

**VETERINARY DIAGNOSTIC SERVICES  
CLINICAL PATHOLOGY  
Updated: January 8, 2016**

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The Clinical Pathology Section of Veterinary Diagnostic Services provides testing in the fields of hematology, urinalysis, cytology, chemistry and endocrinology.

## **HEMATOLOGY**

### **General information**

- Whole blood in EDTA (purple top tube) is the required specimen for mammalian hematology.
- For birds and reptiles, the preferred hematology specimen is whole blood in a lithium heparin (green top) tube.
- EDTA and lithium heparin tubes should be adequately filled in order to provide a proper blood-to-anticoagulant ratio. Overfilling or underfilling tubes can cause inaccurate results. For example, underfilling can result in:
  - An erroneously low hematocrit and RBC count.
  - Inaccurate mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).
  - Altered RBC and WBC morphology.
- Gently mix blood well after collection and refrigerate. Do NOT freeze.
- If the blood sample has clotted, a second blood sample must be collected because specimens that have clotted during blood draw can result in inaccurate cell counts (WBC, RBC and platelets) on the hematology.
- Whole blood EDTA samples degrade rapidly after collection, thus air dried blood smears should be made IMMEDIATELY in order to preserve cellular morphology.
- Do NOT refrigerate smears.
- Ensure that smears are packaged in slide shipping containers.
- If submitting with formalin-containing samples or moisture from cold packs, protect slide shippers by placing them in a sealed bag.

### Preparation of blood smears:

- Properly made blood smears are essential for reliable identification and evaluation of peripheral blood cells.
- Ensure the slides being used for blood smears are clean and free from dirt and grease. Bevelled edge smears with frosted ends (easy labeling) are preferred.
- Write pertinent client information on the smear.
- Gently mix anticoagulated whole blood well.
- Using a half-filled hematocrit tube (without heparin) with well-mixed anticoagulated blood, place a 2-3 mm drop of blood approximately 1 cm from the frosted edge of the smear.
- If right handed, use the thumb and forefinger of the right hand to hold the end of the spreader slide against the surface of the smear with the blood droplet at an angle of 30-45°.
- Draw the spreader slide back to contact the blood and pause to allow the blood to spread and fill the angle between the two slides.
- Push the “spreader” slide toward the end of the slide in one quick, smooth motion.
- Allow smears to air dry on a flat surface and place in slide folders at room temperature until shipping.

### Characteristics of a good quality blood smear:

- The blood film covers about half the length of the slide.
- The blood film should have a smooth, even appearance and be free from ridges, waves or holes.
- Typically, the blood film has the shape of a thumbnail or bullet.
- The end of the smear is smooth and even (the “feathered edge”).

### Causes of poor quality blood smears:

- Hesitation in making the smear
  - There should be no delay in making a smear as it can result in abnormal distribution of the leukocytes (typically accumulating at the feathered edge).

- Too large or too small of a blood drop
- The spreader slide was pushed across the horizontal slide in an abrupt or very slow manner.
  - This can lead to either rupture or uneven distribution of the leukocytes in the blood film.
- Failure to use the appropriate angle
  - Adjusting the angle of the second slide can help compensate for very viscous (hemoconcentrated) or watery (anemic) blood samples.
    - If the patient is anemic, increase the angle of the “spreader” slide.
    - If the patient is hemoconcentrated, decrease the angle of the “spreader” slide.
- Exposure to formalin, insects, moisture, etc.

### **Complete Blood Count (CBC):**

- Includes:
  - Total WBC count, RBC count, Hgb, Hct, MCV, MCH, MCHC, reticulocyte count (if anemic), automated platelet count, manual leukocyte differential and smear evaluation of RBC and WBC morphology and parasite screen. (Freshly made blood smears in-clinic ideal for assessment)
    - Please note: Ensure that blood smears made in-clinic are concurrently submitted with the EDTA whole blood sample, particularly if there is to be any delay in shipping to the lab
  - Fibrinogen is also included (large animals only)
- If the submitted blood sample is clotted or severely hemolyzed (likely attributed to freezing) and if a concurrent blood smear was submitted, we will provide a WBC and platelet estimated and bill accordingly

### **Canine and Feline Coombs':**

- Direct antigen test (DAT)
- Use to determine the presence of anti-erythrocytic antibodies; aids in the diagnosis of immune mediated hemolytic anemia (IMHA)
- Requires a minimum of 2 mL of EDTA whole blood.

