In-Clinic Hematology:

THE BLOOD FILM REVIEW

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A complete blood count (CBC) is a critical component of the minimum laboratory database for evaluating veterinary patients. The proliferation of in-clinic analyzers facilitates rapid turnaround time that can improve patient care.

However, 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.

EVALUATING BLOOD FILMS

Prepare blood films immediately after an atraumatic sample is collected to avoid the potential for pre-analytical error and in vitro artifacts. Hand dipping using well-maintained Diff-Quik type stains is used with success for in-house staining, with a limitation of poor staining of some mast cell granules. New methylene blue should be on hand for highlighting reticulocytes and Heinz bodies. In reference laboratories, automated stainers are often utilized for a slightly more complex process that enhances some cytologic features, such as chromatin patterns, for more advanced diagnostic evaluation.

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

![FIGURE 1. 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 (A). 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 (B). 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 (C).]
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, and be sure to:

1. Perform a manual differential cell count to verify the analyzer results because this data can be misleading, especially when there are morphologic abnormalities.
2. Assess red cell morphology, including the presence of inclusion bodies or parasites.
3. Assess white cell morphology.
4. Note platelet density to verify analyzer data.
5. Note small platelet clumps and the presence of large platelets.
HIGH MAGNIFICATION EVALUATION BY LINEAGE

Red Blood Cells
Numerous red blood cell (RBC) shape changes are described in textbooks, but clinicians should focus on identification and interpretation of the most diagnostically specific features that are occurring in significant numbers. These features are keys to screening for some of the 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. Staining with new methylene blue can highlight smaller Heinz bodies, as well as identify reticulocytes using a manual method.

- **Schistocytes**, in large numbers, are 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, page 46).

**FIGURE 6.** RBC morphology: Note anisocytosis due to presence of spherocytes (arrows) and polychromatophils (arrowheads). Normal erythrocytes have eosinophilic cytoplasm and central pallor. Spherocytes appear smaller, darker, and lack the central pallor typically noted in RBCs. Spherocytes can be difficult to identify in feline patients because their RBCs generally lack central pallor. Additionally, in some instances, all RBCs present appear as spherocytes in dogs, which makes identification difficult since typical RBCs are not present for comparison. Polychromatophils appear more basophilic and are frequently larger than typical RBCs. Wright-Giemsa stain; magnification, 1000x.

**FIGURE 7.** Heinz bodies (arrows) in a cat secondary to metabolic stress. Echinocytes (arrowheads) are spiked, regularly spaced projections off the borders of RBCs. Echinocytes most frequently represent drying artifact, but are also seen with electrolyte derangements, renal disease, or secondary to snake envenomation. Wright-Giemsa stain; magnification, 1000x.
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** that 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 Blood Cells**

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** is a common and diagnostically critical morphologic abnormality indicative of inflammation that may be observed even with normal cell counts (Figure 14). 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 increased production.

**FIGURE 13.** Distemper inclusions in RBCs (arrows) and neutrophils (arrowheads) are displayed. This example shows distemper inclusions visible in both Wright-Giemsa (A) and Diff-Quik (B) stained samples. The inclusions stain more faintly on the Wright-Giemsa stain than the Diff-Quik stain. Be aware that distemper inclusions are more easily visible with Diff-Quik preparations, and can be extremely difficult to identify in Wright’s-Giemsa stained preparations due to poor staining characteristics. Magnification, 1000x.

**FIGURE 14.** WBC morphology: Toxic change revealed in 2 segmented neutrophils; toxic change appears as increased cytoplasmic basophilia, foaminess, and Döhle bodies (arrows). Wright-Giemsa stain; magnification, 1000x.

**FIGURE 15.** WBC morphology: Two band neutrophils that exhibit toxic change. Wright-Giemsa stain; magnification, 1000x.
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.

High power examination of the feathered edge is recommended to optimize identification of infectious agents. Although leukocyte morphology is often distorted, the concentration of cells usually allows visualization of rare agents. Infectious agents can be identified in patients with quantitatively normal CBC results.

**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

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**FIGURE 16.** Canine blood film with 2 reactive lymphocytes with increased size, cytoplasmic basophilia, and perinuclear clearing. Wright-Giemsa stain; magnification, 1000x.

