

CYTOLOGY

Blood Smear Review: A Step-by-Step Guide to Platelets in Dogs and Cats

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Because multiple factors often play a role in hemostatic disorders, components of primary hemostasis (platelet plug formation) and secondary hemostasis (coagulation fortification of platelet plug into fibrin clot) must be evaluated. Hemostatic disorders can include defects in both phases of hemostasis, including insufficient or defective platelets (primary hemostasis) and/or clotting factors (secondary hemostasis). Primary hemostatic disorders are typically exhibited as surface bleeding; secondary hemostatic disorders may be exhibited as bleeding into body cavities.¹

Platelets are essential for the primary phase of hemostasis, during which a platelet plug forms, coagulation factor binding is promoted, and anticoagulant factors are inhibited at the site of vessel

injury.¹ Thus, platelet analysis is helpful for evaluating a patient with a bleeding disorder. Automated hematology analyzers provide a more accurate and precise assessment of platelet count than manual methods; however, various artifacts can reduce their accuracy.² For this reason, a blood smear review is essential for verifying an automated platelet count and detecting any artifacts, especially if thrombocytopenia is reported. If any questions or concerns arise, do not hesitate to reach out to or forward samples (e.g., premade unstained blood smears) to a local reference laboratory.

Platelets, or thrombocytes, originate from megakaryocytes in the bone marrow, yet most platelets are not in circulation and are stored in the spleen. In

Abstract

Platelet evaluation by manual blood smear review is essential for verifying an automated platelet count, which is particularly relevant when thrombocytopenia is reported. A key initial step for diagnosis and treatment of bleeding disorders is platelet evaluation. Hemostatic disorders are typically caused by insufficient or defective platelets (primary hemostasis) or insufficient or defective clotting factors (secondary hemostasis). Hemostatic disorders are often multifactorial, requiring assessment of numerous aspects of hemostasis, which involves sample collection technique and evaluation of platelet clumping, estimated platelet count, and platelet morphology.



Take-Home Points

- Platelet evaluation is an important initial step for the diagnosis of bleeding disorders.
- Thrombocytopenia is often caused by decreased platelet production, increased platelet consumption, and/or increased platelet destruction.
- For patients with a low platelet count indicated by an automated hematology analyzer, a blood smear review should be conducted to determine an estimated platelet count, platelet clumping, and/or other interferences.
- To estimate the number of platelets, count platelets in the monolayer of a blood smear for 10 high-power fields (100× objective), calculate the mean, and multiply the mean platelet count by a factor of 15 000 (dogs) or 20 000 (cats).
- After a platelet count has been performed, hemostatic defects resulting from platelet function and coagulation should be assessed.

most dogs and cats, the lifespan of a platelet is 4 to 6 days but can vary, especially with splenectomized patients. Because the spleen is responsible for removing aged platelets, platelet lifespan in splenectomized patients can be up to 8 days.³

SAMPLE COLLECTION

A platelet count should be performed within 5 hours of collection.² When a clot forms, coagulation factors are activated and platelets are consumed; thus, blood should be collected into a tube with anticoagulant to assess platelet mass, morphology, and function. The preferred anticoagulant for making blood smears and performing automated platelet counts in dogs and cats is EDTA. When the tube is properly filled, EDTA enables optimal sample staining and minimal sample dilution. However, EDTA has been reported to cause platelet clumping; platelet clumping may be reduced by prewarming EDTA samples to 37 °C (98.6 °F).^{4,5} Collecting samples in citrate may decrease platelet clumping in cats but not in dogs.⁶⁻⁸ Of note, samples are diluted by 10% when collected in citrate tubes; platelet counts must be corrected for this dilution.³ Adding amikacin to blood samples may also disaggregate clumps.⁹

Platelet clumping can be enhanced by refrigeration, which is recommended if CBC analysis is not performed immediately. For this reason, 2 unstained, air-dried blood smears should be made at the time of sample collection and submitted with the blood sample. The blood sample should come to room temperature and be mixed thoroughly by gentle inversion immediately before being placed in the hematology analyzer.^{5,6}

PLATELET EVALUATION

The most common cause of artifactually low platelet counts (pseudothrombocytopenia) is clumping. Clumping is also among the most common hematology laboratory artifacts, often attributed to improper sample collection (e.g., delayed transfer of blood to anticoagulant tube, inadequate sample mixing, traumatic sample collection).¹⁰ In vitro platelet clumping can occur during blood collection, especially with cats.³ If platelet clumps are present, an accurate platelet count cannot be determined. Typically, an analyzer will not categorize platelet clumps, but smaller clumps may be counted as white blood cells.¹¹

