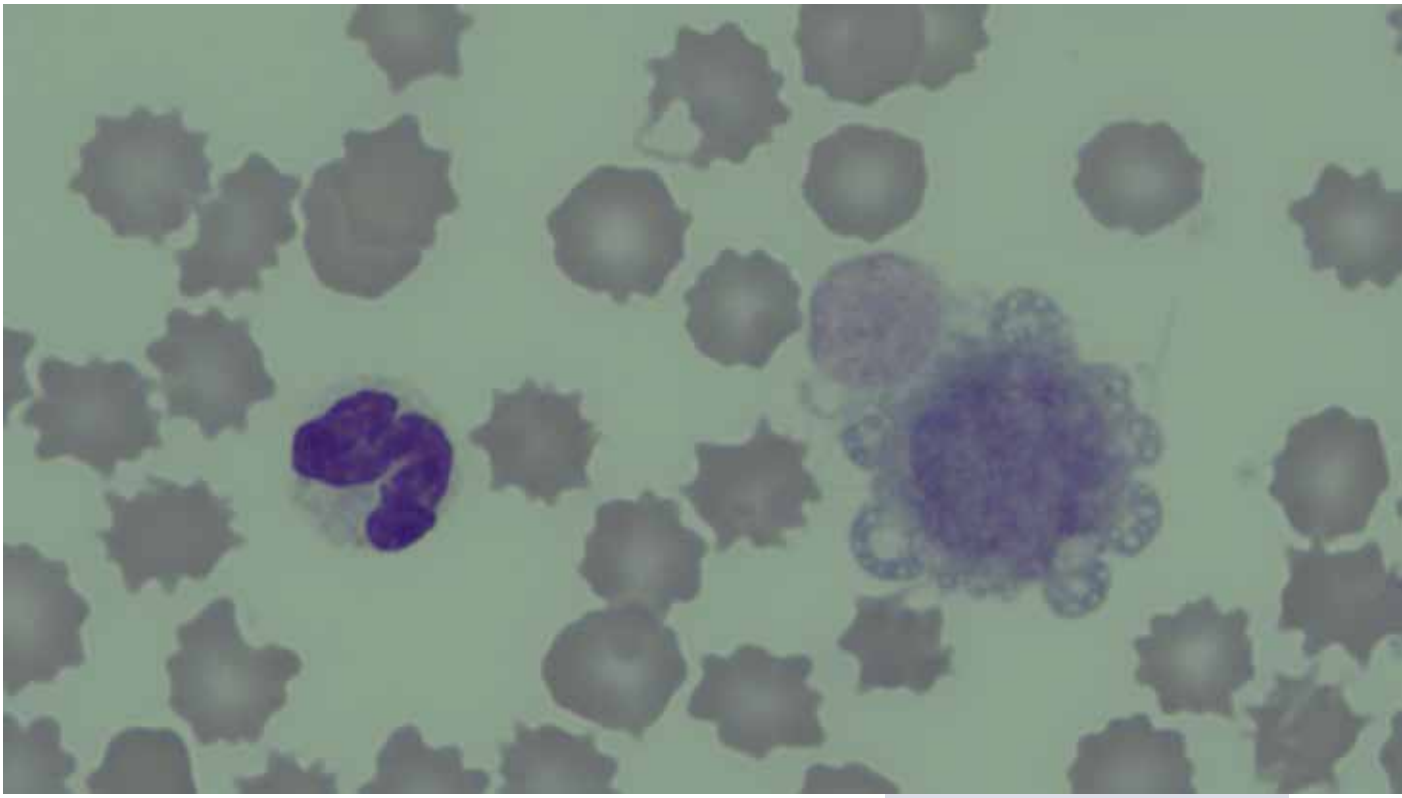

FIGURE 1: A giant platelet in a dog with increased platelet turnover.

The importance *of blood smear examination*

Sandra Forsyth, of SVS Laboratories, argues that you can't rely on your benchtop analyser to always produce error-free results, and offers some helpful tips on how to best make and examine a blood smear.

HOW OFTEN DO you examine a blood smear when a sample goes through the benchtop haematology analyser? Every case? Never? Only when the analyser flags an abnormality? Although modern analysers infrequently produce inaccurate results, they are still just machines that work from software-based algorithms and can produce erroneous results.

