

CYTOLOGY

Blood Smear Review: Erythrocyte Morphology Changes in Dogs and Cats

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Automated hematology analyzers help clinicians identify abnormalities of erythrocyte and hemoglobin concentrations as well as certain erythrocyte morphology changes (e.g., size, volume). Automated analyzers are helpful for diagnosing and classifying anemia; however, they fail to fully assess erythrocyte morphology, which can provide useful diagnostic information about the underlying cause of anemia or other diseases. In particular, certain abnormalities of erythrocyte shape (e.g., acanthocytes, elliptocytes, codocytes, eccentrocytes) and association (e.g., agglutination, rouleaux) may not be detected by current automated technology, leading to possible misclassification of cells and/or false size and volume assessments. Recognizing abnormalities through blood smear review can identify pathologic conditions and

provide critical information about the underlying mechanisms of disease.

This article reviews the most common erythrocyte morphology changes that can be identified by blood smear review and focuses on association, size, color, and shape (**BOX 1**). An article in the *Today's Veterinary Practice* November/December 2025 issue will cover erythrocyte inclusions (e.g., Heinz bodies, Howell–Jolly bodies, infectious agents).

ERYTHROCYTE BASICS

In dogs and cats, mature erythrocytes are rounded, anucleate cells that consist of a plasma membrane and underlying cytoskeleton, hemoglobin, and glycolytic

Abstract

Automated hematology analyzers are frequently used to evaluate erythrocytes and detect changes of concentration, size, and volume; however, analyzers are often not reliable for detecting changes of erythrocyte morphology that may have a clinical effect. For example, changes of associations among erythrocytes could suggest an underlying inflammatory or immune-mediated disease; erythrocyte size and color changes could indicate a regenerative response or iron deficiency; and certain erythrocyte shape changes could point to erythrocyte damage/fragmentation, immune-targeting, or oxidative injury.



Take-Home Points

- Automated hematology analyzers provide quantitative information about erythrocyte number and size and hemoglobin concentration but fail to accurately assess erythrocyte morphology.
- Blood smear review is a crucial component of a CBC, complementing automated analyzer findings and enabling thorough evaluation of erythrocyte morphology.
- Abnormalities of erythrocyte association, size, color, and shape are frequently encountered with veterinary patients, and their detection can lead to improved patient care.
- Rouleaux can be distinguished from agglutination by a saline dispersion/agglutination test and should involve a saline-to-blood ratio that is >1:1.
- Variation of cell size can be determined by blood smear review and/or automated indices (e.g., red cell distribution width, mean corpuscular volume).
- Echinocytes represent a common shape change that is often attributed to artifact but can be associated with pathology.
- Some erythrocyte morphology changes can interfere with analyzer-derived values for erythrocyte concentration, mean corpuscular volume, mean corpuscular hemoglobin concentration, and platelet concentration.

enzymes (**FIGURE 1**). They principally deliver gases to and from tissues via capillaries, which are not much larger than the diameter of an erythrocyte. As a result, the erythrocyte membrane and cytoskeleton maintain a biconcave disc shape that enables flexibility to move through the vessels without rupturing and to provide efficient gas exchange. Hemoglobin is an iron-containing protein that is responsible for binding oxygen and carbon dioxide for transport; it makes up roughly one-third of an erythrocyte's contents (hence the reason why a patient's hemoglobin should be one-third of its hematocrit) and also provides some structural support. Because mature erythrocytes lack organelles, they rely on glycolytic enzymes for energy

BOX 1 Common Erythrocyte Morphology Changes Detected by Blood Smear Review

Association

- Rouleaux
- Agglutination

Size

- Anisocytosis
- Macrocytes
- Microcytes

Color

- Polychromasia
- Hypochromasia

Shape

- Poikilocytosis
- Echinocytes
- Acanthocytes
- Keratocytes
- Schistocytes
- Eccentricocytes
- Spherocytes
- Ghost cells
- Elliptocytes
- Codocytes