Automated platelet counts by well-maintained hematology analyzers are more accurate than manual platelet counts (in mammals). However, impedance counters can fail to differentiate feline platelets from erythrocytes and other cells, particularly when platelet clumping occurs.¹² Thus, impedance counters are often inferior to those that use flow cytometry. Regardless, performing a manual blood smear review is advised to complement any automated platelet count, especially when thrombocytopenia is reported below the limit of sensitivity (5000 to 20 000 platelets/μL).^{2,3} A previous article in this cytology series, “Blood Smear Review: A Step-by-Step Guide to Normal Findings for Cats and Dogs,” in the *Today's Veterinary Practice* January/February 2025 issue provides more in-depth information on how to conduct a blood smear review beyond platelet evaluation (go.navc.com/4fxiLVu).

Step 1: Look for Platelet Clumps

On low magnification (10× or 20× objective), scan the feathered edge and periphery of the smear for platelet

clumps (**FIGURE 1A**). Scan the rest of the smear as platelet clumping can also be found in the monolayer (**FIGURE 1B**). After degranulation, platelets can be difficult to discriminate from basophilic debris (**FIGURE 1C AND 1D**).

Step 2: Estimate the Number of Platelets

A platelet estimate is an essential initial diagnostic tool for evaluating a patient with suspected thrombocytopenia, yet variability can be high due to operator error and/or platelet distribution throughout the smear.¹³ In addition, if numerous and/or large platelet clumps are seen, the slide estimate may be artifactually low.

Platelets are estimated by counting the platelets in the monolayer of 10 high-power fields (100× objective), calculating the mean, and multiplying the mean by a factor of 15 000 (dogs) or 20 000 (cats), which equals the estimated number of platelets per microliter.^{13,14}

Step 3: Assess Platelet Morphology

Platelets are round, anucleate cell fragments with pale blue cytoplasm that contain numerous small, round to irregular, pink to purple granules (**FIGURE 2A**). When platelets are activated, they transform from round to oval due to the formation of filipodia, or cytoplasmic extensions, from their round bodies (**FIGURE 2B**). After platelet activation, the granules often aggregate centrally, which can be misconstrued for a nucleus.

Platelets should be distinguished from cytoplasmic fragments that are often seen in patients with lymphoid leukemias and circulating lymphomas (**FIGURE 2C**). Proplatelets may be infrequently noted as elongated platelets.¹⁵

Platelet size, which is represented by mean platelet volume (MPV) on hematology analyzers, can vary within and among species. In dogs, platelets are much smaller than their erythrocytes (approximately 7 μm), ranging from 2 to 4 μm . In cats, platelets vary in size from 2 μm to approximately the size of their

