CYTOLOGY

Blood Smear Review: Lymphocyte Changes in Dogs and Cats

***Kristina Meichner**, DVM, DECVIM-CA (Oncology), DACVP (Clinical Pathology), and **Samantha Schlemmer**, DVM, MS, DACVP (Clinical Pathology)*
University of Georgia College of Veterinary Medicine, Athens, Georgia

A CBC is an integral part of a minimum database that identifies changes in cellular concentrations and morphologies, which can help diagnose disease and illness. Automated hematology analyzers are helpful for determining cellular concentrations. However, they cannot reliably assess morphologic changes or classify cells.[1] Therefore, blood smear review is recommended to evaluate cellular morphologies and confirm analyzer quantifications. This article focuses on lymphocyte abnormalities, primarily morphologic and concentration changes.

A blood smear review should be performed if lymphocytosis is reported. A few examples of additional analyzer flags that should prompt a blood smear review include monocytosis, suspected blast cells (especially if lymphocytes are more immature or large), and abnormal leukocyte distribution. As previously described in the January/February 2025 issue (go.navc.com/4fxiLVu), systematic review of a properly prepared and stained blood smear is recommended.[1] Because cellular distortions may occur in the feathered edge and body of the smear, lymphocytes should be identified in the monolayer. However, if large lymphocytes are present in low numbers, they may occur predominantly in other parts of the blood smear like the body. Thus, it is recommended to screen the entire smear.

LYMPHOCYTE POPULATIONS

Lymphocytes are the primary mononuclear cells in peripheral blood, and they are responsible for several immune functions. Lymphocytes are derived from bone marrow stem cells that mature into T or B cells.

Abstract

Automated hematology analyzers are routinely used to evaluate lymphocytes and detect changes in concentration (e.g., lymphopenia, lymphocytosis). However, hematology analyzers cannot reliably detect morphologic changes or classify cells (e.g., large lymphocytes). Therefore, manual blood smear review is critical for accurate lymphocyte assessment. This article describes peripheral lymphocyte physiology, variations in peripheral lymphocyte morphology, and conditions that commonly cause variations in lymphocyte concentration.

Take-Home Points

- Blood smear review is recommended to confirm and complement automated hematology analyzer findings.
- In dogs and cats, peripheral blood lymphocytes are mostly T cells but also include a mixture of B cells and null cells (natural killer cells), which cannot be distinguished reliably with light microscopy.
- In healthy animals, lymphocytes are primarily smaller in diameter than neutrophils.
- A mild increase in the number of medium and large lymphocytes can be seen in response to immune stimulation (i.e., reactive).
- Moderate to marked expansion of lymphocytes raises concern for underlying lymphoid neoplasia (e.g., lymphoma, leukemia).
- Lymphopenia is frequently attributed to stress but can also occur with cell loss, lysis, and redistribution or reduced cell production caused by immunodeficiency or high-dose steroid administration.

T cells develop in the thymus, then circulate or accumulate in lymphoid tissues, such as lymph nodes and the spleen. T cells are the primary lymphocytes found in the blood of healthy dogs and cats. They are responsible for cell-mediated immunity and include helper T cells ($CD4^+$) and cytotoxic T cells ($CD8^+$).

Depending on the species, B cells develop in the bone marrow or specialized lymphoid tissue (e.g., ileal Peyer's patches, bursa of Fabricius). B cells are responsible for humoral immunity via production of immunoglobulins, also known as antibodies. There is also a population of null cells that do not express T or B cell markers, which includes natural killer (NK) cells, which play essential roles in the innate immune system, particularly defending against viral infections. The different subsets of lymphocytes cannot be reliably distinguished by light microscopy, yet flow cytometry can identify different cellular markers and receptors (immunophenotyping).[2]

BOX 1 Summary of Peripheral Blood Lymphocyte Cytoplasmic Morphology Changes

Granules
- T or NK (natural killer) cell:
  - Normal (low numbers)
  - Reactive
  - Neoplastic
- Lysosomal storage disease (rare)

Vacuoles
- Prolonged storage (artifact)
- Reactivity
- Lysosomal storage disease (rare)
- Lymphoma (occasional)

Inclusions
- *Ehrlichia canis* (also in monocytes)
- Canine distemper virus (also in erythrocytes and other leukocytes)
- Chediak-Higashi syndrome (very rare; inclusions more commonly seen in neutrophils)

LYMPHOCYTE MORPHOLOGY VARIATIONS

When morphologic characteristics of lymphocytes are evaluated, cell size as well as nuclear and cytoplasmic qualities should be noted (BOX 1). Normal lymphocytes in healthy dogs and cats are small—a distinction made by comparison to a neutrophil. A normal lymphocyte is smaller than a neutrophil; an intermediate lymphocyte is approximately the same size as a neutrophil; a large lymphocyte has a bigger diameter than a neutrophil.