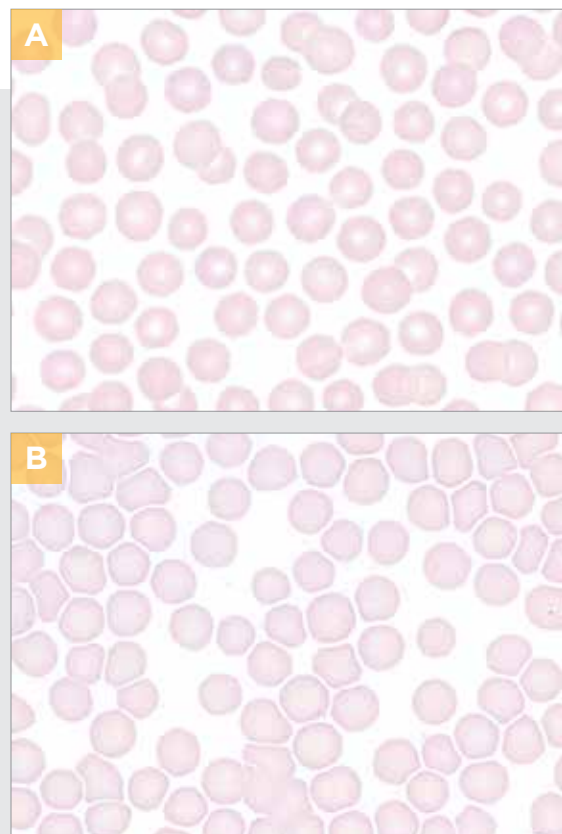


FIGURE 1. Normal erythrocyte morphology and arrangement in a blood smear monolayer. **(A)** Canine blood. **(B)** Feline blood; note lack of prominent central pallor compared with dog blood. Wright-Giemsa stain, 100× objective.

production as well as antioxidants to protect against oxidative damage during oxygen exchange. Alterations to these cellular components can lead to morphology changes. The general approach to evaluating a blood smear and normal erythrocyte morphology has been described elsewhere.¹

ERYTHROCYTE MORPHOLOGY CHANGES

Association

Rouleaux

Rouleaux is a linear arrangement of erythrocytes that looks like a stack of coins (**FIGURE 2**). It is a common finding for healthy cats and is thought to result from an

alteration of the electrical charge on the surface of the erythrocyte, resulting in a weaker electrostatic repulsive force and tendency to aggregate. Prominent rouleaux formation is considered abnormal for dogs and is often associated with hyperproteinemia, namely increased globulins, many of which are immunoglobulins produced by lymphoid cells (e.g., B lymphocytes, plasma cells).² Chronic inflammation causes increased production of high-molecular weight proteins such as fibrinogen (by the liver) and immunoglobulins (by lymphoid cells), which then coat erythrocytes and lower their charge potential, leading to rouleaux formation.² Excess immunoglobulin production may also be associated with certain lymphoproliferative diseases (e.g., multiple myeloma, certain lymphomas/leukemias).

Agglutination

Agglutination is an abnormal aggregation or clumping of erythrocytes into irregular clusters (**FIGURE 3**). It is



FIGURE 2. Normal rouleaux on feline blood smear. Note linearly stacked erythrocytes (**arrow**). Wright-Giemsa stain, 100× objective.

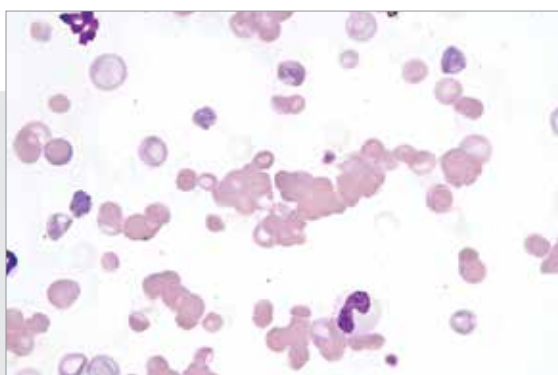


FIGURE 3. Erythrocyte agglutination on blood smear from a dog with immune-mediated hemolytic anemia. Note multiple variably sized aggregates of erythrocytes and several ghost cells. The moderate to marked anisocytosis and polychromasia indicates a regenerative response. Wright-Giemsa stain, 50× objective.