### **Feline IDEXX SNAP® Triple Test®:**

- Detection of feline immunodeficiency virus (FIV) antibody and feline leukemia virus (FeLV) and feline heartworm (FHw) antigen
- Requires a minimum 1 mL of EDTA whole blood, plasma or serum (plain red top tube).
- For additional information, please visit the IDEXX website:  
<https://www.idexx.com/smallanimal/inhouse/snap/feline-triple.html>

### **Canine IDEXX SNAP® 4Dx Plus Test:**

- Detection of antibodies to *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Ehrlichia ewingii*, *Borrelia burgdoferi*, and antigen to *Dirofilaria immitis*
- Requires a minimum of 1 mL of EDTA whole blood, plasma or serum (plain red top tube).
- For additional information, please visit the IDEXX website:  
<https://www.idexx.com/smallanimal/inhouse/snap/4dx.html>

## **URINALYSIS**

- A minimum of 3 mL of fresh urine is required for urinalysis and/or urine protein:creatinine ratio determination.
- Urine should be collected in sterile medical containers and tightly sealed.
  - Refrain from collecting and submitting urine in the following containers:
    - glass or plastic; non-sterile containers, e.g., food jars.
    - syringes with or without the needles attached
    - red top plastic blood collection tubes or any containers with clot activator
      - The crystalline clot activator in these plastic tubes interferes with the microscopic sediment examination.
- Clearly indicate method of collection.
- Refrigerate sample for storage and shipping (ice-pack) and prevent sunlight/UV exposure.

## **OTHER**

### **Fecal Occult Blood:**

- Used in cases of suspected GI ulceration, neoplasia, when microcytic hypochromic anemia is detected as well as anemia with low total protein and no obvious signs of blood loss.
- A special diet needs to be fed to the patient for 3 days prior to the test in order to avoid false positive results. Please contact the laboratory for additional information and testing protocol.

# CYTOLOGY

- Excellent detailed, yet concise history and clinical findings contribute to accurate cytologic interpretation. The following should be included in a cytologic submission:
  - Patient signalment, history and clinical signs
    - Number of masses along with a gross description of each site/mass with specific anatomic location, size, consistency, shape, definition of borders, etc. Radiographic and/or ultrasound findings are always welcome.
    - Any previous results and/or case submission numbers
    - Differential diagnoses
    - Questions you would like answered.
- Includes samples collected by fine needle aspirate or non-aspirate techniques, impression smears, and/or scrapings.
- Prepare slides using the blood smear technique\*\* see guidelines for more information
- Label all smears with unique identifiers (animal and owner names) and site aspirated.
- Submit fresh, air-dried, unstained smears or stained in-clinic.
- Do NOT heat fix slides.
- Keep all slides at room temperature, and protected from moisture, insects, formalin fumes and extreme temperatures.
- Make and submit multiple slides (up to 6 slides per site).
  - This helps to increase diagnostic yield, particularly as some lesions do not exfoliate cells well.

## Smear preparation guidelines:

- Avoid blood dilution
  - This can be attributed to prolonged aspiration as well as using too large of a needle.
- Avoid spraying out the aspirated material onto a slide without spreading it.
  - The small drops of cells sprayed onto the smear yield small clusters of poorly spread-out cells. It is usually impossible to adequately visualize the morphology of these cells
- Avoid making excessively thick smears.
  - Smears that are too thick will not have cells adequately spread out, making the cells virtually impossible to evaluate.

### **Cerebral Spinal Fluid (CSF) Analysis:**

- Includes total nucleated cell count (TNCC), RBC count, total protein and microscopic description of findings

### **Bone Marrow Cytology:**

- Concurrent CBC submission (included with the price) is necessary for accurate cytological interpretation.
- Please contact the duty pathologist for information regarding method of sample collection and smear preparation.

### **Body Fluid analysis:**

- Pericardial, pleural and peritoneal fluid should be collected in EDTA tubes for cell counts and plain, plain red top serum tubes (do NOT use tube with clot activators) for total protein analysis.
- Includes total nucleated cell count (TNCC), RBC count, Total protein, and microscopic description of findings
- Clearly labeled smears (direct, sediment or both) should always be concurrently submitted; particularly if there will be any delay in sample submission.
- If needed, bacterial culture can be performed on the plain serum tube sample without a clot activator.

## **CHEMISTRY**

- A minimum of 1.0 mL of serum or plasma is required for a full biochemistry profile.
  - **Serum** is the best sample for chemistry tests but plasma from lithium heparin tubes can be used for avian/exotics submissions.
  - Plasma from EDTA tubes (purple top) is NOT suitable for chemistry tests.
  - Red top tube with or without clot activator. The sample should be centrifuged within 30 minutes of sample collection and the serum separated into a clean, properly labeled tube for shipping.
  - Serum separator tubes (SST) have a gel that facilitates separation of serum from cells and reduces hemolysis, eliminating the need to transfer the sample to another tube.
    - SST tubes should be centrifuged for 10-15 minutes for proper separation
    - SSTs must not be used for endocrinology testing
- \*\*please see endocrine section for more information

- Proper sampling and handling are very important to avoid hemolysis of serum or plasma.
  - Excessive hemolysis or lipemia can dramatically alter select test results.
- Refrigerate samples or freeze if storage is longer than 3 days.
  - Repeated freeze and thaw cycles are less than ideal for serum samples.
- A full biochemistry profile includes:
  - Feline and Canine: Na, Cl, K, Ca, P, ALKP, ALT, AST, GGT, CK, bilirubin, urea, creatinine, cholesterol, total protein, albumin, globulin, glucose.
    - Amylase & Lipase are included on canine submissions only
  - Large animal: Same plus magnesium, but without amylase and lipase
  - Other add-on analytes at an additional cost include: triglyceride.

