Blood smear

Microscopic evaluation of a blood film is required to not only verify analyzer results but identify critical diagnostic features that analyzers cannot evaluate. Diagnostically essential morphologic abnormalities can be present even in patients with quantitatively normal results for all hematologic parameters. Perform blood smears when you suspect:

- blood-borne protozoal or mycoplasmal conditions
- haemolytic conditions
- anaemia
- haematologic neoplasia.

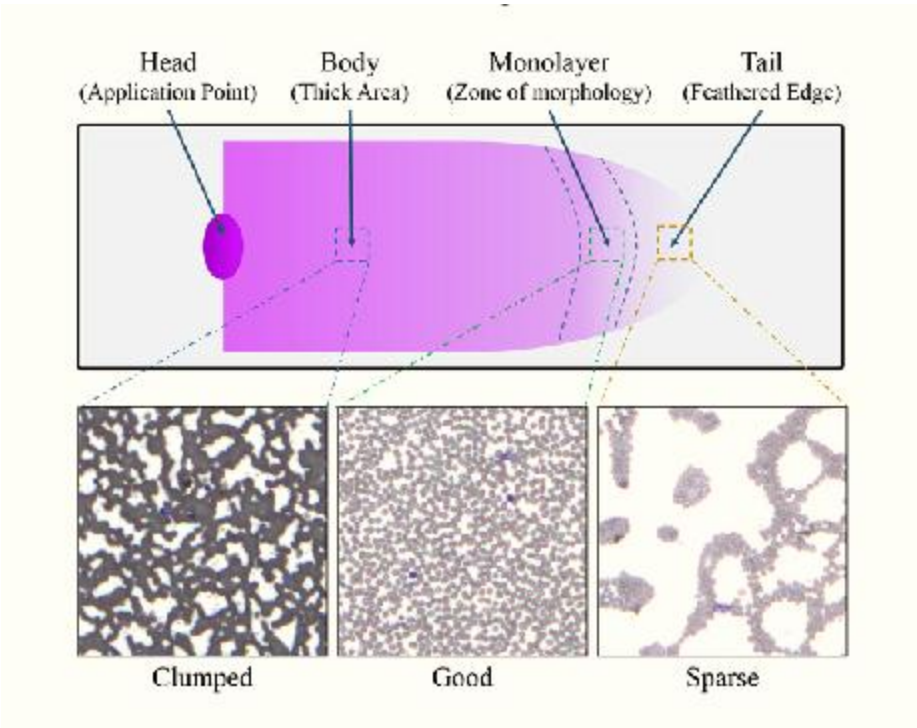
Equipment required for performing blood smears:

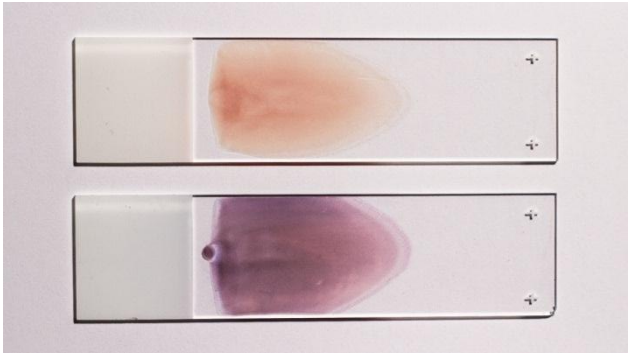
- microscope slides with frosted ends for writing on
- spreader slide – use a specific spreader slide with beveled edges, or a second clean microscope slide
- capillary (microhaematocrit) tubes
- pencil to label the slides
- blood collected in an EDTA tube
- stain (giemsa, wright , leishman or romanowesky)
- alcohol for fixation of blood smear.

Procedure:

1. Collect blood in an EDTA tube and make the smears when back at the clinic.
2. Put a drop of blood by the capillary tube on the slide from one end and spread with another slide at an angle greater than 45°. Note that the blood is dragged over the slide, not pushed.

3. Dipping the slide in 100% methanol for 1 minutes and then remove the slide aside to let dry.
4. Cover the smear with **giemsa** stain, or transfer slide to second coplin jar containing fresh diluted Giemsa stain (1/10) and allow to stain for 15-60 min..
5. Gently wash off the slide under running water for 30 seconds and shake off the excess. Blot dry with bibulous paper.
6. Microscopy You will need the standard objectives for blood smear review; 10x, 40x and 100x oil immersion. Remember, if you are using a 40x objective, you will need to place a coverslip on top of the slide to get a sharp focus. Add oil immersion and exam the stained blood smear with 100X.





