What is flow cytometry?

Flow cytometry is a test that determines the immunophenotype of cells by recognizing normal and abnormal expression of cell surface markers on T cells (helper T cells, cytotoxic T cells), B cells, neutrophils, and monocytes. We offer clinical flow cytometry for cats and dogs.

Clinical flow cytometry is most often used:

1. To help distinguish between reactive and neoplastic lymphocytes
2. To determine if lymphoma/leukemia is of B or T cell origin and in some cases, identify specific subtypes of B or T cell lymphoma/leukemia which will aid in prognosis
3. To differentiate between lymphoid and non-lymphoid (myeloid/monocytic) neoplasia

Clinical flow cytometry is used most often in combination with clinical history/presentation and cytology/blood smear examination for diagnosis of disease. In some cases, additional diagnostics (PARR-PCR for antigen receptor rearrangement, histopathology, immunohistochemistry, immunocytochemistry) are also required.

When might clinical flow cytometry be most helpful?

1. Lymphadenopathy, organomegaly, or mediastinal mass with the following cytology results:
   a. Confirmed or probable lymphoma composed of intermediate to large lymphocytes
   b. Homogeneous expansion of small to intermediate lymphocytes
   c. Suspected lymphoma or thymoma (for mediastinal mass)

   Sample to submit: Fine needle aspirate of lymph node/enlarged organ/mass (see sample submission guidelines)

   Note: Flow cytometry is not indicated on these samples if cytology results report rare to low numbers of atypical/suspicious cells

2. Abnormal CBC findings consisting of:
   a. Peripheral lymphocytosis with increased numbers of small mature, intermediate, or immature lymphocytes
   b. Presence of any immature cells/‘blasts’ with a normal or elevated white blood cell count

   Sample to submit: Peripheral blood (EDTA tube)

   NOTE: If the lymphocytosis is composed of small to intermediate lymphocytes test for Ehrlichia infection in dogs with lymphocyte counts up to ~50k and ‘stress’ in cats with lymphocyte counts up to ~30k prior to running flow cytometry

3. Abnormal bone marrow findings with the following cytology/CBC results:
   a. Increased numbers of blasts or small/intermediate lymphocytes with leukopenia on CBC

   Sample to submit: Bone marrow (EDTA tube) for samples with leukopenia on CBC. Peripheral blood (EDTA tube) is preferred if lymphocytosis is present.

4. Body cavity effusion with the following cytology results:
   a. High numbers of small/intermediate/OR large lymphocytes consistent with or concerning for lymphoma
Sample to submit: Cavity fluid (EDTA tube and red top tube when possible- 0.5ml in each tube, see sample submission guidelines)

Uncertain if flow cytometry will be helpful for your patient? Email vetclinflow@uga.edu or contact (706)542-9430 alt phone: (706)542-5161

Sample shipping guidelines

- Collect samples and ship on the same day overnight for Monday through Friday delivery
- Keep samples refrigerated (DO NOT FREEZE) until shipped
- Ship overnight with an ice pack
- Samples may be submitted via:
  - The Athens Veterinary Diagnostic Laboratory
    - Courier services may be available in your area
    - Contact AVDL (706) 542-5568 or visit http://www.vet.uga.edu/dlab/ for information
  - Or ship directly
    - $10 UPS overnight labels available by email, contact vetclinflow@uga.edu or 706-542-9430, alt- 706-542-5161

Sample collection and submission guidelines

Lymph node, organ or mediastinal mass aspirates

- Put 1 ml of 0.9% saline in a red top tube (no additives, no serum separator)
- Add 0.1ml of serum from the same patient or another patient of the same species
  - Note: the volume of serum should be ~10% of the total volume (this helps preserve cells)
- Using a 22ga needle and 6 or 12cc syringe, aspirate with suction
- Eject the contents into the saline, gently aspirate the saline into the syringe and eject back into the tube to recover all cells
- Aspirate several times (2-3X if possible) repeating the above process until the saline is slightly cloudy

Blood and bone marrow

- Provide a minimum of 0.5ml in an EDTA tube
- A current CBC (within 2days) of the flow sample collection is required
  - Include a copy of your patient CBC
  - OR
  - Check the CBC test request box and include: 1) a minimum of 2.0 ml of blood in an EDTA tube (use the appropriate fill volume for your tube) for the CBC and flow cytometry and 2) one unstained blood smear
    - See pricing schedule for CBC
Body cavity fluid

- Provide a minimum of 0.5ml in an EDTA tube
- If enough fluid is available please provide an additional red top tube (no additives, no serum separator) with a minimum of 0.5ml
- If the total protein is < 5mg/dl please add two drops of serum from the same patient or another patient of the same species (if protein measurement is unavailable, add 2 drops of serum regardless)

**Reporting and pricing schedule**

- Samples will processed on the day they are received or the next day to determine the cell count and cell viability and perform flow cytometry
- *Note: If a sample has too few cells or the cell viability is too low, flow cytometry results will not be accurate and flow cytometry will not be performed.*
  - A fee of $15 will be charged for the initial sample assessment in these cases
- **Pricing Schedule**
  - Canine & Feline Flow Cytometry $115.00
  - Cytology Review $35.00
  - Body Cavity Fluid $40.00
  - CBC $20.00
  - Blood Smear Review $15.00
- **Reporting**
  - Reports will be generated within approximately 3 business days of sample processing

**Questions?**

Email: vetclinflow@uga.edu
Phone: (706)542-9430
Clinical Immunodiagnostic Laboratory: (706)542-5161