

CYTOLOGY

Blood Smear Review: A Step-by-Step Guide to Normal Findings for Cats and Dogs

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A complete blood count (CBC) is an important part of a minimum database, providing information on peripheral blood cells that can help identify underlying disease and illness. Hematology analyzers are commonly used to survey mammalian blood cells, and they perform relatively well for objective cellular quantifications and measurements. However, currently available in-house benchtop analyzers cannot assess morphologic changes and may be subject to interferences and misclassification of cells.¹⁻³ Therefore, concurrent blood smear review is recommended to verify and complement hematology analyzer findings, particularly for sick patients, for patients with flagged analyzer results, and/or if results do not match the clinical picture.

A blood smear is cost-effective, minimally invasive, and relatively easy to perform and review in-house. In addition to verifying analyzer results, a blood smear can confirm a white blood cell (WBC) differential count, reveal morphologic changes of blood cells (e.g., red blood cells [RBCs], WBCs, platelets), and may facilitate detection of hemoparasites. If the results are uncertain after the review is performed, samples can be sent to a veterinary pathologist for further interpretation. In that case, when mailing a CBC blood sample to a reference laboratory, clinicians are advised to submit a freshly made blood smear along with anticoagulated blood (ideally collected less than 48 hours from laboratory receipt) to mitigate any storage-related changes that can occur with transport.

Abstract

A blood smear review is a cost-effective, minimally invasive, and important part of a complete blood count that can be used to identify underlying disease and illness. A systematic approach to manual blood smear review is recommended, starting with a low-magnification scan of the entire smear (to identify cellular patterns and large structures) followed by a high-magnification review of the monolayer (to assess red blood cell, white blood cell, and platelet morphology and numbers). This article describes the steps of a blood smear review with a focus on key features expected in clinically healthy dogs and cats, highlighting species- and breed-related differences to facilitate detection of clinically relevant findings.



Take-Home Points

- Automated hematology analyzers cannot reliably assess morphologic changes and may be subject to interferences and misclassification of cells.
- Blood smear reviews are used to verify and complement analyzer results and may help identify neoplasia, infection, immune-mediated disease, and more.
- For accurate review, the blood smear must be properly prepared and stained.
- The entire smear should first be evaluated on low magnification because large structures and patterns can be missed by going straight to high magnification.
- Key elements identified at low magnification (10× objective) include the 3 divisions of the blood smear (i.e., body, monolayer, and feathered edge), cellular densities/associations, and large structures (e.g., platelet clumps, atypical cells, microfilariae).
- Key elements discerned at high magnification (40× to 100× objective) include white blood cell differential, estimated white blood cell count, estimated platelet count, and assessment of all blood cell morphologies.
- Blood cell morphology differs among some species and breeds, and knowing what to expect in healthy dogs and cats is necessary to recognize abnormalities.

This article describes the steps of performing a blood smear review and highlights normal findings for dogs and cats. Knowing what to expect in healthy animals can help clinic personnel recognize abnormalities. Similar to a physical examination, the approach for a thorough blood smear review should be stepwise and the smear should be properly prepared and stained (how that can be achieved is covered elsewhere).⁴⁻⁷

STEP 1: REVIEW ENTIRE SLIDE AT LOW MAGNIFICATION

Low-magnification scans (10× objective) help identify key divisions of a blood smear and screen for cell patterns and large structures. A blood smear can be divided into 3 parts (**FIGURES 1 AND 2**):



FIGURE 1. (A) Gross image of blood smear and **(B)** highlighted divisions where the body is demarcated in orange, the monolayer in white, and the feathered edge in yellow. Diff-Quik stain.

- **Body (FIGURE 2A):** The thicker base of a smear where cells exhibit significant piling or contraction, which often impedes reliable individual cellular distinctions. Larger structures can infrequently be found here (see feathered edge point below).
- **Monolayer (FIGURE 2B):** The thin, evenly dispersed area between the body and feathered edge, where cells should have minimal to no overlap and be displayed individually without significant distortions. *Identifying the monolayer is key because this is where higher magnification morphologic assessments will be made.*
- **Feathered edge (FIGURE 2C):** The patchy leading edge of a smear, where cells are often distorted or disrupted. Large structures (e.g., abnormal cells, large infectious agents, platelet clumps) can accumulate at the feathered edge.⁸

Other features assessed at low magnification include:

- **Cellular densities/associations:** Crude estimates of RBC and WBC density can be determined on low magnification and correlated with analyzer results. For example, anemic patients will have increased space between RBCs, and leukopenic patients will have sparse WBCs. Those with leukocytosis will have frequent WBCs and may exhibit leukergy, or WBC clumping. RBC associations (e.g., rouleaux versus agglutination; **FIGURE 3**) and platelet clumps (**FIGURE 4**) can also be visualized at this magnification.
- **Atypical cells**, such as cancer cells, mast cells, and macrophages.
- **Large infectious agents**, such as microfilariae.

STEP 2: REVIEW MONOLAYER AT HIGH MAGNIFICATION

Most of the time spent doing a blood smear review will be analyzing the monolayer at high magnification (40× to 100× objective). Individual cellular distinctions (e.g., cell types, morphologic changes) can be made during this step.