Note: there are certain species of birds, e.g. cranes, and reptiles, e.g. turtles, whose blood hemolyzes on contact with EDTA. This hemolysis invalidates the PCV and affects assessment of red blood cell morphology during blood smear examination. For these species, blood can be collected directly from the needle into citrate anticoagulant. However, the correct citrate to blood ratio must be maintained, i.e. 1 part citrate to 9 parts blood.

Common blood smear errors and their cures

FAULT	CURE
Smear too short or small	Use a larger drop of blood and/or Decrease the angle of the spreader slide and/or Decrease the speed of the spreader slide.
Smear too long, extends to end of slide with no feathered edge	Use a smaller drop of blood and/or Increase the angle of the spreader slide and/or Increase the speed of the spreader slide.
Smear has waves and ridges	Relax the wrist holding the spreader slide (too much downward force causes the spreader slide to skip) and/or Increase the speed of the spreader slide. Maintain even contact between the two slides and a smooth motion while pushing the blood forward
Only part of the drop was picked up by the spreader slide	Draw spreader slide completely back through the drop before pushing forward. If one side of the drop was left behind, the edge of the spreader slide was not in contact with the stationary slide - relax the wrist holding the spreader slide.
Smear too thick	Use a smaller drop of blood and/or Decrease the angle of the spreader slide and/or Increase the speed of the spreader slide.
Smear too thin	Use a larger drop of blood and/or Increase the angle of the spreader slide and/or Decrease the speed of the spreader slide.

Evaluation of blood smear:

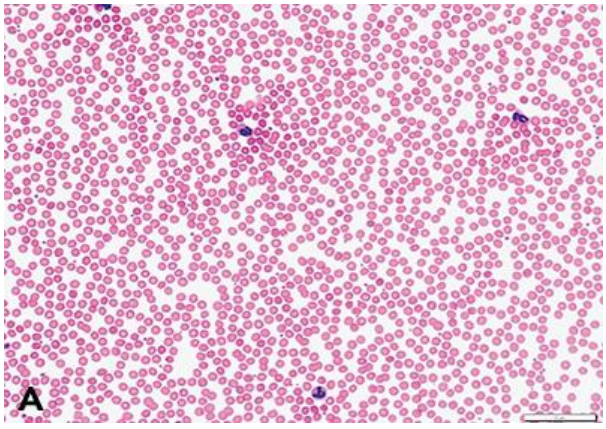
Scan the smear at low magnification (10×), and be sure to:

1. Note red and white blood cell densities in the counting area (Figure 1A), which is a few frames back from the feathered edge (Figure 1B), where cells occur in a monolayer; evaluation deeper in the smear can be more difficult (Figure 1C)
2. Note the presence of:

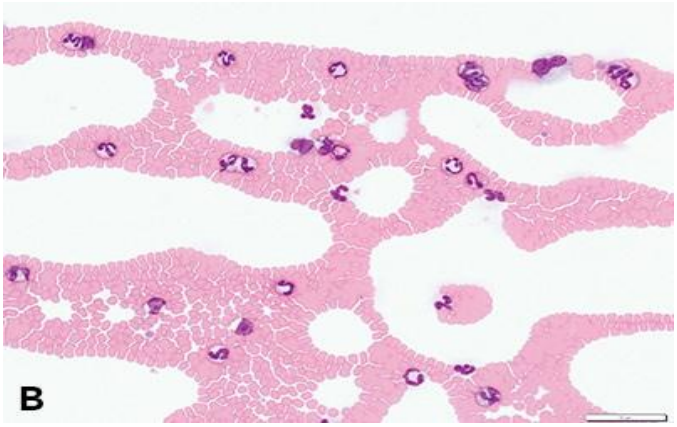
- **Rouleaux** (Figure 2): "Coin stacks" indicative of inflammation or hyperproteinemia that disperse with addition of saline. A mild degree of rouleaux formation is common in cats, and the amount present in Figure 2 would be considered normal in cats
- **Agglutination** (Figure 3): "Grape clusters" or doublets and triplets indicative of immune-mediated interactions that do *not* disperse with saline

3. Observe any bias in cell distribution, such as concentration of leukocytes at the feathered edge that may bias cell count estimates
4. Evaluate for the presence of microfilaria (Figure 4)
5. Identify platelet clumps that might artifactually decrease platelet numbers (Figure 5).

