



PROTOCOL MANUAL

GUIDE TO SAMPLE SUBMISSION TEST PROTOCOLS AND REFERENCE INTERVALS

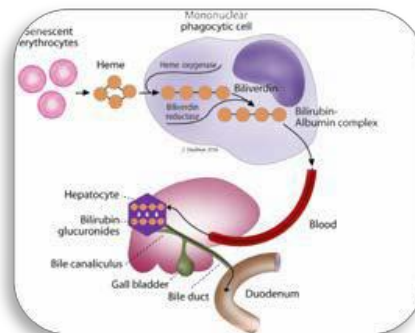
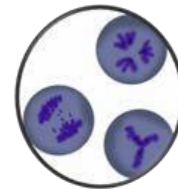


TABLE OF CONTENTS

Shipping of Samples

General Information	1
Hematology and Coagulation	2
Chemistry	3
Urinalysis	3
Cytology	3
Bone Marrow Aspirates/Core Samples	4
Steps in Obtaining Bone Marrow (publication)	6

Hematology and Coagulation

Complete Blood Count	15
Blood Grouping	15
Crossmatch	15
PT/PTT	15
FDPs	16
von Willebrand Factor	16
Platelet Aggregation	16

Serum Chemistry

Ammonia Tolerance Test	17
Bile Acids	18
Blood Gases	19
Calcium, Free (ionized)	19
Cortisol and Endogenous Adrenocorticotropic Hormone (ACTH):	
Cortisol General Information	20
Canine Reference Intervals and Cutoff Concentrations	20
Feline Reference Intervals and Cutoff Concentrations	21
ACTH Stimulation Test (canine and feline)	21
Monitoring Treatment for Canine Hyperadrenocorticism	22
Low Dose Dexamethasone Suppression Test (LDDST) (canine and feline)	22
High Dose Dexamethasone Suppression Test (HDDST) (canine and feline)	22
Endogenous ACTH (canine and feline)	23
Urine Cortisol to Creatinine Ratio (canine and feline)	24
Equine Pituitary Pars Intermedia Dysfunction (PPID)	24
Dexamethasone Suppression Test (equine)	24
Endogenous ACTH (equine)	25
Thyrotropin Releasing Hormone (TRH) Stimulation Test (equine)	25
Equine PPID and Insulin Dysregulation	25
Electrolytes (Fractional Excretion)	26
Thyroid Tests:	
Thyroid Disease in Dogs and Cats	28
Reference Intervals	28
T3 Suppression Test (feline)	28
Endogenous Thyroid Stimulating Hormone (feline)	29
Hypothyroid Therapy Monitoring in Dogs	29
GI Function Tests:	
Trypsin-Like Immunoreactivity	30
Fecal Alpha-1 Protease Inhibitor	30
Cobalamin	31
Folate	31
Pancreatic Lipase Immunoreactivity	31

Urine Chemistry

GGT to Creatinine Ratio	32
Protein to Creatinine Ratio	32
Urolith (Calculus) Analysis	33
Urolith Submission Form	34

Cytology

CSF Analysis	35
Fluid Effusions (Equine Peritoneal)	36
Fluid Effusions (Thoracic, Peritoneal, Pericardial)	37
Lymph Nodes	39
Synovial Fluid	40

Generic Reference Intervals 41

(Note: When available, reference intervals provided with the laboratory report should be used rather than “generic” values.)

Prairie Diagnostic Services Inc. Reference Intervals 49**Tables for SI Conversion** 51

Shipping of Samples

General Information

Clearly label all tubes/slides. If sending both urine and serum in similar tubes, clearly identify which sample is which. Do not cover the entire tube with a label as the contents must be visible.

Complete a submission form (for the specific diagnostic laboratory to which you will be sending your sample) and **provide a brief history** including any treatments administered, particularly those that might affect results. Indicate if the sample represents a fasted or non-fasted state. Proper requisition form completion allows for better data interpretation. Incomplete forms may cause a delay in sample processing.

Provide collection date and time on all requisitions. This is very important. Processing will be delayed if collection date/time are not provided.

Indicate which tests/panels you are requesting.

Indicate if sample is infectious or potentially infectious (e.g. rabies suspect).

Hemolysis affects all tests to varying degrees. To prevent hemolysis:

- a) Collect blood sample.
- b) Remove needle from syringe.
- c) Remove stopper from tube.
- d) Gently expel blood into tube. Mix gently if anticoagulant present.