A study by Cora et al. (2013) found that 20% of smears detected abnormalities that were missed by the analyser on blood smear examination of dogs receiving chemotherapy. Additionally, if you are not regularly using quality control samples or an external laboratory to verify results, the chance of error is even higher. You don't want your patient to be the one with incorrect results!

When scanning a smear, an experienced eye can estimate platelet count, determine if the leukocyte count compares with that from the analyser, and decide if there are abnormalities in erythrocyte or leukocyte morphology that warrant close examination. The skills to examine a blood smear accurately come only with plenty of practice, and the ability to detect abnormalities in cells, platelets and the background heavily relies on the observer knowing what normal looks like. This highlights the need to examine blood smears from every case to become familiar

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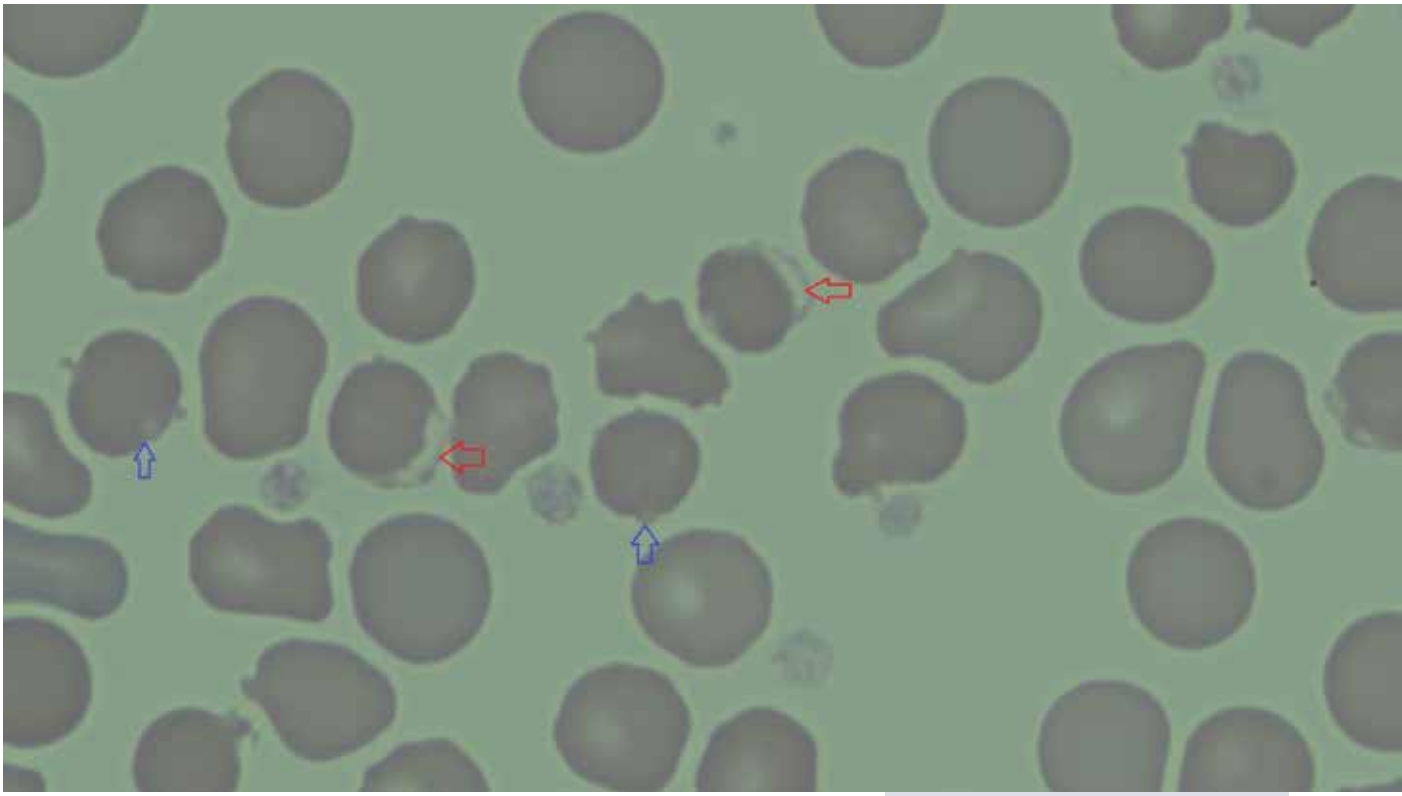

FIGURE 2: Heinz bodies (red arrows) and eccentrocytes (blue arrows) in a dog with allium toxicity.