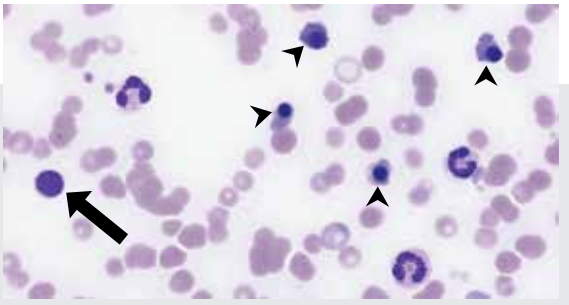

FIGURE 1. Compared to a small lymphocyte **(arrow)**, nucleated red blood cells **(arrowheads)** have more cytoplasm that resembles the color of other erythrocytes and, at later stages, an eccentrically located nucleus with very smooth, condensed chromatin. Dog blood. Modified Wright stain, 100× objective.

A small lymphocyte has a rounded to ovoid nucleus with clumped chromatin, no visible nucleoli, and scant to low amounts of basophilic cytoplasm (**FIGURE 1**). Small lymphocytes should be distinguished from nucleated red blood cells (nRBCs), which are also small cells with round nuclei. However, the cytoplasm of nRBCs is smooth, resembling that of other erythrocytes (orange/pink or polychromatophilic [blue/purple]). Late-stage nRBCs have very smooth nuclear chromatin (**FIGURE 1**). A small number of lymphocytes may contain a few fine, pink granules (origins of T cell or NK cell; **FIGURE 2**), which are best seen with methanolic Romanowsky stains rather than aqueous quick stains (e.g., DiffQuik).

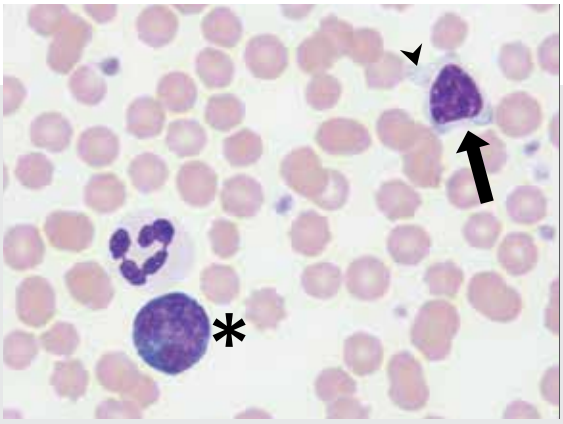

FIGURE 2. A granulated lymphocyte **(arrow)** with fine magenta cytoplasmic granules **(arrowhead)** and a reactive lymphocyte with deeply basophilic cytoplasm **(asterisk)**. Reactive lymphocytes are associated with antigenic stimulation. Dog blood. Modified Wright stain, 100× objective.

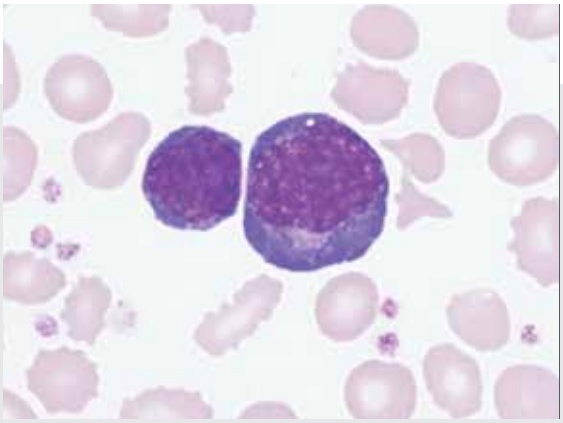

FIGURE 3. Two reactive lymphocytes of different sizes with deeply basophilic cytoplasm. Low numbers of these can be seen with immune stimulation. Dog blood. Wright-Giemsa stain, 100× objective.