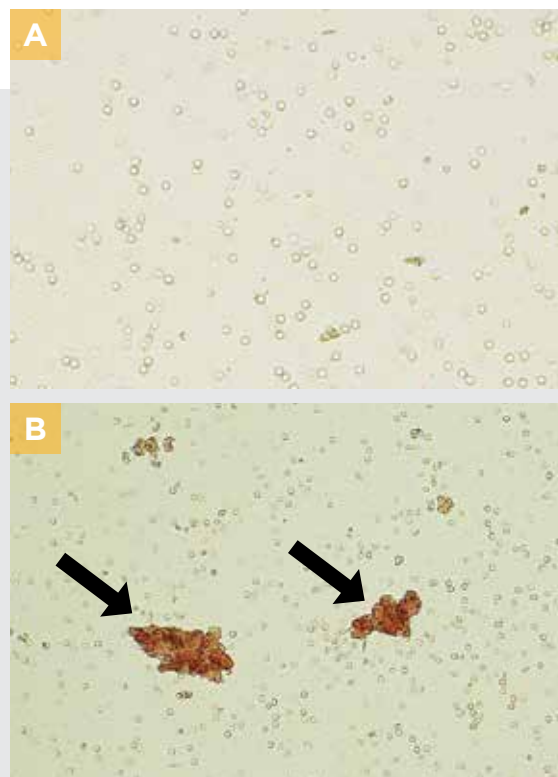


FIGURE 4. (A) Normal saline dilution of canine blood. Note even, individualized distribution of erythrocytes. (B) Abnormal saline dilution of blood from a dog with immune-mediated hemolytic anemia. Note persistent aggregates of erythrocytes (**arrows**) despite dilution with saline, indicating agglutination. Unstained wet mount, 10× objective.

Figure 4 (A and B): courtesy Kristina Meichner, DVM, DECVIM-CA, DACVP



caused by the binding of immunoglobulins to the surface of erythrocytes and is most commonly associated with immune-mediated hemolytic anemia (IMHA).³ Also, some nonpathologic causes of agglutination have been documented with feline blood and EDTA anticoagulant.³ For patients with auto-agglutination, a hematology analyzer may falsely alter red blood cell count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) because erythrocyte clumps may be counted as single large erythrocytes (thus, falsely asserting decreased red blood cell count, increased MCV, and increased or decreased MCHC).²

Rouleaux Versus Agglutination

Rouleaux and agglutination may appear similar and must be differentiated, usually with a saline dispersion/agglutination test, which involves dilution of a blood sample with isotonic saline. To minimize false-positive results, the amount of saline should exceed the amount

of blood (i.e., > 1:1 saline-to-blood ratio); current dilution recommendations can be found elsewhere.⁴ Saline dilution will disperse rouleaux erythrocyte arrangements but not agglutinated erythrocytes (due to their strong antigen–antibody binding) (**FIGURE 4**).²

Size

Anisocytosis

Anisocytosis refers to variation of erythrocyte size (**FIGURES 3, 5A, AND 5B**), such as larger (macrocytic, **FIGURE 5C**) or smaller (microcytic, **FIGURE 5D**) erythrocytes. Anisocytosis can be subjectively classified by blood smear review as mild, moderate, or marked and is generally absent or mild in healthy dogs and cats. A hematology analyzer can also assess erythrocyte size variation, measured as red blood cell distribution width (RDW). A low/narrow RDW is noted with healthy animals, and increased RDW correlates with wider variation of erythrocyte volumes.² Low numbers