White Blood Cells

With regard to WBCs, one of the key components of blood smear review is a manual WBC differential,

which is used to confirm the analyzer differential (if provided) and its morphologic assessments. The number of WBCs in the monolayer can also be estimated by multiplying the average number of WBCs

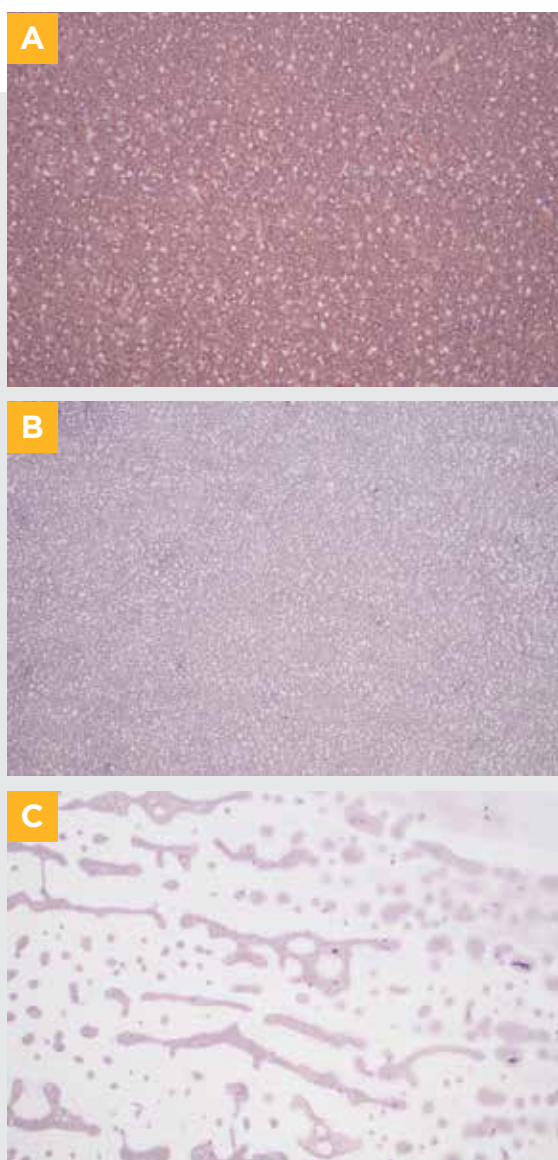


FIGURE 2. Divisions of the blood smear: (A) body, (B) monolayer, (C) feathered edge. Diff-Quik stain, 10× objective.

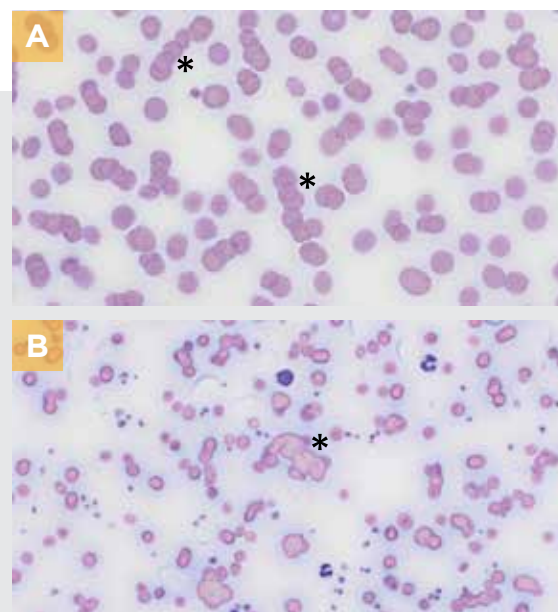


FIGURE 3. Red blood cell associations: (A) feline blood smear showing rouleaux formation (asterisks), which can be a normal finding for this species; (B) canine blood smear showing agglutination (asterisk), which is an abnormal finding for this species. Wright-Giemsa stain, 100× objective.

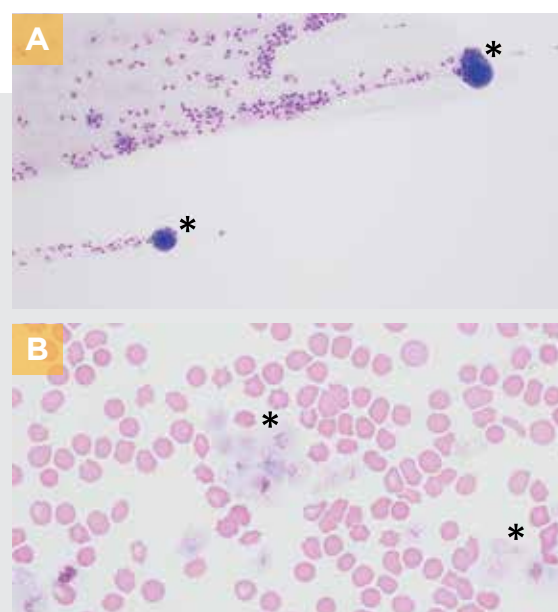


FIGURE 4. Feline blood smear with platelet clumps (asterisks) in the (A) feathered edge and (B) monolayer. Wright-Giemsa stain, (A) 10× and (B) 100× objectives.

per field across 10 nonoverlapping 40× fields by 1600, reported as number of WBCs per microliter.

A WBC differential is determined by classifying at least 100 WBCs by cell type, yielding a percentage that can be multiplied by the total WBC count to determine absolute concentration. While the 100-cell differential is being determined, a tally of nucleated red blood cells (nRBCs) should be kept, reported as number of nRBCs per 100 WBCs. (See **RBC MORPHOLOGY: INCLUSIONS**.)

