

Smear campaign

By Kathryn Jenkins

As a tool for preventing misdiagnoses, blood smear examination is a critical part of any CBC evaluation.

Microscopic blood smear examinations are an important component of a full CBC, and are necessary in identifying morphologic changes, infectious agents, atypical cells and platelet clumping. They help to ensure that critical patient information is neither missed nor misinterpreted.

In the past few years, in-house haematology analysers have had an increasing role in veterinary practice. Most modern analysers provide data in the form of a haemogram, a five-part white blood cell (WBC) differential, and WBC and platelet counts. They also deliver the most accurate data when sampling healthy patients; the accuracy can decrease in samples from sick patients owing to the morphologic changes often seen as a result of disease processes.

While analysers with flow cytometry can flag abnormal results (via an asterisk next to the data in question, for example) every sick animal should have a blood smear examination to ensure an accurate diagnostic interpretation.

Examples from production animals and horses have been used to demonstrate both the importance of blood smears and that the same principles apply across different species.

Fresh blood smears

Blood cells start to deteriorate as soon as they leave circulation, so a blood smear should ideally be prepared immediately after sampling (such as by using a drop left in a syringe after blood-tube filling).

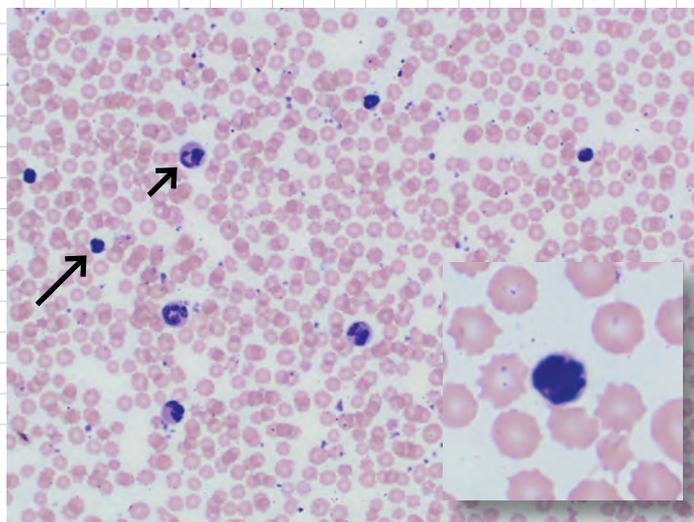


FIGURE 1: Taranaki anaemia in a cow. This image shows four nRBCs (metarubricytes), all with a characteristic wrinkled appearance to the nuclei (long arrow, and inset). They can be easily mistaken for neutrophils (short arrow), which are larger with more abundant cytoplasm. Taranaki anaemia is associated with hypomagnesemia.

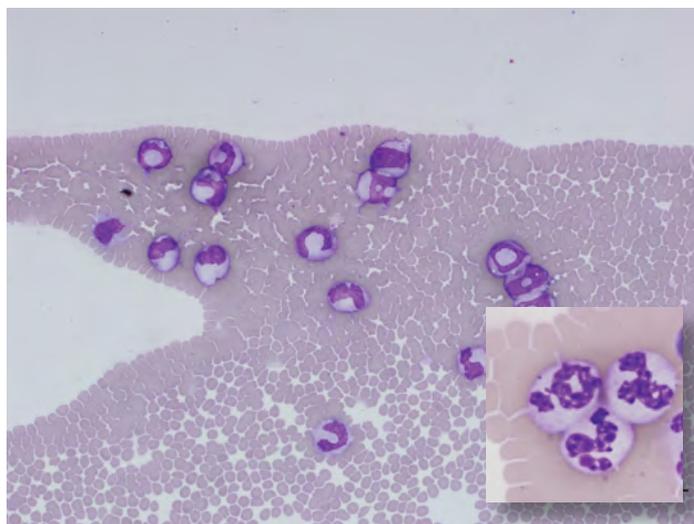


FIGURE 2: Marked left shift in a horse with salmonellosis. These cells are all neutrophils. They have swollen pale nuclei (including bands and doughnut-ring shapes) and basophilic cytoplasm, consistent with a left shift with toxic change. This occurs most commonly as a systemic inflammatory response. The so-called toxic change in these cases represents retained cytoplasmic organelles as a result of accelerated maturation through the bone marrow. Morphologically these neutrophils can mimic monocytes on blood smears, so it is not surprising that analysers make classification errors in cases of marked inflammation. Inset for comparison are normal mature equine neutrophils.