THE SKILLS TO EXAMINE A BLOOD SMEAR ACCURATELY COME ONLY WITH PLENTY OF PRACTICE, AND THE ABILITY TO DETECT ABNORMALITIES IN CELLS, PLATELETS AND **THE BACKGROUND HEAVILY RELIES ON THE OBSERVER KNOWING WHAT NORMAL LOOKS LIKE.**

with 'normal' morphology, such that you are comfortable in knowing that abnormalities will be readily detected.

MAKING A SMEAR

Examining a smear starts with ensuring that the smear is well made and adequately stained. To provide an even smear, the spreader slide should have a cut edge rather than a ground one. The blood should be fresh and at room temperature. Always gently agitate the sample to mix prior to placing a drop on one end of the slide. Spread quickly, but don't place too much pressure on the spreader or many nucleated cells will end up at the feathered edge. The smear should cover about half to two-thirds of the slide. Once made, the smear should be fully dried before staining. The three-pot Diff-Quik system is commonly used for staining cytology and blood smears, and with use it becomes depleted of dye, despite the stains retaining their colour. Adequately stained slides will have nuclei that are a mid- to deep purple, rather than pink or mauve.

EXAMINING A SMEAR

A good-quality microscope is vital when examining blood smears. If your microscope is not up to the job, consider replacing it, getting it serviced by a professional or sending smears to a commercial laboratory. There is nothing quite as frustrating as examining cytological and haematological samples with a dirty, scratched, old or inefficient microscope. When the equipment is of poor quality it isn't used, and samples will not be evaluated microscopically.

Examine the smear using the 10x objective to check that the erythrocytes are sufficiently spread to examine clearly, and that leukocytes are evenly spread