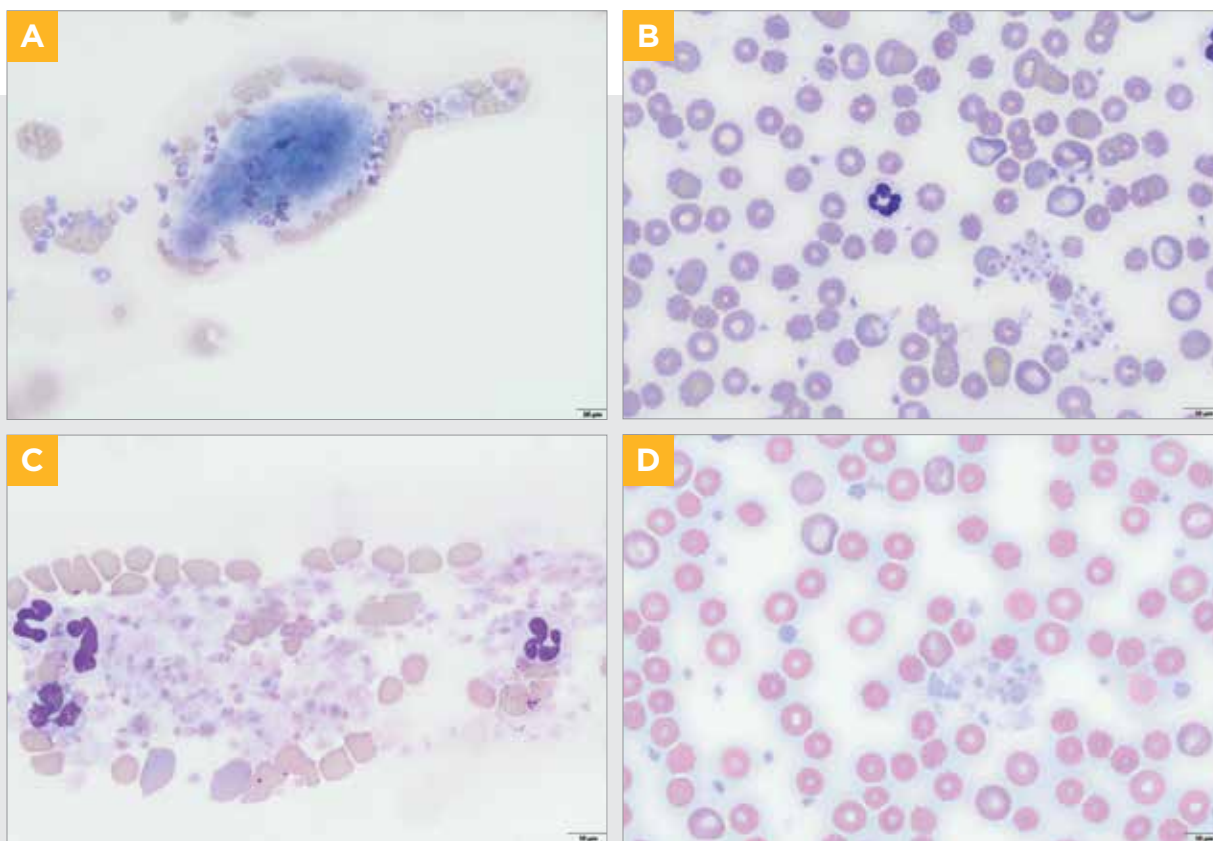


FIGURE 1. Blood smear from a dog. **(A)** A platelet clump on the feathered edge; 200× magnification, Wright-Giemsa stain. **(B)** Platelet clumping within the monolayer; degranulated platelet clumps on the **(C)** feathered edge and **(D)** in the monolayer. 500× magnification, Wright-Giemsa stain.



erythrocytes (approximately 6 μm).¹⁰ Increased MPV may indicate increased thrombopoiesis in cases of non-immune-mediated regenerative thrombocytopenia and inflammatory conditions. Increased MPV has also been noted in patients with myeloid neoplasia and thrombocytopenic feline leukemia virus-positive cats.³ Some dogs of certain breeds (e.g., cavalier King Charles spaniel, Norfolk terrier, Cairn terrier, Akita, beagle) have enlarged platelets resulting from a $\beta 1$ tubulin gene mutation (**FIGURE 2D**).¹⁶⁻¹⁸ These dogs may be thrombocytopenic (i.e., exhibit macrothrombocytopenia); however, no bleeding abnormalities are noted because they have normal platelet masses.¹⁹ Changes in platelet size should be interpreted with the patient's clinical condition in mind. Overall variations in platelet size are unlikely to be helpful for evaluating the cause of thrombocytopenia because many factors can contribute.^{3,20}

Platelet distribution width represents variation in platelet size. Platelet-large cell ratio is the proportion of large platelets. In thrombocytopenic patients, these

2 metrics, platelet distribution width and platelet-large cell ratio, may be more sensitive indicators of increased thrombopoiesis.²¹

HEMOSTASIS DISORDERS

Thrombocytopenia

Generally, thrombocytopenia arises from decreased production, increased consumption, or increased destruction of platelets. The cause can also be multifactorial, which is often the case with infectious agents. In some dog breeds (e.g., greyhounds, Ogar Polski), and those with macrothrombocytopenia as previously mentioned, a lower reference interval for platelet count has been reported.^{16-19,22,23} Assuming platelet function is normal, pathogenic thrombocytopenia (especially when platelet count falls below 30 000 platelets/ μL in dogs or 15 000 platelets/ μL in cats) may be expected if spontaneous hemorrhage is observed.