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Blood left in an anti-coagulant tube will have progressively worsening storage artefacts, obscuring pathologic morphologic changes and potentially leading to a loss of key information or a misidentification of changes on a subsequent blood smear examination. For example, storage artefacts can mimic inflammatory changes, such as neutrophils with swollen nuclei and foamy cytoplasm. Lymphocytes can appear swollen and vacuolated, making it challenging to interpret potential neoplastic cases, and erythrocytes become crenated with time, which can hide Heinz bodies, eccentrocytes, keratocytes and acanthocytes in sick animals.

Nucleated erythrocytes

Nucleated erythrocytes (nRBCs) provide a good example of the hidden information that can be found in blood smears. Haematology analysers often misclassify these immature erythrocytes as lymphocytes, leading to inaccurate differentials and total WBC counts. nRBCs can increase as an expected part of a regenerative response in cases of anaemia (especially in ruminants, alongside basophilic stippling), or can help in diagnosing lead toxicity, erythroid leukaemia and Taranaki anaemia in cattle (see figure 1). Horses are unusual in that nRBCs (like polychromatophils) are very rarely seen in peripheral circulation.

When nRBC numbers are over five per 100 WBC, the total WBC count and differential should be corrected for their presence using the following formula:
Corrected WBC = obtained WBC x (100/[100 + nRBC])

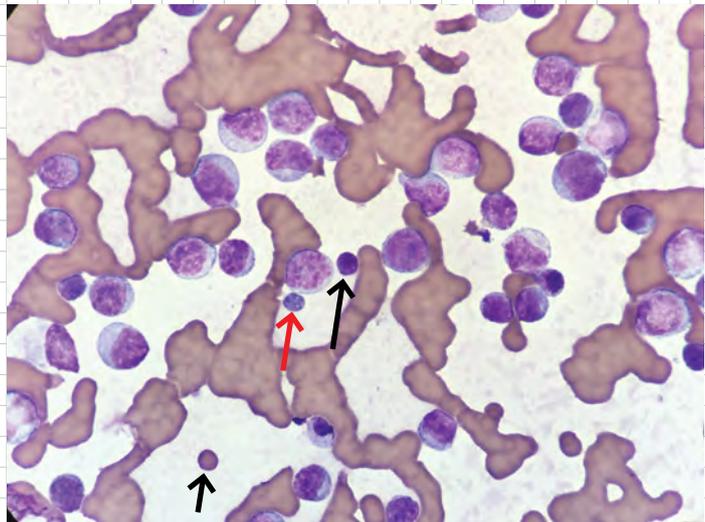


FIGURE 3: Acute leukaemia in a horse. Although the analyser for this case suggested a monocytosis, the blood smear revealed a high proportion of monotypic large round cells (blasts), with a morphology most consistent with lymphocytes. Together with a non-regenerative anaemia and thrombocytopenia in a young horse, this finding supported leukaemia (rather than stage five lymphoma). Compare the large size of the blast cells with an erythrocyte (short arrow) and small mature lymphocyte (long arrow). Note also the cytoplasmic fragment (red arrow) – it is easy to see how analysers can mistake them for reticulocytes.

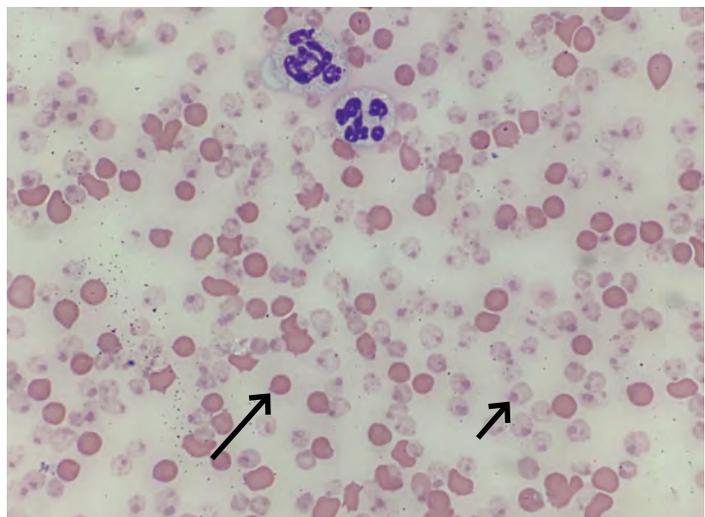


FIGURE 4: Heinz body anaemia in a sheep with copper toxicity. Note the round, pale staining Heinz bodies present in almost all erythrocytes (long arrow). These represent oxidative injury to haemoglobin. The Heinz bodies are more obvious in the numerous pale erythrocytes (short arrow), which are ghost cells. These cells have lost their haemoglobin, indicating significant intravascular haemolysis. Also note the normal small size of ovine erythrocytes compared to the neutrophils (top of picture).