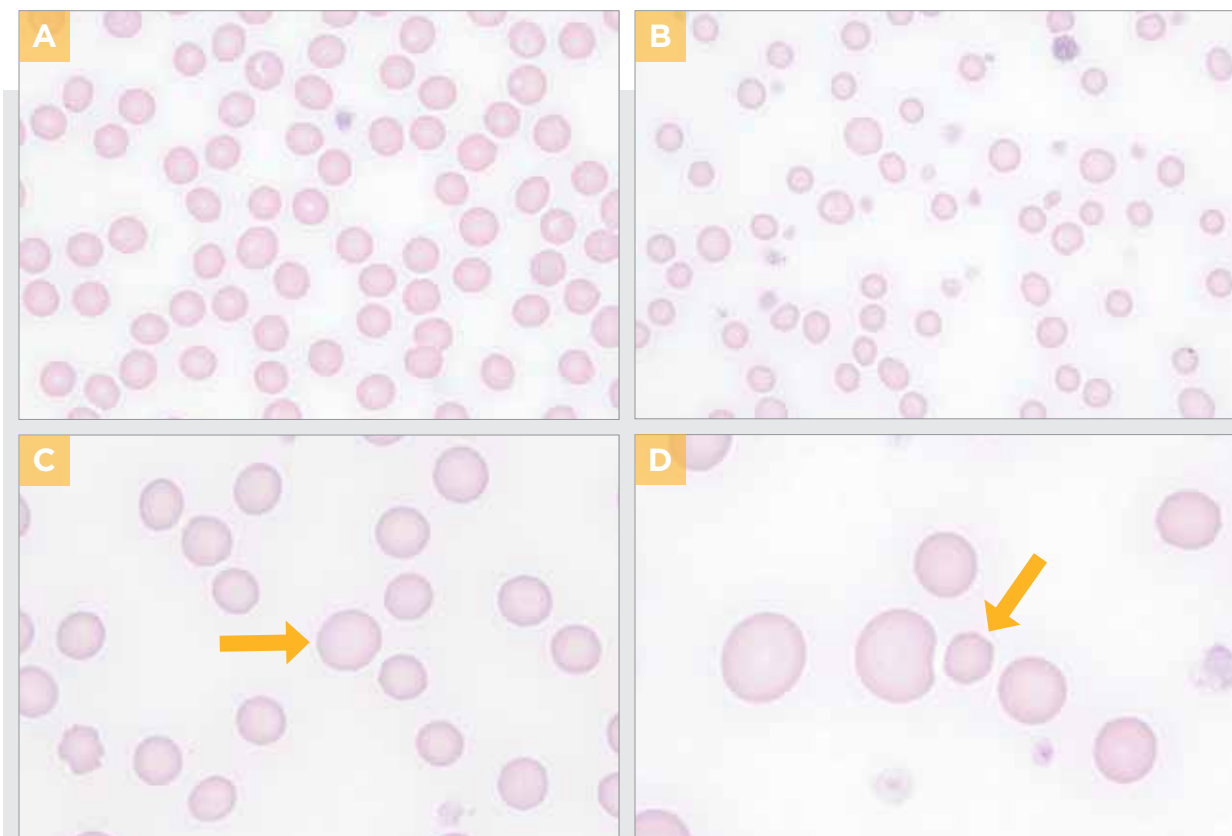


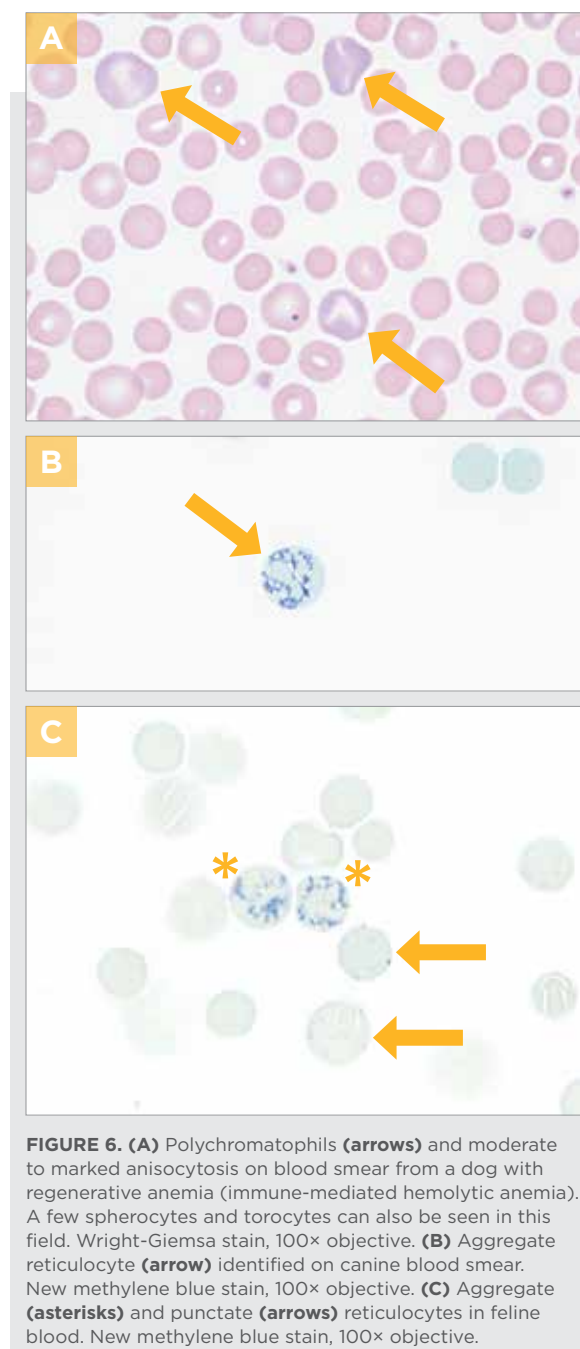
FIGURE 5. (A) Normal canine blood smear with minimal anisocytosis. A single platelet is also in this field. **(B)** Canine blood smear with marked anisocytosis. Several platelets (including 1 large/shift platelet) are also in this field. **(C)** Macrocytic erythrocyte (arrow) on blood smear from a dog with suspected congenital poodle macrocytosis. **(D)** Microcytic erythrocyte (arrow) on blood smear from a dog with a portosystemic shunt. Wright-Giemsa stain, 100× objective.