Signs of reactive change include more abundant cytoplasm that stains deep blue (yet sometimes a pale blue appearance is retained) and a rounded or irregularly margined nucleus (**FIGURES 2 AND 3**). These morphologic changes represent nonspecific antigenic simulation, which can result from inflammation, infection, or other causes. Thus, mild increases in intermediate and rare large lymphocytes can be seen with immune stimulation. A moderate or marked increase in intermediate or large lymphocytes, particularly those with open chromatin and prominent nucleoli, raises concern for lymphoproliferative diseases (e.g., leukemia or lymphoma; **FIGURE 4**).

Rarely, infectious agents are identified in lymphocytes. Canine distemper virus inclusion bodies can occur in leukocytes and erythrocytes, which appear round to irregularly round with single or multiple inclusions. The inclusion bodies are glassy and eosinophilic in aqueous Romanowsky stains (e.g., rapid stains) but often transient in blood (**FIGURE 5**).[3] *Ehrlichia canis* morulae are rarely detected in blood. When morulae are noted in blood, it is typically during the acute stage of infection and predominantly in monocytes, less commonly in lymphocytes. Morulae appear as rounded, granular, basophilic cytoplasmic inclusions.[4] Infrequently, platelets overlay lymphocytes and erythrocytes; thus, fine-focus magnification is key to ensure the object (i.e., infectious inclusion) is associated with the lymphocyte. In addition, clinical and diagnostic findings (e.g., thrombocytopenia, nervous

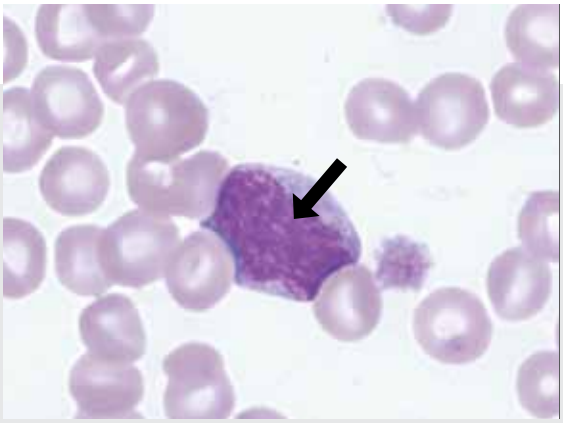

FIGURE 4. Large lymphocyte with moderately basophilic cytoplasm and a slightly irregular nucleus with fine chromatin and a visible nucleolus (**arrow**; slightly paler staining than the surrounding nuclear chromatin) from a dog with stage V lymphoma. Note the morphology is not specific to lymphoma and could also resemble acute leukemia. Dog blood. Wright-Giemsa stain, 100× objective.

system signs) should be considered before diagnosing infection.

Congenital and inherited diseases that lead to lymphocyte morphologic changes are rarely encountered. The term "lysosomal storage diseases" aptly refers to a group of diseases of lysosome dysfunction that result in metabolic disorders and commonly cause nervous system signs. Sometimes, lysosomes and lysosomal contents can be seen in neutrophils and lymphocytes by using light microscopy. In these instances, lysosomes and their contents can be distinct with clear vacuolar changes or basophilic, granular (rounded to rod-shaped) cytoplasmic inclusions. Lysosomal storage diseases that cause vacuolar changes in peripheral blood lymphocytes include mucopolysaccharidosis, GM1 and GM2 gangliosidosis, mannosidosis, fucosidosis, and Niemann-Pick disease. In addition, granular inclusions can be seen in the blood of patients with mucopolysaccharidosis and GM2 gangliosidosis (**FIGURE 6**).[5] Chediak-Higashi syndrome is another rare genetic disorder documented in cats and other species that causes immune dysregulation, depigmentation/albinism, and clotting disorders resulting from defective lysosomal trafficking. In blood from patients with Chediak-Higashi syndrome, common morphologic changes are large, pink to purple inclusions in neutrophils; however, lymphocytes may also contain similar-appearing inclusions.[5] These diseases, again, are rare, and clinical suspicions can prompt diagnosis via genetic or molecular testing.