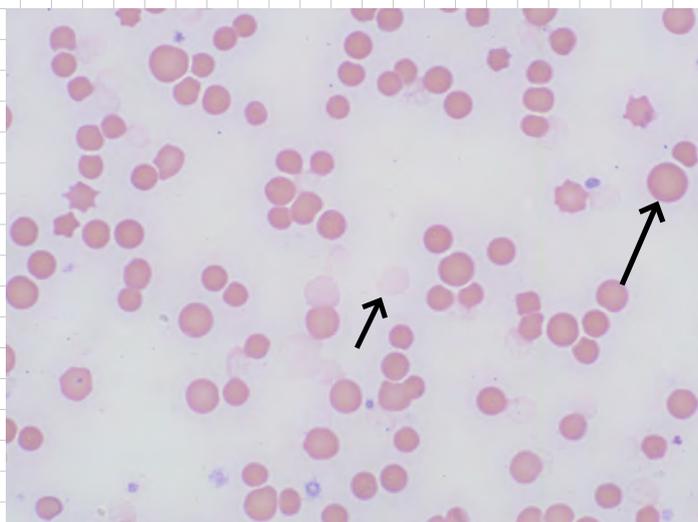


FIGURE 5: Neonatal isoerythrolysis in a foal. Note the reduced density of erythrocytes in the monolayer, supporting anaemia. Macrocytes (long arrow) are a common finding in equine regenerative responses, as this species rarely releases polychromatophils or nRBCs, even in cases of severe anaemia. This case also had numerous erythrocyte ghost cells in the fresh smear (short arrow), indicating intravascular haemolysis.

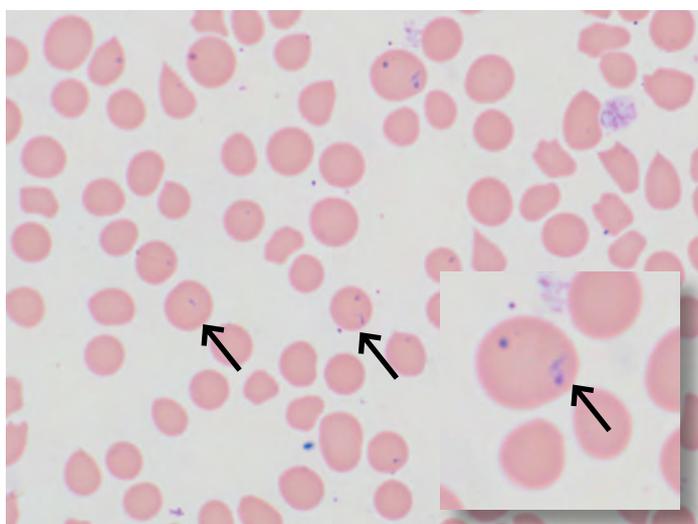


FIGURE 6: Theileriosis in cattle. Note the small, pale-purple intracellular protozoan parasite within several erythrocytes. Theileria organisms are pleomorphic in appearance, with fine comma-like shapes and signet-ring forms. The insert shows both of these protozoal morphologies (arrow), as well as a small, round, dark Howell-Jolly body at 10 o'clock (nuclear remnant). As with Howell-Jolly bodies, haemoparasites must be differentiated from stain precipitate, refractile water artefact and overlaid platelets.

It is important to remember that both the WBC count and the WBC differential may appear unremarkable in cases of acute or severe inflammation. This occurs commonly in production animals and horses, as they have smaller bone-marrow stores than companion animals.

Left shift and toxic change

Inflammatory markers such as left shift (increased immature neutrophils, such as bands) and so-called toxic change (eg, foamy cytoplasmic basophilia and increased Döhle bodies in neutrophils due to accelerated maturation) are readily identified on blood smears (figure 2).

However, it is important to remember that both the WBC count and the WBC differential may appear unremarkable in cases of acute or severe inflammation. This occurs commonly in production animals and horses, as they have smaller bone-marrow storage pools than companion animals. Analysers often misclassify immature and toxic neutrophils as monocytes, or even lymphocytes, in all species. Without a blood smear examination, significant inflammation may be missed.

Anaemia

A blood smear that shows an increase in polychromatophils (immature erythrocytes that appear larger than normal and purple/blue in colour) helps to establish that an anaemia is regenerative and occurring in response to either haemorrhage or haemolysis. Some in-house analysers can provide a reticulocyte count, which is a more accurate assessment of a regenerative response. However, in cases of reticulocytosis, blood smears should always be compared with the degree of polychromasia. Analyser-based false reticulocytosis can occur with leukaemia (due to cytoplasmic fragments – see figure 3), oxidative injury (due to Heinz bodies, figure 4), and giant platelets.

