrocytes are smaller with less central pallor (*Figure 7*). A small amount of polychromasia is normal in dogs and cats, but increased polychromasia (*Figure 8*) or variation in red blood cell shape (*Figure 9*) should be noted.

Next, examine the white blood cells and their morphology. A manual leukocyte differential is performed by counting at least 200 leukocytes and categorising them by type. A tally counter can be used, or mobile phone apps for cell counting are available. Percentages obtained are then applied to the total white blood cell count from the analyser. This process is helpful for detecting abnormal or neoplastic cells and for verifying the accuracy of the individual cell type counts from the analyser, as errors can occur.

Neutrophils (*Figure 10*), lymphocytes (*Figure 11*) and monocytes (*Figure 12*) have a similar appearance in dogs and cats. However, eosinophils and basophils appear somewhat different in the two species. *Figure 13* shows a dog eosinophil and basophil with clearly visible granules. Cat eosinophils and basophils are shown in *Figure 14* and *15*, respectively. Granules in these cell types typically appear finer in cats than in dogs. Commonly observed morphological changes in leukocytes include band neutrophils, which may indicate a left shift, and toxic neutrophils (*Figure 16*).

Unless using an oil immersion lens, try to avoid getting oil onto the objective. If oil gets onto the lens, it should be wiped off with

lens paper immediately as it is harder to remove once it has dried. If oil does dry on the lens, it can be cleaned by applying lens cleaner or isopropyl alcohol with a cotton swab.

Conclusions

Automated analysers can be a vital source of diagnostic information. However, care needs to be taken interpreting these results and they should be complemented with blood smears wherever possible.

KEY POINTS

- Always interpret complete blood count results alongside blood smear examination if possible.
- Sample factors such as haemolysis, sample lipaemia, presence of Heinz bodies, autoagglutination (as seen in immune-mediated haemolytic anaemia) can cause inaccurate red cell parameters.
- Make use of dot plots if provided to assess the accuracy of automated white cell differentials.
- Good technique is essential to produce a quality blood smear of diagnostic value.
- Blood smear provides useful information on cell morphology which may not be apparent on complete blod count results.

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