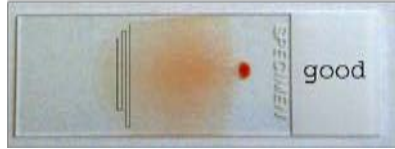
Ensure Proper Packaging:

- a) Blood/urine should be in capped, leak proof containers.
 - b) Enclose in durable outer container with enough packing material to absorb all contents should breakage occur.
- Cytology and blood smears must not have any contact with formalin, even fumes, as cell morphology is destroyed. Pack samples for histopathology separately.

Hematology and Coagulation

CBC

- Send two well-made, air dried, unstained blood smears and an EDTA tube (purple top) of blood.
- Make the smears from the EDTA tube, using a PCV (microhematocrit) tube to dispense a drop of blood, not the needle hub or the inside of a rubber stopper



A thumbnail-shaped blood smear is best for smear evaluation and leukocyte differential (lines show pattern to follow for WBC differential).

- Avoid contact with formalin (even the fumes) which compromises cell morphology; also protect from humidity and flies.
- Avoid freezing of EDTA samples in cold months. Warm packs may help; ask the transporter to ensure samples are weather protected (e.g. keep samples in the passenger area).

See links below for components of the CBC, particularly making blood smears:

1. Manual WBC count: <https://youtu.be/WwHI1d5pqSc>
2. PCV and total protein: <https://youtu.be/cau5wWe4Uds>
3. Making & staining blood smears: <https://youtu.be/nbRUiWI2Qrs>
4. Blood smear evaluation: <https://youtu.be/wIZtvTGJL6M>

PT/PTT/FDP

- Collect blood in blue top (sodium citrate) tube.
- Appropriate volume is indicated on tube and must be followed (1:9 ratio of anticoagulant: blood).
- Centrifuge and remove plasma with a plastic pipette into a plastic tube or syringe made leak-proof.
- Freeze plasma or send ASAP on ice packs.
- Many laboratories prefer to be notified in advance; check with your laboratory.

Chemistry

- a) Separate serum from cells if possible (accuracy of results is greatly improved). If a centrifuge is unavailable, allow sample to clot, and remove as much serum as possible (some RBCs will probably remain in the serum).
- b) If insufficient serum is received, the laboratory technologists may either request more serum or do those tests that are prioritized by the submitter.

****If hemolysis is >3+, you may be advised that accuracy of some tests is compromised.****

Urinalysis

Collect a minimum of 3 ml of urine in a clean, leak-proof container.
Note collection method on the requisition (e.g. cysto, free flow, catheterization).
Urine cytology (cytospin smear examination) can be requested along with routine urinalysis (U/A), and will not generally be performed without a concurrent U/A.

See link below for all aspects of performing a complete urinalysis:

https://www.youtube.com/playlist?list=PLm-jtvx5oGMTmnz0GJvPDzK001_AIFz5z

Cytology

Avoid contact with formalin; send 3-5 unstained slides, when possible.

See links below for various aspects of sample acquisition, preparation & cytology microscopy:

1. Fine needle aspiration & smear making: https://youtu.be/DkXDBom_3JI
2. Fine needle nonaspiration: <https://youtu.be/jgTnqCWewI4>
3. Impression smear making: <https://youtu.be/prggfKrNlbI>
4. Cytology fluid handling: https://youtu.be/Dl_Adsa7mpc
5. Cytology microscopy: <https://youtu.be/vWSD7bjkjs>

a) **Fluids**

- Submit in EDTA, even if the sample does not appear bloody (prevents clotting if blood/fibrinogen present).
- Make direct smears. Line preps are useful for cell poor fluids. If flocculent material is present, make smears from this material as well. This material may be retrieved using forceps and impression smears made. Blotting the material on a gauze pad may be necessary to remove superficial blood/moisture.
- Send air dried, unstained smears.

b) **Impression Smears**

- **Blot the surface** of the sample with a gauze sponge, tissue, or paper towel to remove superficial fluid and/or blood, and make gentle imprints, avoiding smearing and/or suction.
- If the sample is very firm/fibrous and does not seem to exfoliate well, score the surface with a scalpel blade before making impression smears.
- Send air dried, unstained smears.

c) **Fine Needle Aspirate or Nonaspirate Smears**

- Send air dried, unstained smears.