TABLE 1 Size of Major Blood Cells in Dogs and Cats

CELL	DOG	CAT
Red blood cell	6–8 μm	5–6 μm
Platelet	1–3 μm	2–5 μm
Neutrophil	10–12 μm	
Lymphocyte	7–10 μm	
Monocyte	15–20 μm	

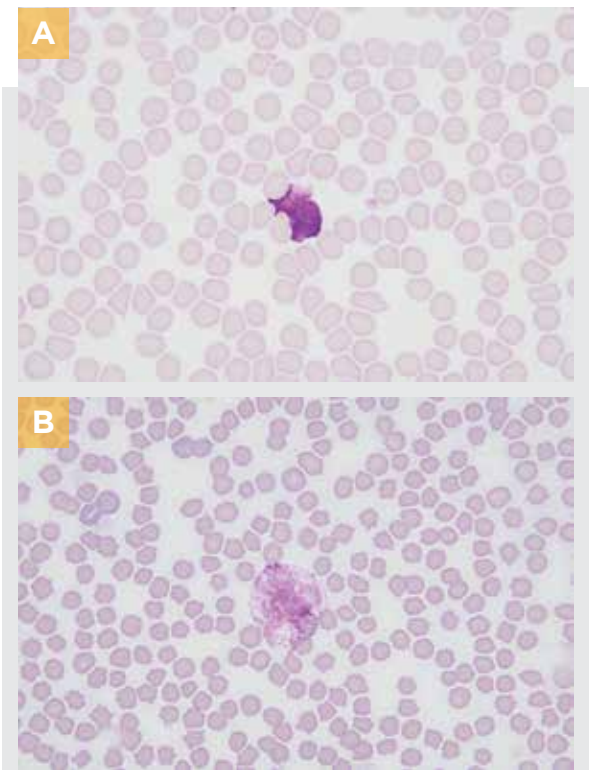


FIGURE 5. Examples of lysed cells (also referred to as smudge or basket cells) in (A) canine and (B) feline blood. Wright-Giemsa stain, 100× objective.

Mammalian peripheral blood WBCs can be distinguished by their nuclear shape (i.e., segmented/polymorphonuclear [granulocytes] versus mononuclear [lymphocytes and monocytes]), cytoplasmic characteristics, and size (**TABLE 1**). Cellular distinctions must be made on intact cells, and lysed cells should be skipped but noted (because numerous lysed cells can alter the differential) (**FIGURE 5**).

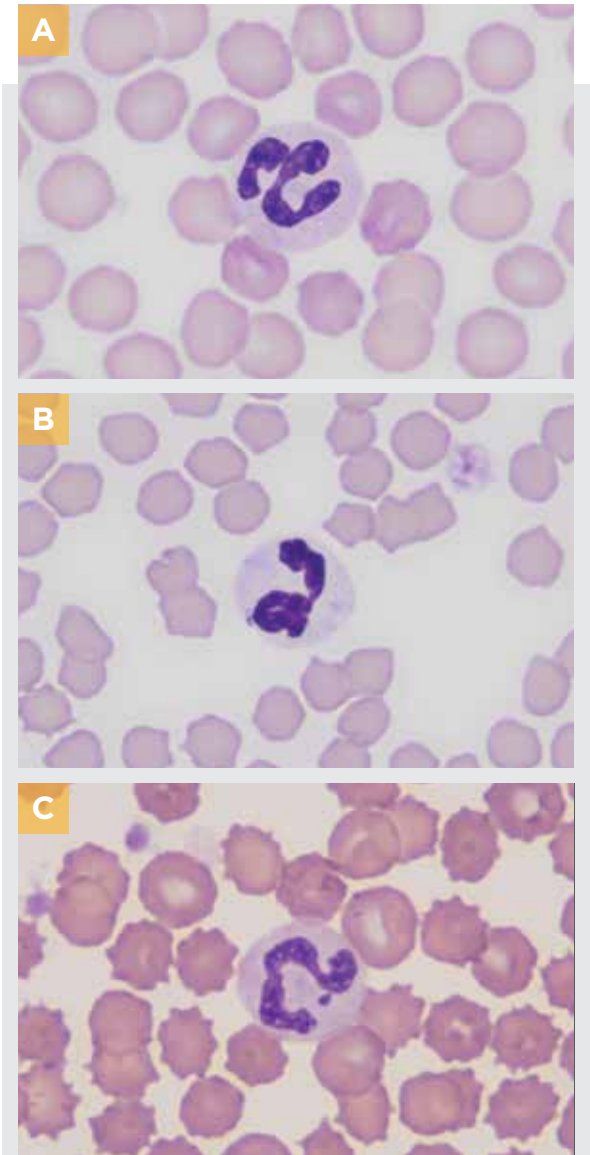


FIGURE 6. Neutrophils in healthy (A) canine and (B) feline blood. (B) Notice the feline neutrophil contains a few small blue cytoplasmic inclusions (Döhle bodies). (C) Neutrophils in female patients may have a single, small teardrop-shaped extension of the nucleus, representing the inactivated X-chromosome (Barr body). Canine blood. (A and B) Wright-Giemsa stain, (C) Diff-Quik stain; 100× objective.