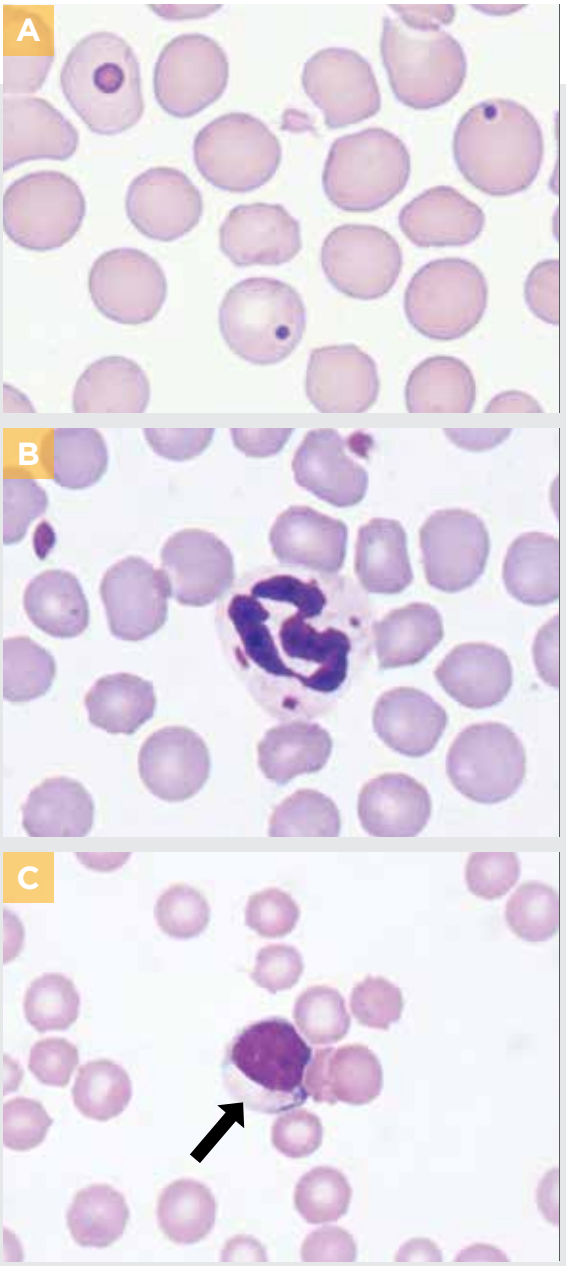

FIGURE 5. Blood smear from a dog with distemper virus infection. Note the purple cytoplasmic inclusions in red blood cells **(A)**, a neutrophil **(B)**, and a glassy pale eosinophilic inclusion in a lymphocyte **(C, arrow)**. Rapid aqueous Romanowsky stain, 100× objective.

LYMPHOCYTE CONCENTRATION CHANGES

The concentration of granulocytes and monocytes in peripheral blood typically reflects the balance between production in bone marrow and usage in tissues (traveling from blood to tissue is usually unidirectional without recirculation). In contrast, lymphocytes can

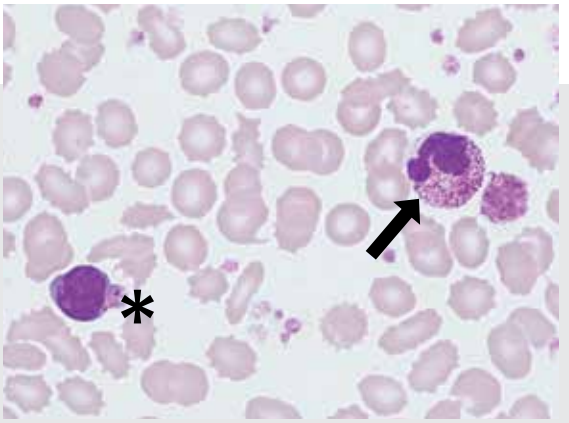

FIGURE 6. Blood smear from a dog with a mucopolysaccharidosis storage disease. Note the basophilic granular cytoplasmic inclusions in the neutrophil **(arrow)** and lymphocyte **(asterisk)**. Wright-Giemsa stain, 100× objective.

recirculate between blood and lymphoid tissue. See **BOX 2** for a summary of these concentration changes.

Lymphopenia

A decrease in absolute lymphocyte concentration (lymphopenia) is most commonly noted as part of a corticosteroid-induced stress leukogram in dogs and cats (i.e., with or without concurrent neutrophilia, monocytosis, and/or eosinopenia). During times of stress, decreased peripheral blood lymphocytes likely result from a combination of lysis and redistribution of lymphocytes from blood to lymphoid tissues.[6] Less common causes of lymphopenia include loss of lymphocyte-rich fluid (e.g., lymphangiectasia, chylothorax), depletion during viral infection, and lysis resulting from high-dose corticosteroids.[6]

In young animals, certain types of congenital immunodeficiencies can affect both B and T lymphocytes, resulting in lymphopenia, immunoglobulin deficiencies, and lymphoid tissue hypoplasia (i.e., severe combined immunodeficiency).[6] Affected animals are healthy at birth, yet fatal infections often quickly develop, especially after circulating maternal antibody concentrations become low.