of smaller or larger erythrocytes may increase RDW before MCV is affected.²

Macrocytosis

Macrocytosis refers to the presence of larger erythrocytes (macrocytes) that can be identified by blood smear review or as increased MCV by hematology analyzer. Macrocytosis is most often associated with regeneration secondary to increased

numbers of immature erythrocytes (i.e., polychromatophils) in circulation that have a larger volume than mature erythrocytes (**FIGURE 6A**).² Macrocytosis may also be associated with poodle bone marrow dyscrasia (i.e., poodle macrocytosis [**FIGURE 5C**]), feline leukemia virus infection, congenital canine stomatocytosis, and myelodysplastic syndromes.^{2,5-7} Artifactual increases of MCV may be associated with agglutination, hyperosmolality (e.g., hyponatremia), and prolonged sample storage.²



Microcytosis

Microcytosis refers to the presence of smaller erythrocytes (microcytes) that can be identified by blood smear review or as decreased MCV by hematology analyzer. Microcytosis commonly results from iron deficiency, which impairs heme synthesis in erythrocytes and, in advanced stages, results in microcytic, hypochromic anemia.⁸ In adult animals, iron deficiency most often results from chronic hemorrhage (e.g., blood-sucking parasites, gastrointestinal ulceration, colonic ectasia) and impaired iron absorption or metabolism. Iron deficiency in young animals (less than 3 months of age) may result from decreased intake of their milk-based diets and low iron reserves.² Microcytosis in patients with congenital or acquired portosystemic shunts may result from liver dysfunction leading to altered iron metabolism (**FIGURE 5D**). Microcytosis without anemia may be exhibited by certain breeds of dog, including Akitas, Shiba Inus, and English springer spaniels.^{8,9} Artifactual decreases of MCV have also been associated with excess EDTA (inadequate amounts of blood in EDTA tubes) and hyposmolality (e.g., hyponatremia).

Color

Polychromasia

Polychromasia refers to the presence of purple/blue-tinged erythrocytes (e.g., polychromatophils) detected by blood smear with Romanowsky-type stains such as Wright-Giemsa and Diff-Quik (**FIGURES 3 AND 6A**). Polychromatophils are immature erythrocytes that stain purple/blue due to residual RNA within their hemoglobinized cytoplasm. Low numbers of circulating polychromatophils may be present in healthy dogs, but polychromasia is often absent in healthy cats. Increased polychromasia is associated with regeneration, which is best evaluated by

an absolute count of reticulocytes (measured by manual or automated methods). Staining with new methylene blue can be used to identify the 2 types of reticulocytes: aggregate reticulocytes that contain more clumped RNA (more immature, **FIGURE 6B AND 6C**) and punctate reticulocytes that contain few dispersed RNA inclusions (more mature, **FIGURE 6C**). Polychromatophils directly correlate with aggregate reticulocytes when stained with new methylene blue.²

Hypochromasia

Hypochromasia, or hypochromia, refers to erythrocytes with insufficient hemoglobin concentration, which can be identified by blood smear review as erythrocytes with increased central pallor or by automated analyzer as decreased MCHC (**FIGURE 7A**). Hypochromic erythrocytes must be distinguished from torocytes, which are artifacts with a similar morphologic appearance. Torocytes are most easily recognized by

Microcytosis commonly results from iron deficiency, which impairs heme synthesis in erythrocytes and, in advanced stages, results in microcytic, hypochromic anemia.⁹

their abrupt shift from central pallor to hemoglobinized cytoplasm (**FIGURE 7B**), whereas the transition is more gradual in hypochromic erythrocytes.² Hypochromasia is most often associated with iron deficiency but may also be seen with lead toxicity and vitamin B₆ deficiency.^{2,10} Polychromatophils also often look hypochromic on blood smear review due to their lower hemoglobin concentration.