Granulocytes

Granulocytes can be distinguished by their cytoplasmic granules. The predominant granulocytes in healthy dogs and cats are neutrophils; eosinophils and basophils are rarely encountered in healthy dogs and cats.

■ **Neutrophils (FIGURE 6)** contain granules that stain neutrally or indistinctly, making the cytoplasm appear clear to faintly pink. Feline neutrophils may contain a few small, rounded basophilic cytoplasmic inclusions (Döhle bodies, FIGURE 6B). For certain cat breeds (e.g., Siamese, Himalayan, Birman), reddish-pink cytoplasmic granules are sometimes noted.^{9,10} Mature neutrophils should have 3 to 4 nuclear segments; hypersegmentation (more than 5 segments) can occur as an age-related change due to prolonged blood storage, excess glucocorticoids (endogenous or exogenous), and/or other pathologic conditions. Female patients may have a small teardrop-shaped extension of a neutrophil nucleus, which represents an inactivated X-chromosome (Barr body, FIGURE 6C) and should not be confused with infectious agents such as morulae. Rare immature neutrophils (typically less than 5% of WBCs) with C- or U-shaped nuclei

(i.e., bands) can be seen in blood smears from healthy dogs and cats. An inherited defect in granulocyte nuclear segmentation (i.e., Pelger–Huët anomaly) in dogs (e.g., Australian Shepherds)¹¹ and cats¹² has been documented, and granulocyte nuclei of affected animals will appear peanut-shaped, similar to bands, or rounded. Increased incidence of Döhle bodies (in addition to cytoplasmic basophilia and vacuolation) and band neutrophils can be markers of inflammation (e.g., toxic change and left shift, respectively).

■ **Eosinophils (FIGURE 7)** are slightly larger than neutrophils and contain pink granules, which are rod-shaped in cats and irregularly rounded in dogs. “Gray” eosinophils have been reported in some dogs (e.g., greyhounds)¹³ and cats¹⁴ and have pale blue/gray cytoplasm with very pale/clear granules and may be difficult to distinguish from neutrophils.

■ **Basophils (FIGURE 8)** are slightly larger than neutrophils; those of cats contain numerous lavender granules and those of dogs sometimes contain few distinct purple granules. Basophils can be distinguished from monocytes by their nuclear segmentation and lack of distinct cytoplasmic

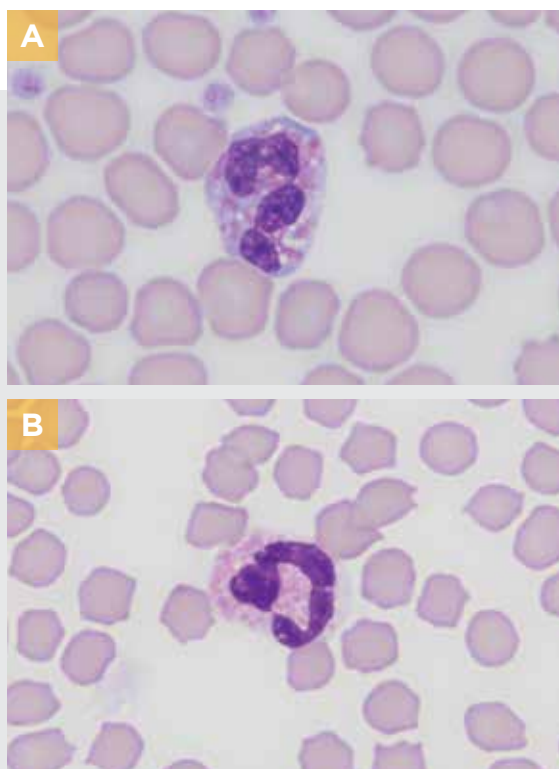


FIGURE 7. Eosinophils in (A) canine and (B) feline blood. Canine eosinophils have irregularly round, patchy granules while feline eosinophils contain many small rod-shaped granules. Wright-Giemsa stain, 100× objective.



FIGURE 8. Basophils in (A) canine and (B) feline blood. Canine basophils contain variably distinct small purple granules while feline basophils contain many large, lavender granules. Wright-Giemsa stain, 100× objective.



vacuolation. Basophils should not be confused with mast cells, which have round nuclei and several more diffusely distributed purple granules.

Mononuclear Cells

In healthy dogs and cats, the predominant mononuclear cells are lymphocytes; monocytes are present in low numbers. Unlike granulocytes, the appearance of mononuclear cells in dogs and cats is similar.

- **Lymphocytes (FIGURE 9)** range in size but are typically small, measuring roughly the same size as RBCs to slightly smaller than neutrophils. They have a rounded to ovoid nucleus with condensed chromatin and scant to low amounts of pale blue cytoplasm. Low numbers (less than 5% to 10%) of lymphocytes may contain a few fine pink cytoplasmic granules (T and NK [natural killer] cells).
- **Monocytes (FIGURE 10)** are the largest peripheral blood WBCs. They have an ovoid to lobulated to

indented nucleus with blue-gray cytoplasm that occasionally contains few distinct, clear vacuoles.

Red Blood Cells

Critical components of blood smear review also include evaluation of individual RBC morphology and the way RBCs are interacting with one another on the slide.