Bone Marrow Aspirates/Core Samples

Steps in Obtaining Bone Marrow (also see publication that follows)

- a. Sedate or anesthetize animal. Use aseptic technique.
- b. If the animal is sedated only, inject local anesthetic deep into site as close as possible to periosteal surface of bone.
- c. Use a bone marrow biopsy needle, and bone marrow collection technique as shown on the following pages. Aspirate sample. **Do not withdraw more than ½ mL**; larger volumes indicate dilution of the bone marrow by peripheral blood and sample quality is compromised.
- d. Expel sample into a flat plastic container, such as a weigh boat or Petri dish, on ice and containing a drop of 10% EDTA (prevents clotting).
- e. Use fine forceps to pick up marrow tissue particles (small glistening white/red flecks) along with sufficient blood to facilitate smear-making. (Note: these are not “spicules”; bone spicules are not aspirated when the bone marrow is sampled.)
- f. Make smears using gentle “slide over slide” technique - no pressure on your part is required.

- g. Air dry. Leave unstained.
- h. Place any excess marrow into an EDTA tube.
- i. Core sample: gently roll the core sample on a glass slide to make a smear for cytologic evaluation prior to placing in formalin.

Bone marrow biopsy needles can be purchased from:

Cardinal Health Canada

1000 Tesma Way
Vaughan, Ontario, L4K 5R8
Phone: (877) 878-7778

Must set up an account to order.

13 gauge (Jamshidi needle for bone marrow core samples): approx. \$300/case of 10; PDJ3513P 15 gauge (Illinois needle for bone marrow aspirates): approx. \$200/case of 10; PDIN1515P

If purchasing 1 Jamshidi needle for core samples, the cost is approx. \$47.00. If purchasing 1 Illinois needle for aspirates, the cost is approx. \$30.00. Both needles can be sterilized and reused as long as the tip is not damaged and the instrument is not bent.

The steps in performing a bone marrow aspiration and core biopsy

Hesitant to aspirate bone marrow or collect a core biopsy? Dr. Relford's review will reacquaint you with the basic techniques of these diagnostic procedures.

ROBERTA L. RELFORD, DVM, MS
Department of Small Animal
Medicine and Surgery
College of Veterinary Medicine
Texas A&M University
College Station, Texas 77843

BONE MARROW EXAMINATION is a straightforward diagnostic tool that can reveal valuable information and, under certain conditions, such as histoplasmosis, malignant mastocytosis, lymphosarcoma, and multiple myeloma, enable practitioners to render a definitive diagnosis. Indications for a bone marrow examination are numerous and include any unexplained alterations of the peripheral blood, such as nonregenerative anemia, neutropenia, thrombocytopenia, pancytopenia, and myeloproliferative changes. Other findings that commonly warrant a bone marrow examination include fever of unknown origin, neoplastic disease, hypercalcemia, and hyperproteinemia.

Collecting bone marrow for cytologic or histopathologic evaluation is a relatively simple, quick, and inexpensive procedure that can be done by aspiration, core biopsy, or both. Both bone marrow aspiration and core biopsy allow cytologic examination, evaluation of iron stores, and identification of pathogenic organisms. Cytologic preparation and examination of a bone marrow aspirate can be performed in a clinical setting, and the ade-

quacy of the sample can be determined immediately. Further processing and a special laboratory are not needed. In addition, multiple slides can be prepared from a single aspirate, and cellular morphology is better preserved than with a core biopsy.

In general, a core biopsy is performed when an aspirate has been unsuccessful in retrieving an adequate sample, and histopathologic examination is needed. The disadvantages of a core biopsy revolve around the processing and handling of the sample. Histologic processing of a core biopsy requires several days in a pathology laboratory and interpretation by a pathologist. The process needed to demineralize the bone also causes artifacts of the cells due to shrinkage. These artifacts can hinder critical evaluation of cellular morphology. The major advantages of core biopsy are the ability to study the architecture of the bone marrow and to evaluate a greater number of cells. Certain disease states, such as myelofibrosis, can only be definitively diagnosed by core biopsy.

