Blood smear basics: From preparation to evaluation

Mike Rosser, DVM, MS, DACVP (Clinical Pathology)
Clinical Assistant Professor, University of Illinois College of Veterinary Medicine

Blood smear preparation

Preparation and evaluation of a diagnostic quality blood smear is an important complementary test to the complete blood count (CBC). Methods for blood smear preparation vary, although the common goal is to generate a monolayer region in which individual WBC and RBC morphology can be evaluated. A capillary tube is recommended to generate an appropriately sized drop of blood, which should be placed ~1 cm from the frosted slide label. A spreader slide is applied to the sample slide at a 30-45° angle, pulled back into or through the drop of blood, and then pushed forward as blood spreads along the edges of the spreader slide. Care should be taken to maintain gentle, even pressure between the spreader and sample slides throughout the process. A well-made smear will have a curved shape and a refractile monolayer region when held against a light source.

Blood smear evaluation

Regions of the blood smear in ascending order of distance from the origin point are the body, monolayer, and feathered edge. A low power (10x objective) evaluation is performed first, which should include scanning of the feathered edge for platelet clumps, large/blast cells, and microfilariae. Subjective evaluation of RBC and WBC density can be determined by scanning the monolayer. Mid-power evaluation (40 or 50x objective) allows for completion of a 100-cell leukocyte differential count. Evaluation of WBC and RBC morphology, platelet estimation, and identification of most hemoparasites is best perfomed at high-power (100x oil objective). Platelet estimation is determined by counting the average number of platelets across 10 high-power fields and multiplying by 15-20,000. The presence of platelet clumping should be noted, as this may falsely decrease the platelet estimate.

Red blood cell morphology

Evaluation of red blood cell size, shape, and hemoglobin content may provide valuable diagnostic information. In anemic patients, blood smears are typically evaluated for evidence of regeneration. This may include anisocytosis (variability in red blood cell size), polychromasia (blue-purple staining of cells with lower hemoglobin content), and in some cases, nucleated red blood cells (metarubricytes). Larger, polychromatophilic erythrocytes correlate with reticulocytes, and provide evidence for accelerated red blood cell production in the bone marrow. Polychromatophils are not expected in the peripheral blood of horses, and basophilic stippling is a common additional feature of regeneration in anemic ruminants.

Echinocytes are a common form of RBC morphologic change, characterized by evenly sized and spaced spicules on the RBC surface. Echinocytosis is often an artifactual change secondary to prolonged smear drying, EDTA excess, etc., but may also occur with electrolyte derangements and other disease processes. Irregular projections on the RBC surface may support red blood cell fragmentation injury, especially if schistocytes are identified. Heinz bodies are associated with oxidative injury to red blood cells, and may be accompanied by eccentrocytes. Up to 5% of red blood cells in healthy cats may contain Heinz bodies, and increased numbers of Heinz bodies without anemia in cats typically supports metabolic disease. In other species, Heinz body anemia suggests toxic insult (i.e. onions/garlic, zinc, acetaminophen, copper in sheep and some goats, red maple leaves in horses). Spherocytes and agglutination are the classic findings in cases of immune-mediated hemolytic anemia (IMHA).

White blood cell morphology

Band neutrophils and cytoplasmic toxic change to neutrophils are associated with inflammatory disease. Band neutrophils have a “U”, “C”, or “S”-shaped nucleus with no segmentation. Suspected band neutrophils should be differentiated from monocytes, which will appear larger in size. Manifestations of toxic change include a blue-gray appearance to the cytoplasm (basophilia), foamy cytoplasmic vacuolation, and Döhle bodies which appear as small, round, pale blue inclusions. Abnormalities in lymphocyte morphology may be described as “reactive” (increased cytoplasmic basophilia) or “atypical” (increased cytoplasmic volume). Blast cells are differentiated from reactive or atypical lymphocytes based on their large cell size and presence of nucleoli.

Suggested reading

Harvey, J. 2011. Veterinary Hematology: A Diagnostic Guide and Color Atlas. Philadelpha: Elsevier-Saunders.