RBC Associations

In most species, RBCs are individualized, but some are prone to rouleaux formation. RBCs demonstrating rouleaux line up in a column, resembling stacks of coins (**FIGURE 3A**). This pattern is common in cats and needs to be distinguished from the more pathologic agglutination indicative of antigen–antibody binding (**FIGURE 3B**). Agglutinated RBCs resemble grape clusters or disorganized aggregates of RBCs. Rouleaux formation, not agglutination, may disperse when blood is mixed with saline. Details about the

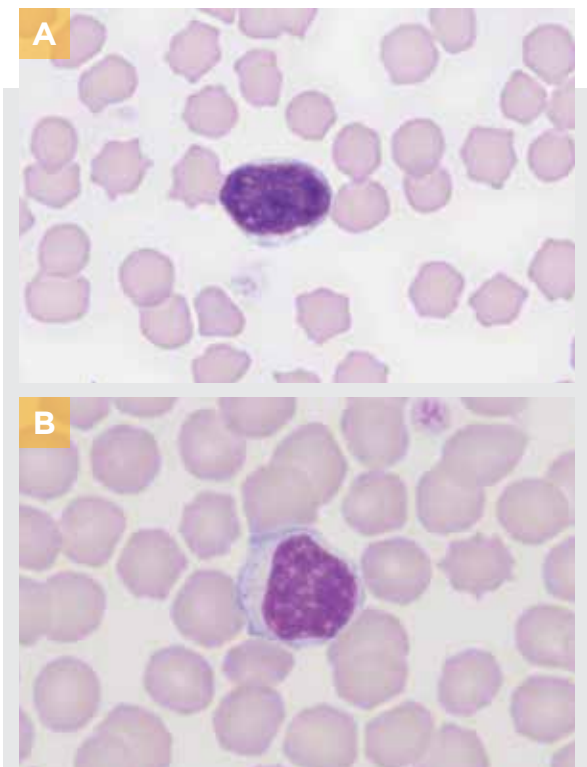


FIGURE 9. Lymphocytes appear similar in dogs and cats. Pictured are **(A)** typical small mature lymphocyte with scant cytoplasm and condensed chromatin (cat) and **(B)** small lymphocyte with punctate pink cytoplasmic granules (T or NK [natural killer] cell origin; dog). Wright-Giemsa stain, 100× objective.

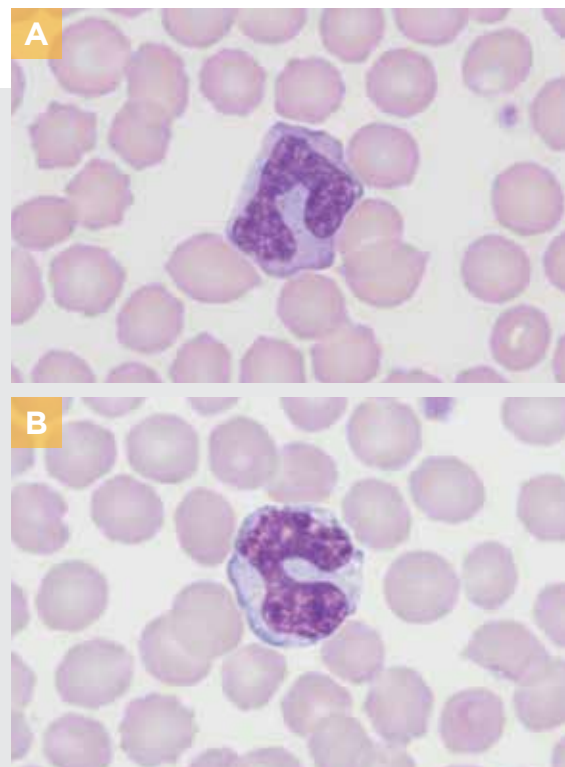


FIGURE 10. Monocytes appear similar in dogs and cats and can vary in appearance. They are large, containing a lobulated **(A and B)** to sometimes ovoid to indented nucleus and deeper basophilic cytoplasm that may contain distinct, clear vacuoles **(B)**. Canine blood. Wright-Giemsa stain, 100× objective.

current recommendations for saline-to-blood ratios can be found elsewhere.¹⁵

RBC Morphology

The 4 categories of RBC morphology assessments are size, color, shape, and inclusions.

- **Size:** Mild anisocytosis (variation in cell size) is common for healthy dogs and cats (TABLE 1 AND FIGURE 11A). The degree of anisocytosis can increase with a variety of conditions, particularly RBC regeneration (FIGURE 11B).
- **Color:** Central pallor, the central area of lightened color in RBCs, is most prominent in dogs and minimal in cats (FIGURE 12A AND 12B). Although increased central pallor can indicate a pathologic condition such as iron deficiency, RBCs with increased central pallor that have a distinct, punched-out appearance (i.e., torocytes) are more likely the result of artifact (FIGURE 12C). Most healthy patients will have

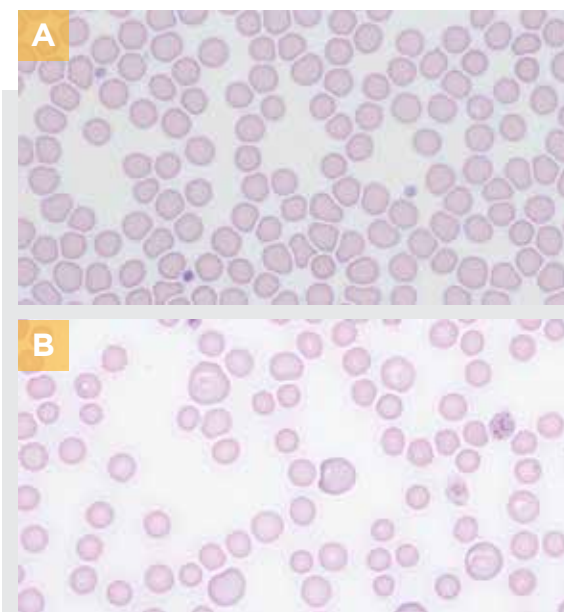


FIGURE 11. Red blood cell size: (A) canine blood smear with mild anisocytosis; (B) canine blood smear with marked anisocytosis and moderate polychromasia due to regenerative anemia. Wright-Giemsa stain, 100× objective.