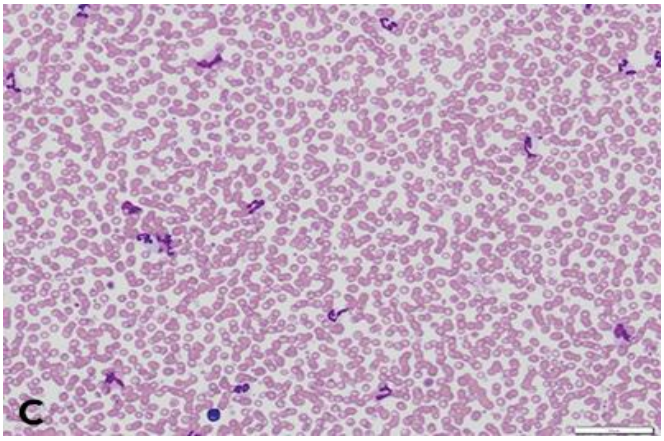
Next, move to a higher magnification (50× or 100×) within the counting area



a) Low magnification canine blood film (200×, Wright-Giemsa stain) illustrating the counting area of the slide, which contains red blood cells (RBCs) in a monolayer with minimal overlap; leukocytes present are minimally distorted. The image is taken at 200× instead of 100× due to distortion caused by the microscope imaging program



b) Canine blood film at the feathered edge of the slide is too distorted to easily evaluate cell morphology. White blood cells (WBCs) can become distorted and RBCs can appear as spherocytes



c) Representative field of the body of a canine blood film, which is too thick to evaluate individual RBC and WBC morphology; RBCs are stacked on each other, with leukocytes compressed or distorted

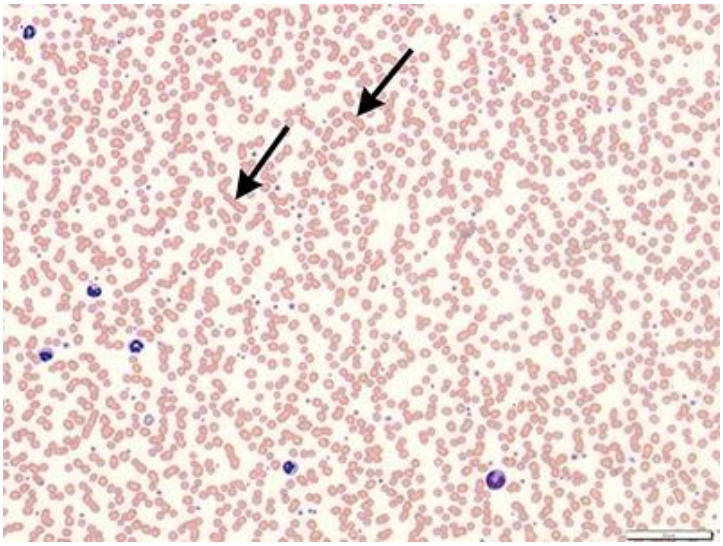


FIGURE 2. Feline blood film with rouleaux (arrows) present, which appear as stacked erythrocytes (similar to a column of coins); this amount of rouleaux is normal in cats. Wright-Giemsa stain; magnification, 200×.

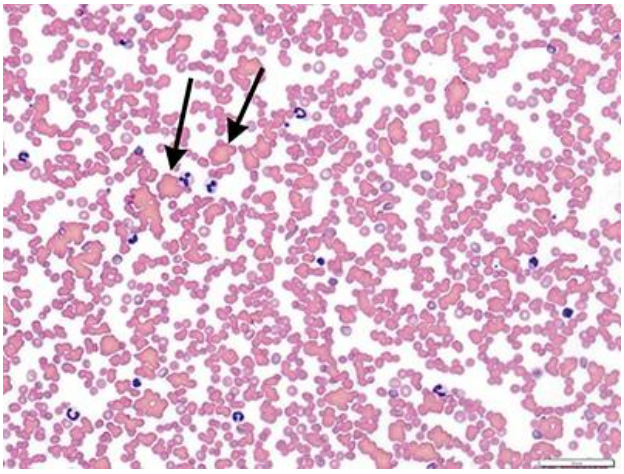


FIGURE 3. Grapelike clusters of agglutinated RBCs (arrows) can be seen in canine patients with immune-mediated hemolytic anemia. Wright-Giemsa stain; magnification, 200×.