## **ENDOCRINOLOGY**

- Testing includes Progesterone, Cortisol, Phenobarbital, T4, TSH and Free T4
  - TSH & Free T4 are canine specific
- **Serum** is the required sample for endocrinology tests
  - minimum of 0.5 mL of serum is required
- Red top tube with or without clot activator. The sample should be centrifuged within 30 minutes of sample collection and the serum separated into a clean, properly labeled tube for shipping.
  - **SSTs cannot be used for Phenobarbital or progesterone analysis**
- Refrigerate samples or freeze if storage is longer than 2 days.
  - Repeated freeze and thaw cycles are not good for serum samples.

	Monday	Tuesday	Wednesday	Thursday	Friday
Total T4	✓	✓	✓	✓	✓
Thyroid panel (T4, TSH and/or FT4)					✓
Cortisol (ACTH stim, LDDST, HDDST)		✓		✓	
Phenobarbital		✓		✓	
Progesterone**	✓	✓	✓	✓	✓

\*\*Please notify the lab prior to submitting the sample for progesterone analysis

- All samples MUST be received before 3:00PM for the above mentioned tests to be analyzed on the allocated day.

# Endocrine Testing Protocols

## ACTH Stimulation Test

### Canine

- **Synthetic aqueous ACTH** (Cortrosyn<sup>\*\*</sup>™, Synacthen®<sup>\*\*</sup> (non –depot form) generic cosyntropin; 0.25 mg/mL
  - 1) Obtain a baseline (0 hr) blood sample, spin, separate and label appropriately.
  - 2) Inject ACTH
    - Inject 5 µg/kg IV or IM (Cortrosyn™, Synacthen® (non –depot form) ONLY) OR
    - Inject 0.25 mg (one vial) of synthetic IM regardless of dog's weight
      - DO NOT INJECT SYNACTHEN DEPOT® IV
        - **\*\*NOTE:** 0.25 mg vials of these two synthetic ACTH can be divided into four aliquots (62.5 micrograms) and frozen at -20°C in plastic syringes for up to 6 months.
        - Keep syringes frozen until prior to administration
  - 3) Collect a blood sample 1 hour after infection, spin, separate and label appropriately.
- **Repository corticotrophin (ACTH gel 40 IU/mL)**
  - 1) Obtain a baseline (0 hr) blood sample, spin, separate and label appropriately.
  - 2) Inject 2.2 IU/Kg IM (maximum dose of 40 IU)
  - 3) Collect blood sample 2 hours after injection, spin, separate and label appropriately

### Feline

- **Synthetic aqueous ACTH** (Cortrosyn™, Synacthen® (non –depot form) generic cosyntropin; 0.25 mg/mL
  - 1) Obtain a baseline (0 hr) blood sample, spin, separate and label appropriately.
  - 2) Inject 0.125 mg of synthetic ACTH (Cortrosyn™ or Synacthen®) IM
  - 3) Obtain blood sample 30 minutes and 60 minutes after injection, spin, separate and label appropriately

## Dexamethasone Suppression Tests

### Canine

- **Low Dose Dexamethasone Suppression Test (LDDST):**
  - Obtain a baseline (0 hr) blood sample, spin, separate and label as 0 hour or “pre”.
  - Inject 0.01 mg/kg dexamethasone IV
  - Obtain a 3 or 4 hour post and 8 hour post injection blood samples, spin, separate and label appropriately (3 or 4 hour post and 8 hour post).
- **High Dose Dexamethasone Suppression Test (HDDST):**
  - Obtain a baseline (0 hr) blood sample, spin, separate and label as 0 hour or “pre”.
  - Inject 0.1 mg/kg dexamethasone IV
  - Obtain a 3 or 4 hour post and 8 hour post injection blood samples, spin, separate and label appropriately (3 or 4 hour post and 8 hour post).

### Feline

- **Low Dose Dexamethasone Suppression Test (LDDST):**
  - Obtain a baseline (0 hr) blood sample, spin, separate and label appropriately.
  - Inject 0.1 mg/kg dexamethasone IV
  - Obtain a 3-4 hour and 8 hour post injection blood samples, spin, separate and label appropriately.
- **High Dose Dexamethasone Suppression Test (HDDST):**
  - Obtain a baseline (0 hr) blood sample, spin, separate and label appropriately.
  - Inject 1.0 mg/kg dexamethasone IV
  - Obtain a 3-4 hour and 8 hour post infection blood sample, spin, separate and label appropriately.

### Equine

- **Dexamethasone Suppression Test:**
  - Obtain a baseline blood sample (0 hr - between 4:00 – 6:00 PM), spin, separate and label appropriately
  - Inject 40 µg/kg dexamethasone IM.
  - Obtain blood sample between 19 hours post (11:00 AM – 1:00PM the following day), spin, separate and label appropriately.