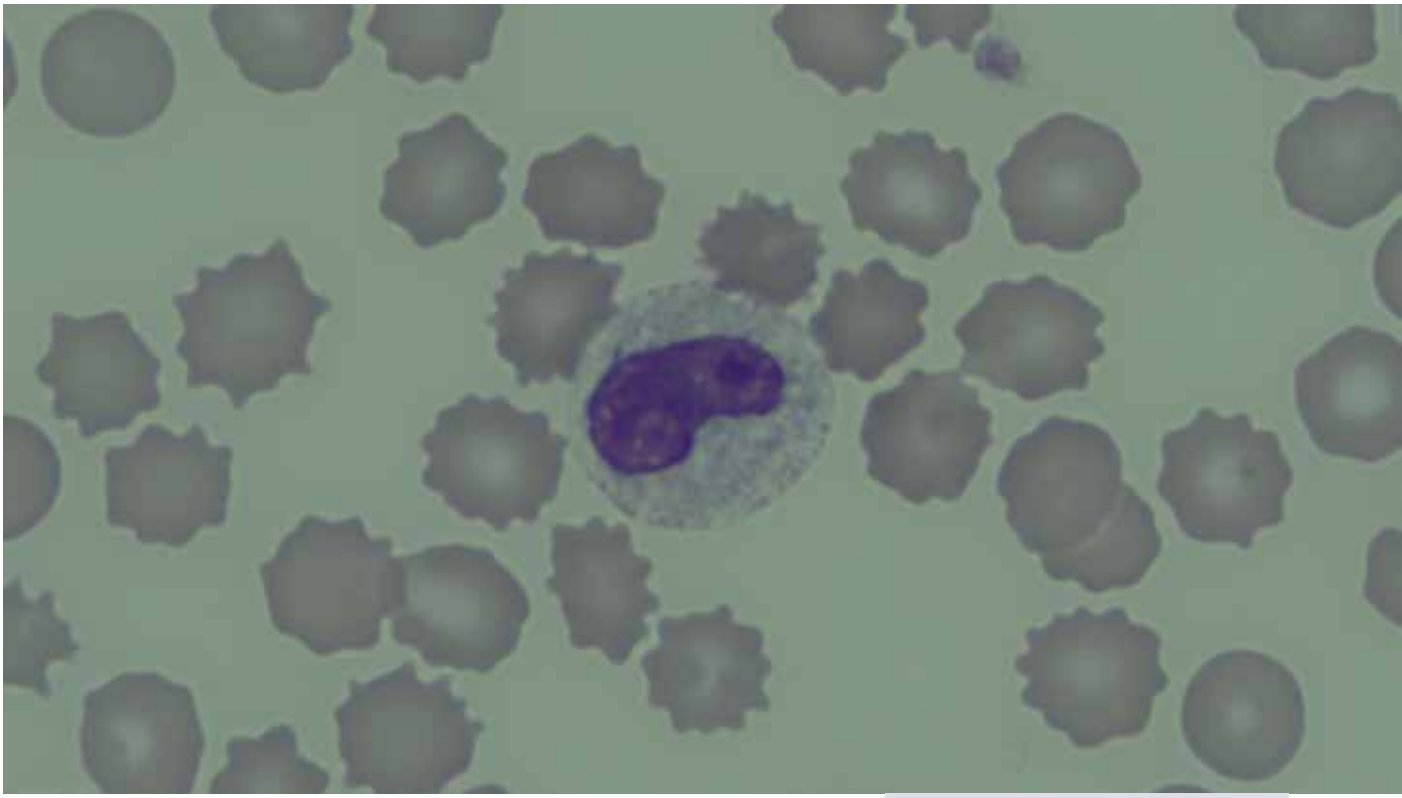

FIGURE 3: A metamyelocyte in a dog with an inflammatory leukogram.

so that when carrying out a differential cell count the results won't be biased by uneven spreading. Develop a pattern for blood smear examination so that platelets, leukocytes and erythrocytes are all evaluated, as discussed below.

PLATELETS

Starting with platelets, look along the feathered edge to see if platelet clumps are present. If none is seen, examine the body of the smear because small platelet aggregates (10 to 20) may only be observed in this area. When platelet clumps or aggregates are present, you can be guaranteed that the count provided by the analyser will be lower than the true count. Decide if the platelets are larger than usual, which can denote increased platelet turnover or a breed-related effect (eg, Cavalier King Charles Spaniels, as in Figure 1). Cats frequently have variably sized platelets, which are sometimes larger than the red cells. Depending on the type of analyser that you have, large platelets may be counted as red blood cells. Similarly, microcytic red blood cells can be counted as platelets.

ERYTHROCYTES

Examine the erythrocytes by looking at variations in size and colour, and note if any inclusions are present. Heinz bodies, spherocytes, ghost cells and *Mycoplasma haemofelis* can be missed by an analyser, and cell fragments (keratocytes and schizocytes) may or may not be detected (Figure 2). Look also for nucleated red blood cells (nRBCs): the analyser may note their presence when they are absent, or they may be missed by the analyser when present. nRBCs are often counted as leukocytes, and as such produce an inaccurate white blood cell count, which must be corrected before a differential count is carried out. Most commonly, nRBCs consist of metarubricytes. Less mature forms such as rubricytes and prorubricytes must be identified because their presence may signal a different disease process.

LEUKOCYTES

Finally look at the leukocytes and determine if the differential count provided by the analyser is correct. Assess the cells for toxic change, immaturity, reactivity and/or neoplastic features that can be missed by an analyser (Figure 3).

SUMMARY

Using data obtained from a haematology analyser without looking at a smear is akin to taking a single-view radiograph: vital information can be missed and an incorrect diagnosis made. VS

REFERENCE:

Cora MC, Neel JA, Grindem CB, Kissling GE, Hess PR. Comparison of automated versus manual neutrophil counts for the detection of cellular abnormalities in dogs receiving chemotherapy: 50 cases (May to June 2008). *Journal of the American Veterinary Medical Association* 242,1539–43, 2013