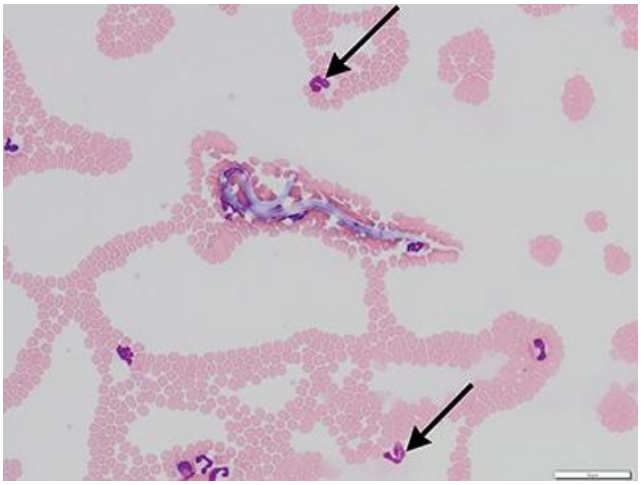


FIGURE 4. *Dirofilaria immitis* microfilaria at the feathered edge of a canine peripheral blood smear. This patient was positive on heartworm antigen testing. Note that many RBCs have lost central pallor along the feathered edge of the sample, making their appearance very similar to that of spherocytes; the leukocytes appear distorted (arrows). The loss of central pallor is likely secondary to thinning of the blood smear at the feathered edge, and leukocyte distortion is likely secondary to dragging of cells to the feathered edge that can occur during slide preparation. Wright-Giemsa stain; magnification, 200×.

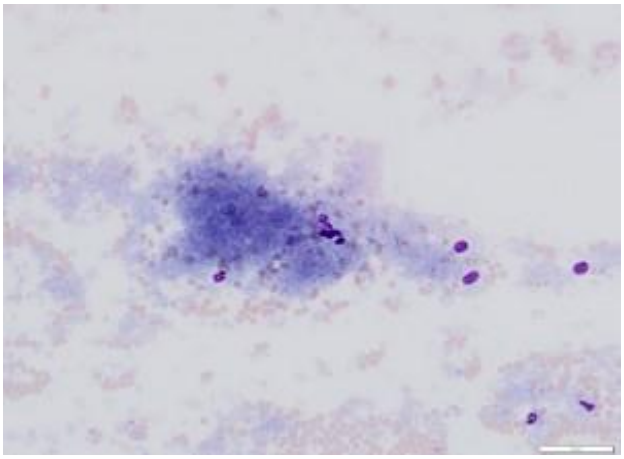


FIGURE 5. Diffuse large platelet clumping along the feathered edge of a feline blood film. Occasionally, platelets stain extremely pale, making them difficult to identify. Wright-Giemsa stain; magnification, 200×.

Red Blood Cells

most important red cell morphologic abnormalities, including:

- **Anisocytosis** and **polychromasia** are indicative of regeneration. Reticulocyte counts are considered the reference standard for identification and quantitation

of a regenerative response, but polychromasia can be used as an estimate, especially in dogs (Figure 6). A low level of polychromasia is normal.[1]

- **Spherocytes**, in large numbers, suggest immune-mediated anemia, zinc toxicity, or bee envenomation. Spherocytes are characterized by loss of central pallor and increased cytoplasm density, and appear smaller than other red cells due to their shape change (Figure 6). They are easier to identify and more common in dogs than in cats, and their presence in small numbers is usually nonspecific. Always evaluate for evidence of agglutination (Figure 3) and, if absent, a Coombs test may be indicated.
- **Heinz bodies** in larger numbers (up to 75% of RBCs) are observed in feline metabolic stress, such as diabetes mellitus, hepatic lipidosis, and other conditions, generally without significant hemolysis; however, in dogs, even small numbers of Heinz bodies are considered pathologic. Large Heinz bodies are relatively easy to identify as knob-like extensions emerging from the margin of red cells (Figure 7), but smaller Heinz bodies can be difficult to identify and may appear as small refractile areas of cytoplasm. **Heinz bodies** form as the result of oxidative damage and can be important indicators of exposure to toxic amounts of acetaminophen, zinc, onions, garlic, and leeks, among other toxic substances. A careful history that evaluates potential exposure is critical, and radiographs to identify metal foreign bodies may be indicated. Eccentrocytes—red cells with hemoglobin that has pulled away from the cell membrane—also indicate oxidative damage and may accompany Heinz bodies (Figure 8).
- **Schistocytes,** in large numbers, are often reflective of vascular pathology, including potential for disseminated intravascular coagulation and hemangiosarcoma. These fragmented erythrocytes, which can occur nonspecifically in small numbers, are also associated with fragmentation anemia (Figure 9).