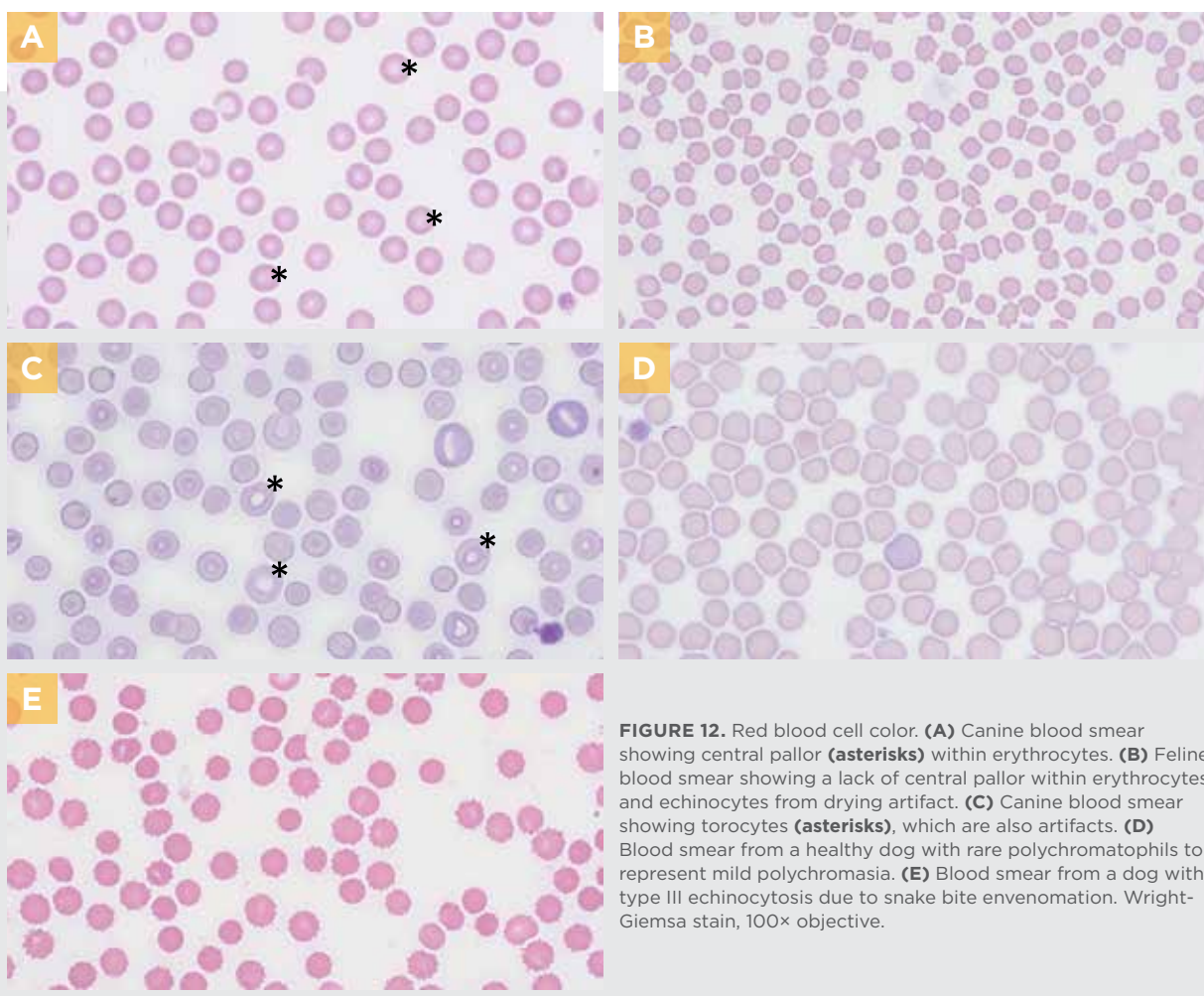


FIGURE 12. Red blood cell color. (A) Canine blood smear showing central pallor (asterisks) within erythrocytes. (B) Feline blood smear showing a lack of central pallor within erythrocytes and echinocytes from drying artifact. (C) Canine blood smear showing torocytes (asterisks), which are also artifacts. (D) Blood smear from a healthy dog with rare polychromatophils to represent mild polychromasia. (E) Blood smear from a dog with type III echinocytosis due to snake bite envenomation. Wright-Giemsa stain, 100× objective.



low numbers of circulating polychromatophilic RBCs (immature RBCs that stain bluish-red due to remaining ribosomal RNA as well as hemoglobin); therefore, mild polychromasia (less than 1.5% of all RBCs) is often noted (**FIGURE 12D**). Polychromasia is expected to be more profound in patients with regenerative anemia (**FIGURES 11B AND 13A**).