Hyperchromasia

Hyperchromasia, or hyperchromia (i.e., increased MCHC), should always be interpreted as presence of artifacts due to erythrocytes' inability to store excess hemoglobin. Hyperchromasia can be associated with hemolysis (in vitro or in vivo), Heinz bodies, auto-agglutination, and lipemia resulting from analyzer interference.^{2,11}

Shape

Poikilocytosis describes the presence of erythrocytes of varied shapes, and its clinical significance depends on the morphologic abnormality, emphasizing the value of identifying the type of poikilocyte.² Erythrocyte shapes can be varied, and this article discusses the most common variations.

Echinocytes

Echinocytes are spiculated erythrocytes that have many blunt or sharp projections of uniform size and even distribution (**FIGURE 8**). Echinocytes can be further subclassified into type I, II, or III depending on their morphology.² Most often, echinocytes represent an artifactual finding (called crenation) that results from prolonged sample storage, excess EDTA in underfilled tubes, or blood smear drying artifact. Pathologic

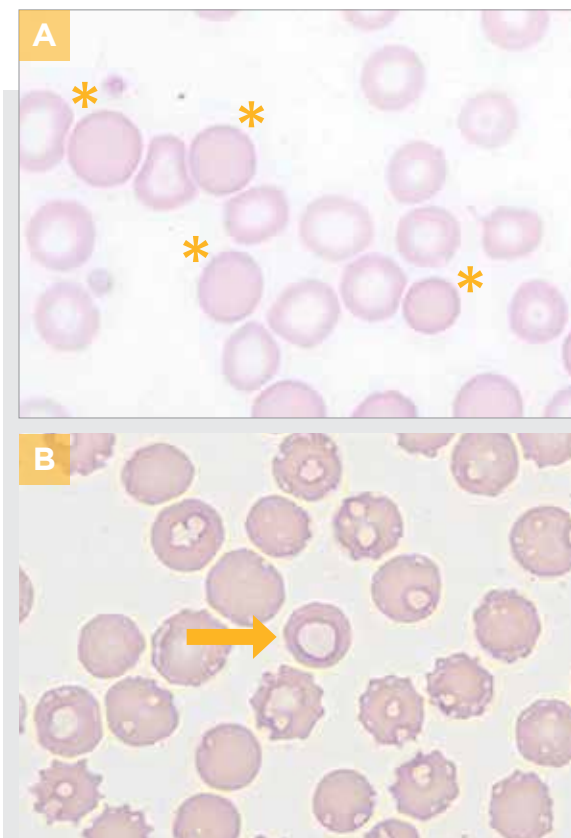


FIGURE 7. (A) Hypochromasia on blood smear from a dog with iron deficiency anemia. Normal erythrocytes indicated by asterisks. Wright-Giemsa stain, 100× objective. **(B)** Torocyte (arrow) on canine blood smear with prominent water artifact. Note abrupt transition from central pallor to hemoglobinized cytoplasm. Diff-Quik stain, 100× objective.

echinocytosis has been reported for patients with electrolyte depletion, glomerulonephritis, snake or bee sting envenomation, pyruvate kinase deficiency (dogs), doxorubicin administration, and certain neoplasms (e.g., lymphoma, hemangiosarcoma).¹²⁻¹⁵

Acanthocytes

Acanthocytes are also spiculated erythrocytes; however, they have irregular, often blunt or club-shaped projections that are variably sized and unevenly distributed (**FIGURE 9**). Acanthocyte formation can result from increased cholesterol content within the erythrocyte membrane, as noted in dogs and cats with liver disease.^{16,17} Acanthocytes may also be a result of erythrocyte fragmentation injury, stemming from conditions like disseminated intravascular coagulation, glomerulonephritis, gastrointestinal disease, and certain neoplasms (e.g., hemangiosarcoma, lymphoma,

osteosarcoma).^{2,16} Fragmentation injury is supported by the presence of keratocytes and schistocytes (see **KERATOCYTES** and **SCHISTOCYTES**).