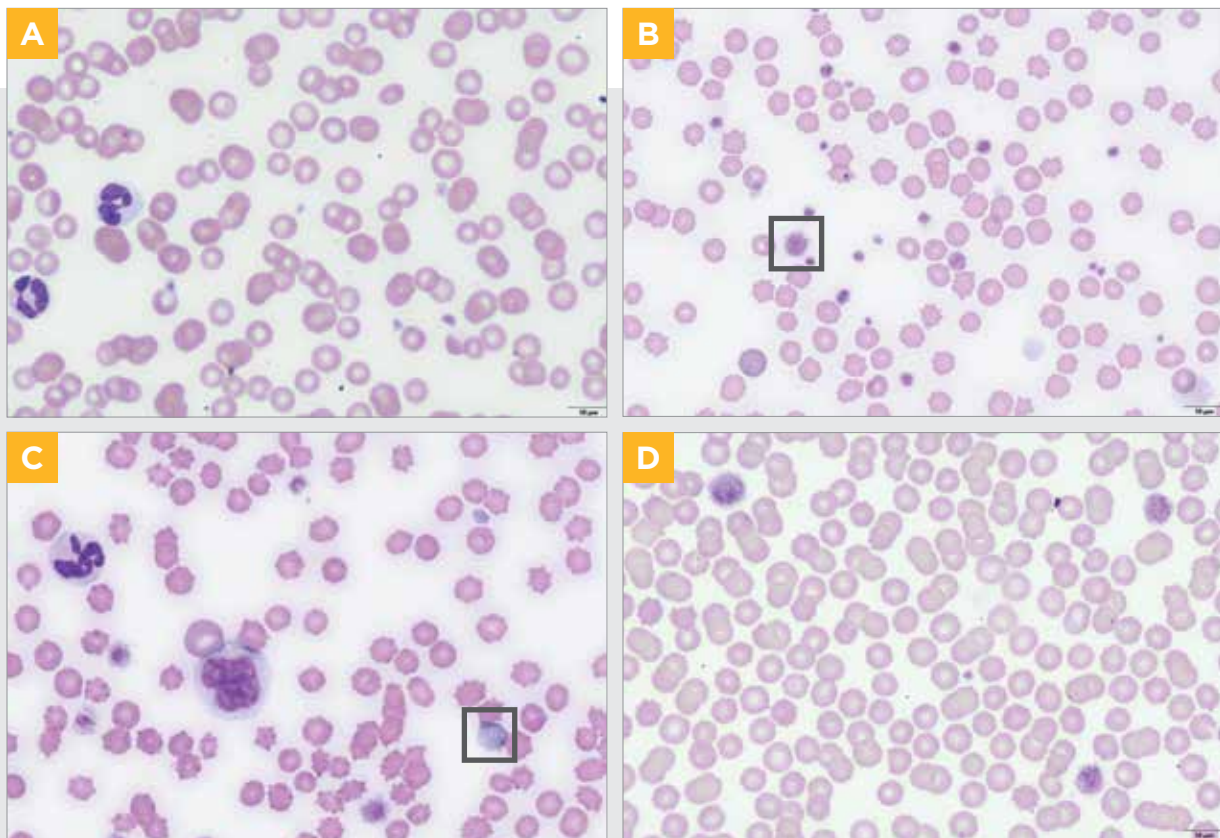


FIGURE 2. (A) Platelets in the monolayer of a canine blood smear. (B) Platelets in the monolayer of a feline blood smear including activated platelets (**box**). (C) A cytoplasmic fragment (**box**) in the blood smear of a cat with leukemia. (D) Macrothrombocytes in a blood smear of a Cavalier King Charles spaniel. 500 $\times$ magnification, Wright-Giemsa stain.

Thrombocytosis

Because the spleen is a storage pool for platelets and platelet removal, splenectomy can result in thrombocytosis persisting for several months. Reactive thrombocytosis can occur secondary to inflammatory and neoplastic conditions as well as acute anemia, iron deficiency anemia, or vincristine administration. Erythrocyte fragments, cytoplasmic fragments, lipemia, bacteria, and/or cryoglobulins can interfere with attaining an accurate count, resulting in pseudothrombocytosis. If these other causes for thrombocytosis have been excluded and the elevated platelet count (often > 1 million platelets/ μ L) persists, a neoplastic process (e.g., essential thrombocythemia, paraneoplastic syndrome) may be considered; however, these conditions are quite rare.³

SUMMARY

Platelets play a critical role in hemostasis. Automated hematology analyzers can estimate platelet mass, yet they are not always reliable. Platelets should also be counted via manual blood smear review, particularly for patients with bleeding disorders. A blood smear review is a simple, inexpensive test that enables evaluation for various interferences like platelet clumping, which frequently decrease the accuracy of automated measurements. **TVP**

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