One of the benefits of blood smear examination is the numerous morphologic clues that can help determine the causes of anaemia, including spherocytes, microangiopathic changes (keratocytes, schistocytes and acanthocytes), eccentrocytes and Heinz bodies indicating oxidative injury (figure 4), and ghost cells indicating intravascular haemolysis (figures 4 and 5). It is important to remember that horses are unique, as they do not typically release polychromatophils in regenerative anaemia. In these cases, we can look for macrocytes and other clues to establish the cause of anaemia (figure 5).

Infectious agents

Although New Zealand benefits from having few endemic haemoparasites, it is still useful to be able to identify infectious causes of anaemia rapidly via blood smear examination. These include *Theileria orientalis*, the Ikeda variant in cattle (figure 6) and *Mycoplasma ovis* infection in sheep. Note that haemoparasites such as *Mycoplasma* are epierythrocytic and will fall off erythrocytes in aged samples; this highlights the importance of preparing fresh blood smears.

Atypical cells

Haematology analysers often miss or misclassify atypical cells in circulation. These include neoplastic cells such as haematopoietic neoplasia, including leukaemia (figure 3), and stage-five lymphoma. Any blood smear of a case with leukocytosis must be examined to confirm the nature of the cell types involved.

Note, however, that atypical cells can be present without obvious leukocytosis. Mast cells can also be found in circulation, especially in companion animals, and may be misclassified by automated analysers as basophils, or missed altogether.

Platelet clumps

False diagnoses of thrombocytopenia are among the more common analyser-based errors seen in the laboratory, due to the presence of platelet clumping.

When analyser data suggests thrombocytopenia, it is critical to confirm it by examining a blood smear for platelet clumps (figure 7). While the clumps are most commonly found in the smear's feathered edge, the whole smear (including the application point) must be examined, especially if pathologic thrombocytopenia is suspected.

In addition, if blood has been placed in an EDTA tube, it is recommended that the sample be checked for clots (using a wooden orange stick) to reduce

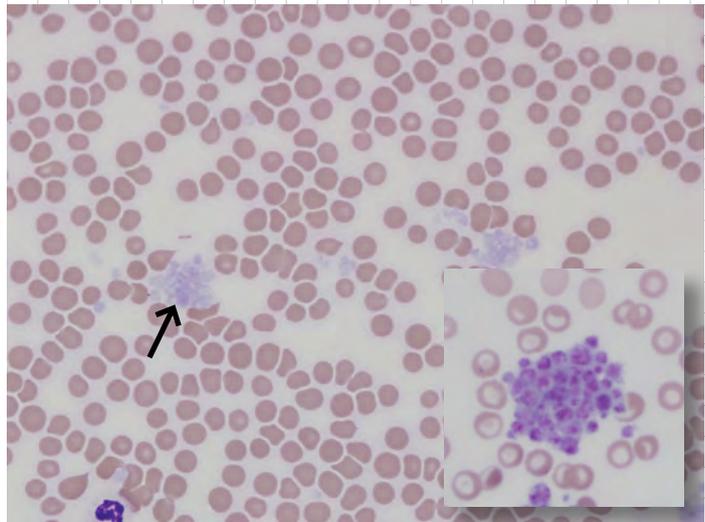


FIGURE 7: Platelet clumps in a horse. Platelet clumping (arrow) can reduce the analyser platelet count, causing false thrombocytopenia. This artefact can be easily detected on blood smear examination. Note the pale-staining platelets in horses compared with those in dogs (inset).

potential errors in platelet counting. Although cats are notorious for platelet clumping (due to platelet activation during collection), it happens in all species.

Microscopic blood smear examinations are a critical component of a full CBC, and a fresh blood smear should be prepared and examined for every sick patient, as a minimum standard. This can be performed either in-house, if you have the knowledge and experience, or can be sent to a reference laboratory for pathology review. The Gribbles Veterinary team values freshly made blood smears submitted to the laboratory, so they can provide their clients with as much useful and accurate information as possible.

Making a blood smear is easy once you have learned some simple tips. The Gribbles team can help you improve your technique, or you can use one of the good instructional videos available online (see below). 

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FURTHER READING AND RESOURCES:

eClinPath. An online textbook on Veterinary Clinical Pathology. [eclinpath.com https://eclinpath.com/hematology/instructional-videos](https://eclinpath.com/hematology/instructional-videos)

Thrall MA, Weiser G, Allison RW, Campbell TW (eds). *Veterinary Hematology and Clinical Chemistry*. 2nd Edtn. Wiley-Blackwell, Hoboken, New Jersey, USA, 2012