**FIGURE 17.** Variably shaped *Anaplasma* morulae (arrows) within segmented neutrophils are present. The patient was antibody negative at time of organism identification. Note that morulae are lighter than neutrophil chromatin. Wright-Giemsa stain; magnification, 1000x.

**FIGURE 18.** Numerous large lymphocytes with occasional visible nucleoli (arrows) present in a canine patient with acute lymphoblastic lymphoma. Wright-Giemsa stain; magnification, 1000x.

**FIGURE 19.** Feline blood film with 2 mast cells and a basophil on the feathered edge; a distorted cell lacking discernable margins is also present. Note presence of dark granular debris that is artifact from slide preparation (arrow). Wright-Giemsa stain; magnification, 1000x.
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; in cats, the presence of mast cells typically indicates visceral mast cell disease, increasing the concern for neoplasia.

Platelets
Identification of platelet clumps, including visual estimation by examination of a blood film, is essential to verify the accuracy of automated platelet counts because technical problems can interfere with accurate platelet counting.

Large platelets are evidence of platelet turnover, which can reflect destructive or consumptive processes, such as immune-mediated thrombocytopenia, inflammation, and disseminated intravascular coagulation. Keep in mind that an expanding number of breeds have congenital macrothrombocytopenia initially characterized in Cavalier King Charles spaniels; these breeds include Norfolk and Cairn terriers, Chihuahuas, Labrador retrievers, poodles, English toy spaniels, shih tzu, Maltese, Jack Russell terriers, Havanese, boxers, cocker spaniels, bichons frises, and some mixed breeds.

• Due to the fact that the platelet mass in these breeds appears to be relatively normal, with platelet size compensating for the low number of platelets, clinical bleeding is not a characteristic of the syndrome.

• Presence of persistent moderate thrombocytopenia, appearance of large platelets, and lack of history of unusual bleeding likely indicates this syndrome.

• When a plateletcrit is available, it should be normal or just below the reference interval.

• Genetic testing can be performed at Auburn University to confirm this diagnosis in breeds in which this genetic mutation has been characterized.

COMMON PATTERNS TO RECOGNIZE
Regenerative Anemia
Regenerative anemia in the absence of clinical evidence of hemorrhage should prompt careful evaluation of red cell morphology for causes of hemolysis. There is a tendency to presume that hemolysis is the result of immune-mediated hemolytic anemia in dogs, which causes clinicians to overlook less common, but important and treatable, causes that are identified with blood film review, such as zinc toxicity and oxidative and Heinz body anemias (induced by, for example, onions, garlic, leeks, skunk musk, and RBC parasites). Inflammatory leukograms often accompany hemolytic anemia, and platelet numbers should be scrutinized to identify concurrent immune-mediated thrombocytopenia or the potential for disseminated intravascular coagulation.

Nonregenerative Anemia
While mild nonregenerative anemia is frequently a nonspecific response to chronic disease, more severe nonregenerative anemia can indicate bone marrow disease. In addition to evaluating the patient for other cytopenias, it is important to examine the blood film carefully for morphologically abnormal cells that may signal malignancy.

Other Morphologic Abnormalities
Patients with significant inflammation and circulating neoplastic cells may have normal total white cell counts, and automated analyzers perform poorly when identifying morphologic abnormalities. Be sure to avoid the pitfall of assuming normal
numbers equate with normal cells and look carefully for:
• Toxic change
• Left shift
• Reactive cells
• Presence of cells, such as lymphoblasts, mast cells, and histiocytes, that signify the need for more diagnostic investigation.

CONCLUSIONS & RECOMMENDATIONS
Blood film review is a critical component of in-clinic hematology. A cursory review may be sufficient for healthy animals, while more detailed analysis is required for sick patients. Key components every practice should have in place for in-clinic hematology include:
1. Written guidelines to determine when samples should be sent to a reference laboratory for evaluation
2. Staff training in blood film review, with attention to ongoing educational activities
3. Quality assurance checks, performed by routinely sending samples to a reference laboratory to compare their results with those generated in the practice. Address any significant discrepancies with additional training and re-evaluation of guidelines for reference laboratory verification of results.

Following the guidelines outlined in this article will ensure that in-clinic hematology is a safe and effective tool in your practice.

CBC = complete blood count; RBC = red blood cell; WBC = white blood cell

References