Lymphocytosis

A transient increase of lymphocytes (as much as 2 to 3 times normal values) can result from an acute surge of epinephrine, such as during a time of fear, excitement, or exercise. This type of lymphocytosis is called epinephrine-induced, or physiologic, because it is suspected to result from splenic contraction and subsequent release of lymphocytes into the blood. Mature neutrophilia (increased segmented neutrophils) may also occur.[6] Physiologic lymphocytosis is commonly noted in younger, otherwise healthy cats, but also in older cats that are very stressed during blood collection. Physiologic lymphocytosis is less commonly seen in dogs in general but more frequently among very young dogs and excitable dog breeds (e.g., toy breeds). Typically, it is short lasting, until the epinephrine surge wanes (within minutes to a few hours). In contrast, other differentials for lymphocytosis usually involve a

BOX 2 Differentials for Changes in Peripheral Blood Lymphocyte Concentrations

Lymphocytosis
- Physiologic response*
  - Stress (epinephrine)
  - Young dogs and cats (<6 months)
- Antigenic stimulation/inflammation (e.g., *Ehrlichia canis*)*
- Hypoadrenocorticism
- Neoplasia or neoplasia-like
  - Lymphoma/leukemia*
  - Polyclonal B-cell lymphocytosis (English bulldogs)
  - Thymoma

Lymphopenia
- Stress (corticosteroid)*
- Immunodeficiency
  - Congenital
  - Acquired
- Lymphoid loss or lysis
  - Chylothorax
  - Lymphangiectasia
  - Viral infection
  - High-dose corticosteroids

**Relatively common*

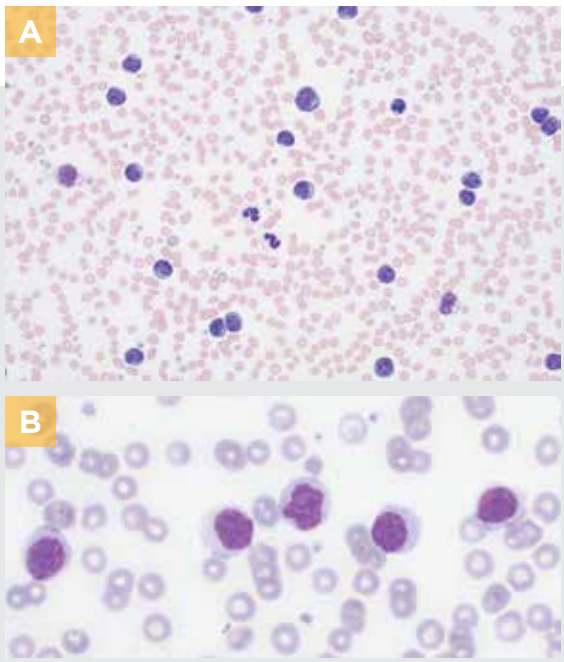

FIGURE 7. (A) A blood smear from a cat with CD4⁺ T cell chronic lymphocytic leukemia. The lymphocytes are predominantly small (compared to the size of a neutrophil) with unremarkable morphology and markedly increased in number. Modified Wright stain, 50× objective. **(B)** Intermediate lymphocytes from a dog with CD8⁺ T cell chronic lymphocytic leukemia. The lymphocytes have mildly expanded pale basophilic cytoplasm with very fine magenta granules and occasionally an irregularly shaped nucleus. Wright-Giemsa stain, 100× objective.

more sustained, chronic underlying condition (persistent lymphocytosis). Lymphocytosis for approximately 2 to 3 months is deemed persistent. Differentiating non-neoplastic lymphocytosis from neoplastic lymphocytosis can be challenging when small to intermediate-sized lymphocytes are only mildly to moderately increased in blood (fewer than 20 000 cells/µL).[2,7] Mild to moderate increases in lymphocytes can occur with chronic antigenic stimulation and is most commonly noted in dogs with chronic *E canis* infections, which can cause a reactive expansion of $CD8^+$ T lymphocytes. Chronic antigenic stimulation is a less common cause of lymphocytosis in cats but can occur with immune-mediated hemolytic anemia.[7] Other causes for a mild lymphocytosis include hypoadrenocorticism in dogs (often with concurrent eosinophilia); hyperthyroidism in cats; and following vaccine administration, especially in younger animals.[7]