Keratocytes

Keratocytes are erythrocytes with a blister-like vesicle that may rupture, leaving 1 to 2 horn-shaped projections (**FIGURE 10**). Low numbers of keratocytes may be clinically insignificant, but increased numbers may result from erythrocyte fragmentation injury (similar to acanthocytes and schistocytes [see **ACANTHOCYTES** and **SCHISTOCYTES**]) as well as oxidative injury (which can be supported by the presence of Heinz bodies, eccentrocytes, and pyknocytes). Keratocytes have also been associated with hepatic lipidosis in cats and iron deficiency anemia (resulting from increased erythrocyte fragility), cardiac disease, neoplasia (e.g., hemangiosarcoma), and doxorubicin administration in dogs and cats.^{15,17-19}



FIGURE 8. Echinocyte (**arrow**) on canine blood smear. Wright-Giemsa stain, 100× objective.



FIGURE 9. Acanthocytes (**arrows**) on canine blood smear. Wright-Giemsa stain, 100× objective.



FIGURE 10. (A) Prekeratocyte, or blister cell (**arrow**), on blood smear from a cat with hepatic lipidosis. (B) Keratocyte (**arrow**) on blood smear from a cat with hepatic lipidosis. Wright-Giemsa stain, 100× objective.



Schistocytes

Schistocytes are small, irregular fragments of erythrocytes that form secondary to direct shearing after contacting fibrin in circulation or turbulent blood flow (**FIGURE 11**). Schistocytes, like acanthocytes and keratocytes, indicate erythrocyte fragmentation injury and may be seen with various conditions as previously described. The detection of schistocytes bears clinical relevance because they may be misclassified as platelets (due to their smaller size) and an analyzer may report spurious thrombocytosis.²⁰

Eccentrocytes

Eccentrocytes, or hemighosts, have an eccentrically placed area of hemoglobin that is surrounded by an area of pallor with a faint cytoplasmic rim (**FIGURE 12**).

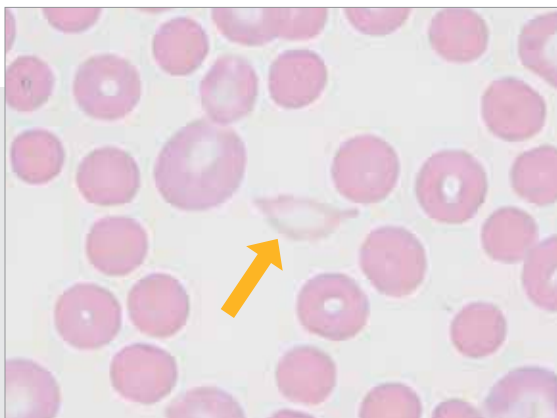


FIGURE 11. Schistocyte (**arrow**) on blood smear from a dog with immune-mediated hemolytic anemia. There are also a few spherocytes and moderate to marked anisocytosis and polychromasia. Wright-Giemsa stain, 100× objective.



FIGURE 12. Blood smear from a dog with prominent oxidative injury. Note many eccentrocytes and few pyknocytes (**asterisks**). Wright-Giemsa stain, 100× objective.

Pyknocytes are eccentrocytes that have lost the thin rim of clear cytoplasm, resulting in a small cell that lacks central pallor, resembling a spherocyte (**FIGURE 12**). Eccentrocytes are associated with oxidative damage that results in the crosslinking of hemoglobin and fusion of opposing cellular membranes. Among other causes, oxidative damage may be present in patients with onion or garlic toxicosis, neoplasia (e.g., lymphoma), diabetic ketoacidosis, and babesiosis (in dogs), or after administration of acetaminophen or propofol.^{2,21,22}

