Clinical Flow Cytometry FAQs

Department of Pathology
College of Veterinary Medicine
UNIVERSITY OF GEORGIA

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.

Clinical flow cytometry is most often used for cats and dogs:

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 ~20k (rarely up to 30k) and ruleout 'stress' in cats with lymphocyte counts up to ~20k (rarely 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

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)

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 0.1-0.15ml of serum from the same patient or another patient of the same species
    - *Note: the volume of serum should be ~10-15% of the total volume (this is critical to 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) 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 3 days of collection of the flow cytometry sample 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

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)

(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

High cellularity, adequate serum (minimum 10%), cold sample temperature (refrigerated and shipped with ice packs) and prompt overnight shipping or courier transport (same day of collection) are critical for an adequate sample.

- Samples can be transported via AVDL courier or with overnight AVDL UPS labels for morning delivery

- *Contact the Athens Veterinary Diagnostic Laboratory (706)542-5568 to inquire:*
  - If courier services are available in your area
  - Or to obtain UPS overnight labels by email from AVDL

Reporting and pricing schedule

- **Pricing: $125/sample**

- **Reporting**
  - **Reports will be generated within approximately 3 business days of sample processing**

- Samples are processed on the day they are received or the next day to determine the cell count and cell viability and perform flow cytometry
- For pricing of additional tests (e.g. CBC, cytology) please see the Athens Veterinary Diagnostic Laboratory Website: *https://vet.uga.edu/diagnostic-service-labs/veterinary-diagnostic-laboratory/*

Questions?

Contact Kristina Meichner DVM, DECVIM-CA (oncology), DACVP (clinical)

Email: meichner@uga.edu Phone: (706)542-9430 Alternate phone: (706)542-5161