If the morphology of most circulating lymphocytes is atypical, lymphoid leukemia should be considered. Lymphoid leukemia should also be considered if flow cytometric analysis demonstrates a phenotypically homogeneous expanded lymphocyte population (with or without aberrant antigen expression), markedly increased lymphocytes (more than 20 000 cells/µL, **FIGURE 7A**), or an increased lymphocyte subtype typically present in low numbers (e.g., B-cell chronic lymphocytic leukemia [CLL] in dogs).[7,8] Dogs should be screened for *E canis* infection before other diagnostics are performed. *E canis* infection can cause homogeneous expansion of $CD8^+$ T cells and can also rarely cause clonal expansion of T lymphocytes. CLL in dogs is predominantly of T cell origin, mostly $CD8^+$ T cells. For many patients, microscopy will show granular lymphocyte morphology (fine magenta cytoplasmic granules, **FIGURE 7B**).[7-9] Most cases of CLL in cats are of T cell origin as well, but with $CD4^+$ T helper lymphocytes as the predominant subset.[10] B-cell CLL is more common in small-breed dogs, and approximately half of affected dogs have enlarged lymph nodes and/or splenomegaly.[11] Most peripheral blood B-cell expansion in cats is polyclonal; therefore, B-cell lymphocytosis in a cat is less likely to be neoplastic in origin.[12] Polyclonal, non-neoplastic or preneoplastic B-cell expansion in English bulldogs has been reported.[13] Because of similarities in clinical presentation and flow cytometry findings, additional testing is required to differentiate between B-cell CLL and English bulldog B-cell lymphocytosis syndrome, specifically to determine clonality through PCR for antigen receptor rearrangement (PARR).[11,13,14] Onset of CLL is slow, and the protracted clinical course is usually stable over several months to even years.[8-10,14,15] In patients with circulating neoplastic cells from lymphoma (stage V) or acute lymphoblastic leukemia, an increased concentration of large lymphocytes, particularly with open, pale, delicate, or lacey chromatin and visible nucleoli, can be seen. Differentiating stage V lymphoma and subtypes of acute leukemia on the basis of cell morphology alone can be difficult to impossible. The distinction usually requires a thorough clinical and hematologic examination in addition to immunophenotypic evaluation by flow cytometry.[16]

SUMMARY

Changes in canine and feline peripheral blood lymphocyte morphology and concentration should be correlated with clinical findings (e.g., age of patient, signs of infection, recent drug or vaccine administration, abnormal/enlarged lymphoid tissues). Lymphopenia is commonly noted as part of a corticosteroid stress response. Lymphocytosis is commonly noted with nonspecific antigenic stimulation (reactivity) and lymphoproliferative diseases (e.g., leukemia, lymphoma). In patients with lymphocytosis, particularly those with persistently increased numbers of small to intermediate-sized lymphocytes, screening for infectious agents (e.g., *E canis,* feline leukemia virus, feline immunodeficiency virus) is recommended. To determine if lymphocyte proliferation is of neoplastic origin, immunophenotyping via flow cytometry and clonality testing through PARR is recommended. TVP

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Kristina Meichner

Dr. Meichner received her DVM degree from the Ludwig-Maximilian University in Munich, Germany. Following a small animal rotating internship, she completed a residency in medical oncology (ECVIM), followed by a residency in clinical pathology (ACVP) at North Carolina State University. Dr. Meichner is currently an associate professor and lab director at the University of Georgia College of Veterinary Medicine. Her research interests focus on immunology and canine cancer.

Samantha Schlemmer

Dr. Schlemmer is an assistant professor of clinical pathology at the University of Georgia. She received her DVM degree from the University of Florida. Following small animal rotating and oncology internships at the Animal Medical Center in New York City, she completed a clinical pathology residency and a master's in biomedical sciences degree at Texas A&M University. Subsequently, she completed the Seeker Oncology Postdoctoral Research Fellowship at Colorado State University's Flint Animal Cancer Center. She is passionate about educating and collaborating with trainees, clinicians, and the veterinary team. Her interests include diagnostic clinical pathology and oncology.