In this article, I will describe several routinely used methods for

DIAGNOSTIC MEDICINE

Bone marrow aspiration and core biopsy (cont'd)

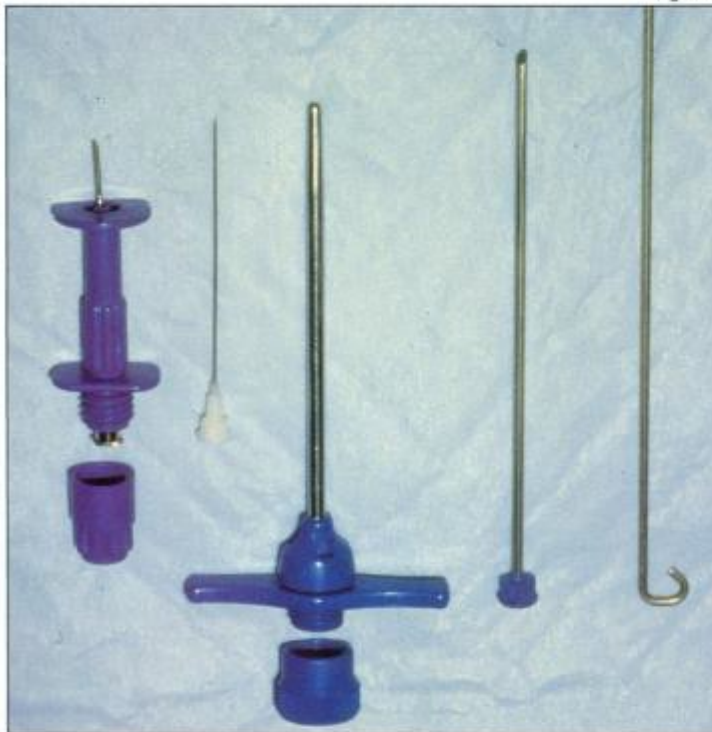


Figure 1

1. Shown here (left to right) are a Jamshidi 17/8-in., 15-ga. disposable Illinois sternal/iliac aspiration needle (American Pharmaseal Company, Valencia, Calif.) with the stylet removed, and a Jamshidi 4-in., 11-ga. disposable bone marrow biopsy/aspiration needle with the stylet and probe removed.

aspirating bone marrow and collecting a core biopsy in dogs and cats.

Selecting the site

Though several sites are suitable for bone marrow aspiration, the most commonly selected sites are the proximal humerus, the proximal femur, and the iliac crest. The preferred site for a core biopsy is the wing of the ilium. These sites have greater hematopoietic activity than other possible sites and are readily accessible. The particular site chosen is often determined by the conformation and size of the patient. I prefer the proximal humer-

us for bone marrow aspiration in most dogs and cats. Alternatively, the iliac crest is the most common site for aspiration in large dogs.

Preparing for aspiration and core biopsy

Surgically prepare the biopsy site, and drape the patient. (The surgical drape has been omitted from this article's photographs and drawings so that the procedures can be clearly seen.) Aspiration and core biopsy usually require only a local anesthetic, though sedation is helpful in some patients. After administering local anesthetic to the overlying skin, subcutaneous tissue, muscle layers, and periosteum of the aspiration site, make a small stab incision through the skin with a No. 11 scalpel blade. This stab incision should only be large enough to allow needle entry through the skin. Therefore, suturing the incision is usually not required.

A variety of bone biopsy needles can be used for aspiration and core biopsy. Two examples are shown in Figure 1. Additional equipment needed includes the No. 11 scalpel blade used to make the stab incision, a 12-ml syringe, several glass slides, and 10% formalin. Optional equipment includes 10% EDTA, a Pasteur pipette, and a Petri dish.

Aspirating bone marrow from the proximal humerus

Collecting the bone marrow

To aspirate bone marrow from the proximal humerus, place the animal in lateral recumbency. Surgically prepare a small area (approximately 6 x 6 cm) over the scapulohumeral joint and proximal

DIAGNOSTIC MEDICINE

Bone marrow aspiration and core biopsy (cont'd)



Figure 2A

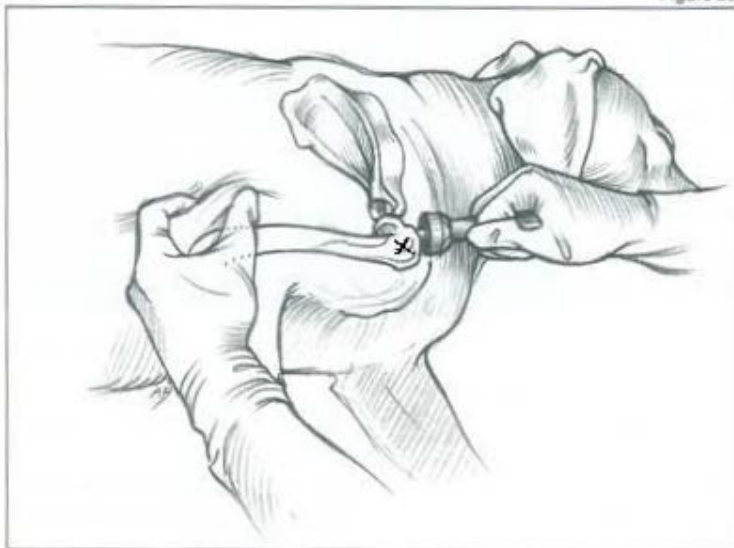


Figure 2B

2A & 2B. The aspiration needle is placed into the proximal humerus so that it maintains a parallel course within the shaft of the humerus.

humerus. Drape the patient.

