AHL LabNote Number 69 July 2025

What is Flow Cytometry? Why and How do I Submit Samples?

Dorothee Bienzle (DVM, PhD, Dipl. ACVP), Katherine Morrison (DVM, Dipl. ACVP), James Mori (DVM, DVSc), Emma Stacey (DVM, MSc); Department of Pathobiology, University of Guelph

Introduction

Flow cytometry (FC) is a laboratory technique used to analyze characteristics of individual cells or particles as they flow in a fluid stream through a beam of light generated by lasers.

Each cell passes single file through the laser light, and detectors measure:

- **Forward scatter (FSC)** – corresponds to cell size
- **Side scatter (SSC)** – reflects cell internal complexity or granularity
- **Fluorescence** – if cells are tagged with fluorescent antibodies, specific markers are detected

Typically, 20,000 to 50,000 cells are assessed, and by analyzing the combination of light scatter and fluorescence, cells can be classified as lymphocytes, granulocytes, or other cells, and the types of lymphocytes can be determined. Such information is useful for typing leukemia or lymphoma, and to indicate prognosis.

Lymphoma

There are many different **types of lymphoma**. Some are *high-grade*, meaning that they will lead to death without treatment in weeks to months, while others are *low-grade*, meaning that they will progress only slowly or not at all. The most common type of lymphoma in dogs is **diffuse large B-cell lymphoma** (DLBCL). There is some variation in the response to multi-agent chemotherapy among DLBCL, but most DLBCL are high-grade. Low-grade B cell lymphomas in dogs are rare and are of small cell size. **T-cell lymphomas** consist of high-grade (e.g. lymphoblastic lymphoma), intermediate-grade (e.g., CD8+/MHCII+), and low-grade (T-zone lymphoma) types. Analysis of cell size in combination with marker expression allows typing of >90% of canine lymphoma samples.[1,2]

Lymphoma affecting lymph nodes is less common in **cats** than dogs, but FC is nevertheless useful for characterizing nodal and some of the extranodal (i.e., renal, thymic, subcutaneous) lymphomas. **Intestinal lymphoma in cats is not suitable for cytology diagnosis or FC typing** since tissue architecture has to be evaluated (incisional or excisional biopsy). Splenic lymphoma in cats is often challenging to diagnose by cytology or type by FC since the spleen contains many different cell types (plasma cells, macrophages, rubricytes, immature granulocytes) in addition to possible lymphoma cells.[3]

Leukemia

Leukemias fall broadly into *lymphoid* and *myeloid* types, and within each type are *acute* (marked cytopenia, severely ill) and *chronic* (slowly increasing leukocyte count, not very ill) types. **Chronic lymphocytic leukemia (CLL)** is far more common than acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML), and chronic types of myeloid leukemia are rare. Dogs with CLL may have marked lymphocytosis (>300 x10^9/L) with little clinical illness. **In dogs, T-CLL is more common than B-CLL**. B-CLL is more likely than T-CLL to progress to cause cytopenia or organ infiltration, and to require treatment. **Lymphocytosis in mature or older dogs is most often due to CLL**, although in younger dogs, mild lymphocytosis due to physiologic responses (epinephrine) and systemic inflammation also occurs.

In young **cats**, physiologic lymphocytosis is most common; in middle-aged and older cats, inflammation (enteritis, nephritis, etc.) and CLL are more common as causes of lymphocytosis. Almost all **CLL in cats are slowly or non-progressive CD4+ T-cell type**.

Lymphocytic effusion

FC is useful to differentiate inflammatory from neoplastic lymphocytes in pleural, peritoneal, and other effusions, and in CSF.