- **Shape:** RBCs in dogs and cats are disc-shaped (i.e., discocytes) and should appear rounded on a blood smear. Poikilocytosis, a general term applied to abnormally shaped RBCs, is nonspecific and not necessarily indicative of a cause. If observed, specific RBC shape changes (e.g., spherocytes, acanthocytes, codocytes) can provide more direct insight as to a pathologic condition or contributing artifact.⁸ RBCs with irregular or angular projections are called echinocytes and are a drying artifact from blood smear preparation in a humid environment (**FIGURE 12B**). Echinocytes can be subclassified (types I, II, and III) according to differences in morphologic features such as spicule number and length.¹⁶ When significant numbers of echinocytes are present and an artifact has been ruled out, then pathologic causes of echinocytosis, such as envenomation, should be considered (**FIGURE 12E**).¹⁷
- **Inclusions:** Frequent RBC inclusions in blood of healthy cats are Howell-Jolly bodies and Heinz bodies.

Howell-Jolly bodies are small, round, and deeply basophilic inclusions that represent nuclear remnants from early (i.e., nucleated) RBCs (**FIGURE 13B**). Howell-Jolly bodies in the blood of healthy cats are commonly encountered in low numbers due to nonsinusoidal spleens. When found in dog blood, Howell-Jolly bodies typically indicate increased RBC turnover or altered splenic function because the spleen is responsible for removing those remnants. nRBCs are rare in the circulation of healthy dogs and cats (normal is less than 5 per 100 WBCs), and the presence of late-stage nRBCs (metarubricytes) typically corresponds to other evidence of RBC regeneration (**FIGURE 13A**). Metarubricytes look very similar to small, mature lymphocytes and can affect automated analyzer WBC counts; when nRBCs exceed 5 per

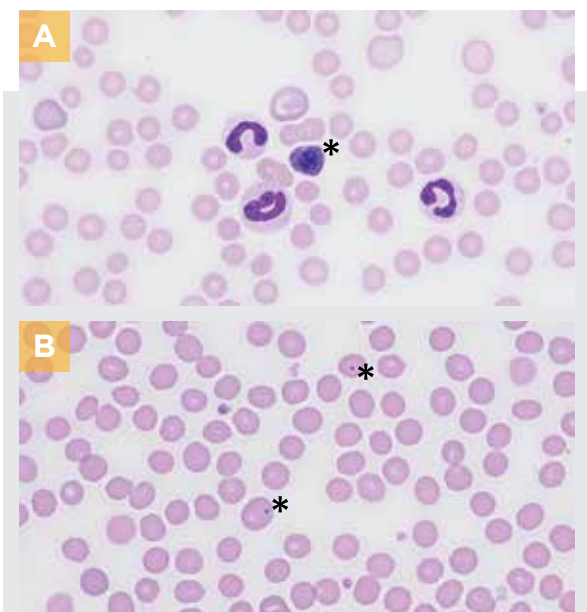


FIGURE 13. Red blood cell inclusions: **(A)** canine blood smear showing a nucleated red blood cell (**asterisk**), moderate anisocytosis, and few polychromatophils; **(B)** canine blood smear showing 2 Howell-Jolly bodies (**asterisks**). Wright-Giemsa stain, 100× objective.

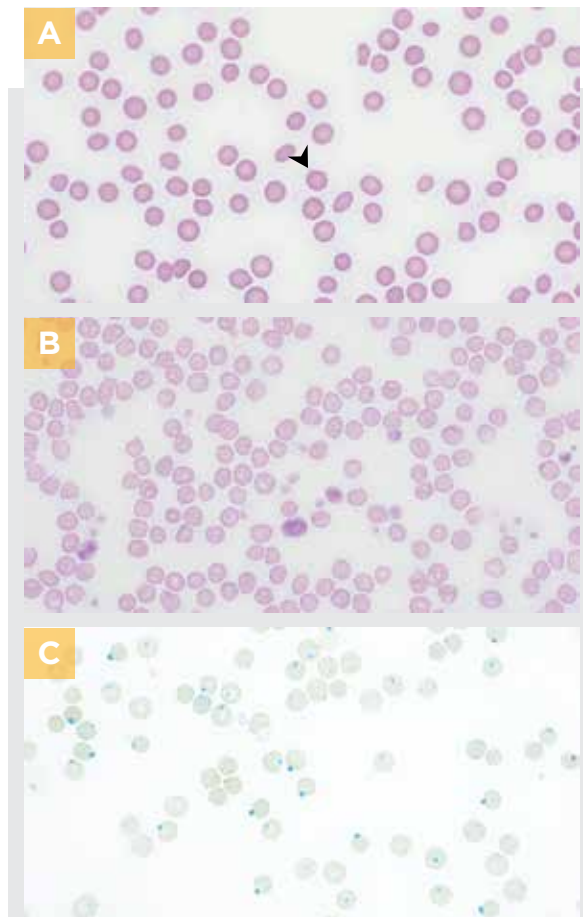


FIGURE 14. Red blood cell inclusions. **(A)** Blood smear from a healthy cat showing rare Heinz bodies (**arrowhead**). **(B)** Blood smear from a cat with oxidative damage showing numerous Heinz bodies. **(C)** Blood smear from a cat with oxidative damage; dark blue aggregates on the edges of the erythrocytes are Heinz bodies. **(A and B)** Wright-Giemsa stain, **(C)** new methylene blue stain; 100× objective.