- **Ghost cells** are observed with intravascular hemolysis. These cytoplasm-free membranes are more rarely seen as artifacts (Figure 10).
- **Acanthocytes,** red cells with irregular projections, are associated with many underlying conditions, including metabolic derangements, vascular abnormalities (eg, hemangiosarcoma), and liver disease (Figure 9).
- **Echinocytes** are often associated with drying artifact or electrolyte abnormalities. These cells have numerous, even sharp, cytoplasmic projections. They may be present in large numbers, but are not critical from a diagnostic perspective (Figure 7).
- **Nucleated RBC** precursors are released from the marrow as part of a regenerative process, but may also signal endothelial damage (ie, sepsis, thermal), lead toxicity, architectural disruption of hematopoietic organs (eg, spleen), or hematopoietic neoplasia (Figure 11). Large numbers—in the absence of a regenerative response or obvious sepsis or hyperthermia—indicate that a pathologist should evaluate the blood film. Typically, these are metarubricytes, though earlier precursors can sometimes be observed.
- **Inclusions t**hat may be present:

- ***Howell-Jolly bodies***, dense, round purple inclusions, which are common and represent retained nuclear material normally observed in cats, or associated with increased red cell turnover or decreased splenic function.
- ***Red cell parasites***, which may appear as pyriform (*Babesia* species), flat, or round inclusions on the cell surface that may detach with time if smears are not prepared immediately after sample collection (*Mycoplasma* species) (Figure 12).
- ***Viral inclusions,*** such as those occasionally seen in the acute phase of canine distemper, which are rare but diagnostically invaluable (Figure 13).

White Cells / Leucocytes

Morphologic observations of white blood cells (WBCs) are made while performing a 100-cell differential cell count at high magnification within the counting area.

- **Toxic change of neutrophils** indicates acute, systemic inflammation and, when moderate or marked, is most closely associated with toxemia from bacterial infections. Slight toxic change is common in cats and slight to mild toxic change can be seen in a variety of disease processes that are not necessarily infectious (immune mediated disease, neoplasia, drugs) or bacterial (fungal disease, protozoal infection, viruses). Common components include cytoplasmic basophilia, vacuolization, and presence of Döhle bodies—small irregular inclusions that, in small numbers, can be present in healthy cats.
- **Left shift** indicates the presence of granulocyte precursors, mostly band forms in which nuclear segmentation is incomplete (**Figure 15**). Left shift often occurs along with toxic change, which indicates the release of granulocyte precursors due to an intense demand for inflammatory cells in peripheral tissues.
- **Reactive lymphocytes** are nonspecific indicators of antigenic stimulation. They are characterized by a slightly larger size (in some cases, approximately the size of a neutrophil), increased amounts of cytoplasm that can have enhanced cytoplasmic basophilia, prominent perinuclear clear zone, a few small clear punctate vacuoles, and/or small magenta granules (**Figure 16**); nucleoli should *not* be present.
- **WBC inclusions** may indicate an infectious agent. Distemper inclusion bodies can be visualized in leukocytes and erythrocytes (**Figure 13**); other relevant infectious agents include tick-borne diseases caused by *Anaplasma* species (**Figure 17**). Rarely bacteria can be seen within neutrophils or monocytes in septic patients.

- **Lymphoblasts (Figure 18), mast cells (Figure 19), and malignant histiocytes** are abnormal cells seen with some frequency but not specifically identified by analyzers**.**
- ***Immature lymphocytes*****,** in small numbers, are occasionally seen in septic patients; therefore, a pathologist should always review smears containing immature lymphocytes.
- ***Mast cells*** are often easiest to identify by examining the feathered edge of the smear, but poorly granulated forms can be challenging to identify. In dogs, mast cells are associated with a number of diseases as well as mast cell neoplasia[2]; in cats, the presence of mast cells typically indicates visceral mast cell disease, increasing the concern for neoplasia**.**

Platelets:

Large platelets are evidence of platelet turnover, which can reflect destructive or consumptive processes, such as immune-mediated thrombocytopenia, inflammation, and disseminated intravascular coagulation.

High globulin levels

When globulin levels are significantly increased, the protein will be present as a pale pink background. In thicker areas of the slides, the protein background will fold over on itself as it dries creating protein crescents or 'finger-nail clippings'.

Reference:

- https://www.agric.wa.gov.au/livestock-biosecurity/blood-smear-technique-veterinarians?page=0%2C0
- https://www.vet.cornell.edu/animal-health-diagnostic-center/laboratories/clinical-pathology/samples-and-submissions/hematology
- https://www.vin.com/apputil/content/defaultadv1.aspx?id=5328156&pid=11349&