Isolate and stabilize the humerus by grasping the elbow and flexing the humerus until a flattened area can be felt between the greater tubercle and the head of the humerus. Once a small stab incision is made, insert the biopsy needle with the stylet in place. Advance the needle through the muscle and fascia until contact is made with the cortex. As the needle contacts bone, apply a firm rotating pressure while directing the needle toward the elbow (*Figures 2A & 2B*). Pay particular attention to maintaining the needle in a parallel course within the shaft of the humerus. The distance the needle should be advanced after contacting the cortex will vary depending on the size of the animal. Advance it about 2 cm in cats and small dogs, and about 3 to 4 cm in medium- to large-sized dogs. To ensure adequate placement of the needle into the marrow cavity, flex and extend the scapulo-humeral joint; the biopsy needle should follow the movement of the humerus.

Next, remove the stylet, attach a 12-ml syringe, and begin aspiration. Apply suction until blood is seen within the hub of the syringe (*Figure 3*). Stop aspiration at this point to prevent further breakdown of capillaries and contamination of the sample with peripheral blood. Place a drop of the sample onto each of several clean slides. Two or three slides can be prepared using this method.

An alternative method of collection using an EDTA-coated syringe offers the following advantages: 10 to 20 slides can be made from a single sampling, greater amounts of

bone marrow can be collected, and high-quality slides can be prepared more consistently because there is less peripheral blood contamination. I prefer this method of collection, though extra equipment is necessary. To perform this technique, aspirate approximately 0.25 ml of bone marrow into an EDTA-coated syringe. Immediately expel and swirl the sample in a Petri dish containing several drops of EDTA to prevent coagulation. Tilt the Petri dish to examine the sample for bone marrow particles that have adhered to the bottom (*Figure 4*). The tan, irregular bone marrow particles should be distinguished from the floating, clear, spherical lipid droplets. While tilting the Petri dish to decrease peripheral blood contamination, aspirate the marrow particles with a Pasteur pipette and place a small drop of the sample onto each of several slides. The excess sample in the Petri dish may be placed in a tube and submitted to the laboratory along with the slides in case the slides are inadequate.

Preparing the slides

After collecting a sample using either of the two methods discussed, quickly prepare the slides using either the vertical or the horizontal "pull-apart" technique. To make a vertical pull-apart slide, place a small drop of the sample onto the center of a slide. Tilt the slide to allow excess peripheral blood to roll off the slide. With a second clean slide placed 90 degrees to the first slide, gently press the slides together and pull them apart vertically (*Figure 5*). Because neoplastic cells are fragile,

Figure 3



Figure 4



3. Suction is applied until blood is seen within the hub of a 12-ml syringe. 4. Tilting the Petri dish will allow the run off of excess peripheral blood. The tan, irregular bone marrow particles that have adhered to the bottom of the dish can then be distinguished from the clear, spherical lipid droplets.

We do not recommend the vertical pull-apart method. Suction tends to rupture cells.

DIAGNOSTIC MEDICINE

Bone marrow (cont'd)

the vertical pull-apart technique is preferred if neoplasia is suspected.

To make a horizontal pull-apart slide, place a small drop of the sample toward one end of a slide. Again, tilt the slide to allow excess peripheral blood to roll off the slide. With a second clean slide placed 90 degrees to the first slide, gently press the slides together and drag them apart horizontally at a 90-degree angle (Figure 6). The horizontal pull-apart technique leaves marrow particles intact, which aids in determining the degree of marrow cellularity and the myeloid to erythroid ratio.

Aspirates should routinely be prepared using both the vertical and horizontal pull-apart techniques. Often the clinical signs and lab findings that prompt bone marrow examination cannot help pinpoint whether the disease is neoplastic or non-neoplastic.