Some dog breeds (e.g., Cavalier King Charles spaniels) may be born with a genetic defect that results in production of fewer large platelets (macroplatelets), but their total volume of platelets is similar to that in other dogs.

100 WBCs, a WBC count correction is performed.¹⁶ Heinz bodies are small, button- or knob-like projections that extend from RBCs and represent aggregated hemoglobin (FIGURE 14A). Moderate to high numbers of Heinz bodies indicate oxidative damage (FIGURE 14B), but low numbers of small Heinz bodies (less than 5% of RBCs affected) can be found in the blood of clinically healthy cats. Heinz

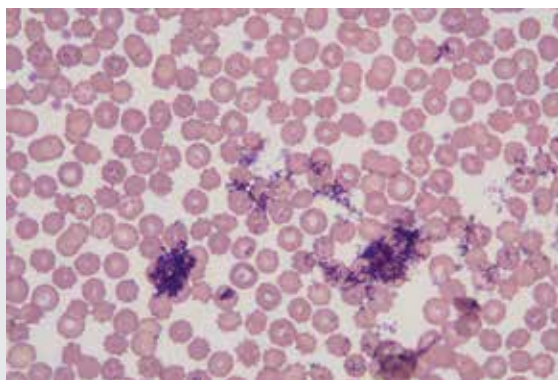


FIGURE 15. Canine blood smear showing stain precipitate, refractile granular material that is overlying red cells (i.e., in a different plane of focus). Diff-Quik stain, 100× objective.

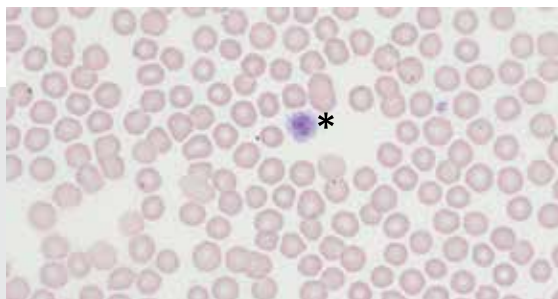


FIGURE 16. Canine blood smear showing a giant platelet (asterisk). Wright-Giemsa stain, 100× objective.

bodies can be challenging to detect on Wright-Giemsa blood smears; new methylene blue stain can be mixed with blood to accentuate Heinz bodies by staining them blue-green (FIGURE 14C). When looking for inclusions on a blood smear, know what to disregard. Refractile (shiny) precipitate or suspected inclusions or debris that is not in the same plane of focus as the erythrocyte is likely an artifact (FIGURE 15).

Platelets

When platelets are reviewed on a blood smear, a useful task is identifying platelet clumping (FIGURE 4). Clumps are often found on the feathered edge of a smear, but they can also occur within the monolayer or body. Platelet clumping is particularly common in cats yet also results from challenging venipuncture and delays in sample processing for all species. Depending on the degree of clumping, automated platelet counts can be falsely lower. When clumping is present, automated and manual platelet counts reflect the minimum possible number for a sample. Because platelet clumps do not evenly disperse throughout a blood sample, estimating platelet count based on the number of clumped platelets is not possible. Platelet number may be estimated from a blood smear by multiplying the average number of platelets per 100× (oil immersion) field across 10 fields by 15 000, yielding an estimated number of platelets per microliter. Manual platelet counts are less accurate and precise than automated methods, but they can provide insight in the absence of other data and when automated errors are suspected.

Regarding morphology, platelets can vary in size between patient species (although they are typically smaller than RBCs; TABLE 1), and they range from pale to quite granular. Large or giant platelets (FIGURE 16) are evidence of platelet turnover/regeneration. Some dog breeds (e.g., Cavalier King Charles spaniels) may be born with a genetic defect that results in production of fewer large platelets (macroplatelets), but their total volume of platelets is similar to that in other dogs. Thus, reference intervals derived from other breeds may make these patients seem thrombocytopenic in comparison, but they typically do not have any clinical evidence of thrombocytopenia.¹⁸ Because of the method used by many automated analyzers to distinguish RBCs from platelets, sometimes larger platelets cannot be distinguished from small RBCs, or even cytoplasmic fragments or debris. Manual evaluation of platelets and RBCs in these patients can be useful.



SUMMARY

Automated hematology analyzers are useful pieces of equipment that are becoming increasingly prevalent in veterinary clinics, but they cannot reliably detect morphologic changes and can be subject to interferences and misclassification of cells. As such, blood smear review is recommended to confirm analyzer cell counts and evaluate blood cell morphology, providing insight into underlying conditions and informing next diagnostic steps. Blood smears can be analyzed in-clinic, and personnel can be trained to make, stain, and review blood smears.

A blood smear review should be approached systematically, starting with a low-magnification scan of the entire smear (to identify cellular patterns and large structures) followed by a high-magnification review, particularly in the monolayer (to thoroughly assess cell morphology and numbers). Because blood smear findings may differ by patient species and breed, knowing what to expect in healthy dogs and cats can help personnel recognize abnormalities. When in doubt, samples can be easily forwarded to a local reference laboratory; do not hesitate to reach out with questions. **TVP**

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