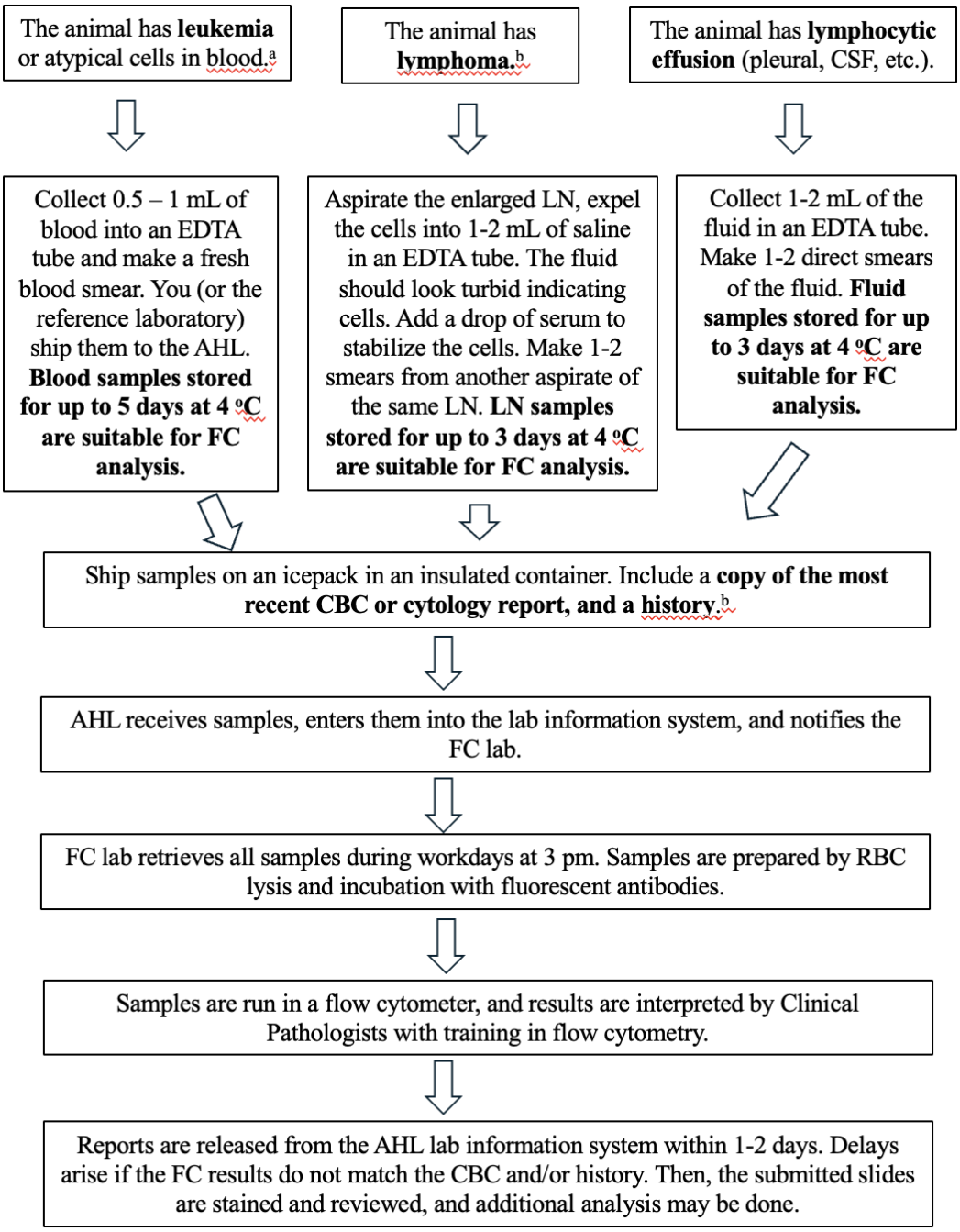
Instrumentation and reagents

We have a state-of-the-art spectral flow cytometer that allows concurrent assessment of multiple fluorescent markers. Each species-specific marker panel is titrated and calibrated, and controls are run each day. Our current panels consist of 16 fluorescent markers for dogs, 9 for cats, and 8 for horses. We are engaged in testing and validating new antibodies as they become available, in standardization, and in multi-institutional collaborations involving flow cytometry.[4]

References

1. Rao S, et al. Class II major histocompatibility complex expression and cell size independently predict survival in canine B-cell lymphoma. J Vet Intern Med 2011;25:1097-1105.
2. Deravi N, et al. Specific immunotypes of canine T cell lymphoma are associated with different outcomes. Vet Immunol Immunopathol 2017;191:5-13.
3. Bienzle D, et al. Tumors of the hematolymphoid system. In: Meuten DJ, Munday JS, eds. Tumors of Domestic Animals, 6th ed, Wiley; *In press*, 2026
4. Meichner K, et al. Multicenter flow cytometry proficiency testing of canine blood and lymph node samples. Vet Clin Pathol 2020;49:249-257.

5. Flow cytometry workflow



[a] If an animal has **lymphoma and lymphocytosis, the blood sample is preferred for FC.**

[b] If multiple LNs are enlarged, pool aspirates from several LNs into 1 tube and submit **only that 1 tube**. We have limited patient information, and if you submit several tubes, we don't know which one to run.

[c] A **meaningful history** includes the breed, sex, and age of the animal, a brief summary of the duration of illness, major laboratory and imaging abnormalities, and information on tumor burden (e.g., how many LNs are enlarged, how large are the LNs).