Spherocytes

Spherocytes are erythrocytes that have lost a portion of their cellular membrane, resulting in a rounded shape and loss of central pallor (**FIGURE 13**). They should be verified in the monolayer of a blood smear as smear preparation may cause erythrocytes near the feathered edge to look like spherocytes. Spherocytes from healthy cats are often difficult to identify because the amount of visible central pallor is variable. IMHA in dogs is a common cause of moderate to marked spherocytosis, resulting from antibody-mediated phagocytosis or complement fixation leading to partial membrane loss and change in shape. Given the immune-targeting of erythrocytes in patients with IMHA, ghost cells and schistocytes may also be seen (**FIGURES 3, 11, AND 14**). Low numbers of spherocytes can also be seen with erythrocyte fragmentation injury (see **ACANTHOCYTES**, **KERATOCYTES**, and **SCHISTOCYTES**); after blood transfusion; envenomation; and, less frequently, inherited erythrocyte deficiencies/defects (e.g., pyruvate kinase deficiency).²



FIGURE 13. Spherocyte (**arrow**) and polychromatophil (**asterisk**) on blood smear from a dog with immune-mediated hemolytic anemia. Wright-Giemsa stain, 100× objective.

Ghost cells

Ghost cells are ruptured erythrocytes that have lost their hemoglobin, which can result from hemolysis as an in vitro artifact (secondary to improper or prolonged sample storage) or by in vivo/intravascular hemolysis, the latter of which is supported by concurrent hemoglobinuria. Common pathologic causes include IMHA (e.g., primary/nonassociative, secondary/associative) and oxidative injury (**FIGURES 3 AND 14**). Similar to schistocytes, ghost cells may also result in spurious thrombocytosis (see **SCHISTOCYTES**).²³

Elliptocytes

Elliptocytes, or ovalocytes, are elongated erythrocytes that may be seen in low numbers as an artifact of smear preparation (**FIGURE 15**). Pathologic elliptocytosis may be seen with myelofibrosis, myelodysplastic syndromes, glomerulonephritis, and phenobarbital administration in dogs.^{2,6,24} Elliptocytosis has been documented for cats with liver disease (e.g., hepatic lipidosis, portosystemic shunts), myeloproliferative disorders, neoplasia, and after doxorubicin administration.^{2,15,17,25,26} Congenital causes of elliptocytosis have also been documented for dogs with protein 4.1 deficiency or β -spectrin abnormalities.^{27,28}

Codocytes

Codocytes, also called leptocytes or target cells, have a distinct bull's-eye appearance that results from an increased surface-to-volume ratio, creating a centrally located disk of hemoglobin within an area of central pallor (**FIGURE 16**). Codocytes may be associated with

cholestatic liver disease, iron deficiency, recent splenectomy, and regenerative anemia in cats and dogs as well as dogs with dyserythropoiesis.^{2,29}

SUMMARY

Despite technologic advances with automated hematology analyzers, blood smear review remains a critical diagnostic tool for assessing erythrocyte morphology. Blood smear review enables a clinician to confirm automated findings and detect erythrocyte morphology changes that may not be detected by an analyzer. Detection of such abnormalities can provide crucial information regarding mechanisms of disease and can be used when developing future diagnostic and therapeutic plans. **TVP**

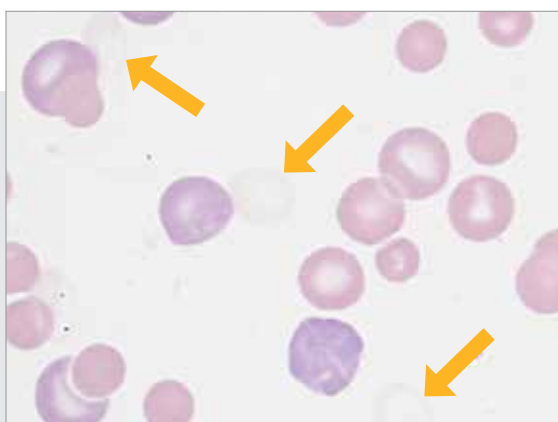


FIGURE 14. Ghost erythrocytes (**arrows**) on blood smear from a dog with immune-mediated hemolytic anemia. There are also a few spherocytes and marked anisocytosis and polychromasia. Wright-Giemsa stain, 100× objective.



FIGURE 15. Elliptocyte (**arrow**) on canine blood smear. Wright-Giemsa stain, 100× objective.



FIGURE 16. Codocyte or target cell (**arrow**) on canine blood smear. Wright-Giemsa stain, 100× objective.



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