Air dry the slides and stain some of them for cytologic examination. Stains routinely used for blood smear examinations are suitable. Several slides should be left unstained in case special stains are required. Special stains can be used to evaluate iron stores in determining anemia of chronic inflammation (Prussian blue), identifying organisms (Giemsa, Gomori's methenamine silver, periodic acid-Schiff), or differentiating leukemic cell types (immunohistochemical stains, myeloperoxidase stains). These special stains can be applied to samples taken either by aspiration or core biopsy.

Collecting aspirate from the proximal femur

Bone marrow from the proximal fe-

Figure 5

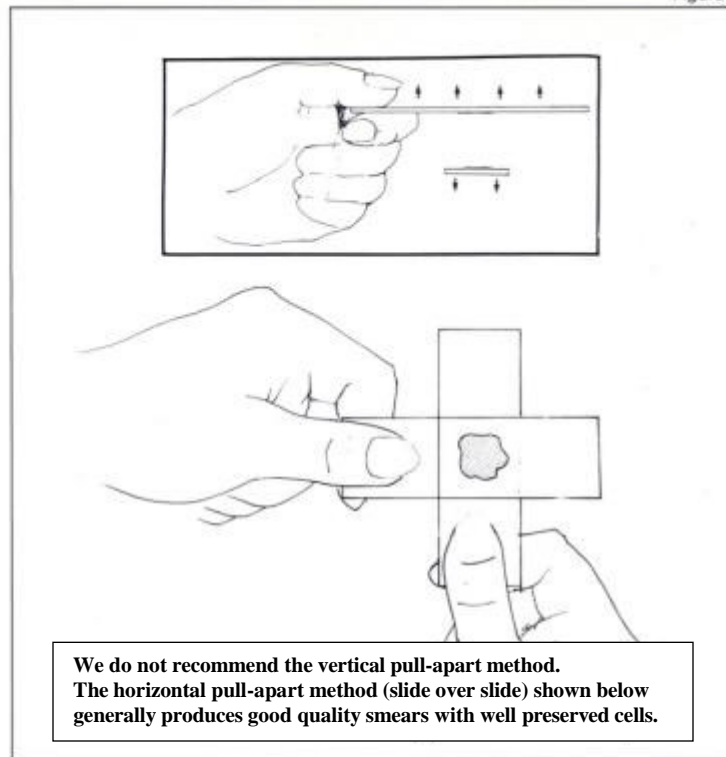
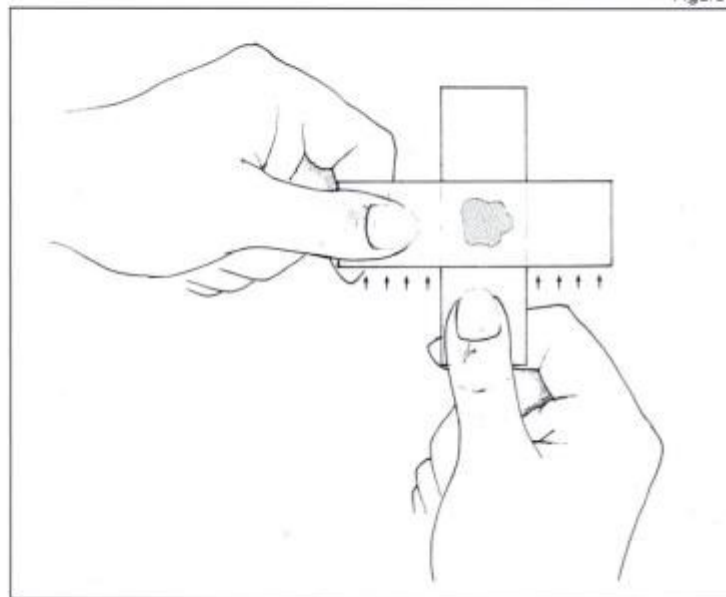


Figure 6



5. To prepare a vertical pull-apart slide, place a small drop of the sample onto the center of a slide. Tilt the slide to allow excess peripheral blood to roll off the slide. Place a second clean slide at 90 degrees to the first slide, gently press the slides together, and pull them apart vertically. 6. To prepare a horizontal pull-apart slide, place a small drop of the sample toward one end of a slide. Tilt the slide to allow excess peripheral blood to roll off the slide. Place a second clean slide at 90 degrees to the first slide, gently press the slides together, and drag them apart horizontally at a 90-degree angle.

DIAGNOSTIC MEDICINE

Bone marrow aspiration and core biopsy (cont'd)



Figure 7A

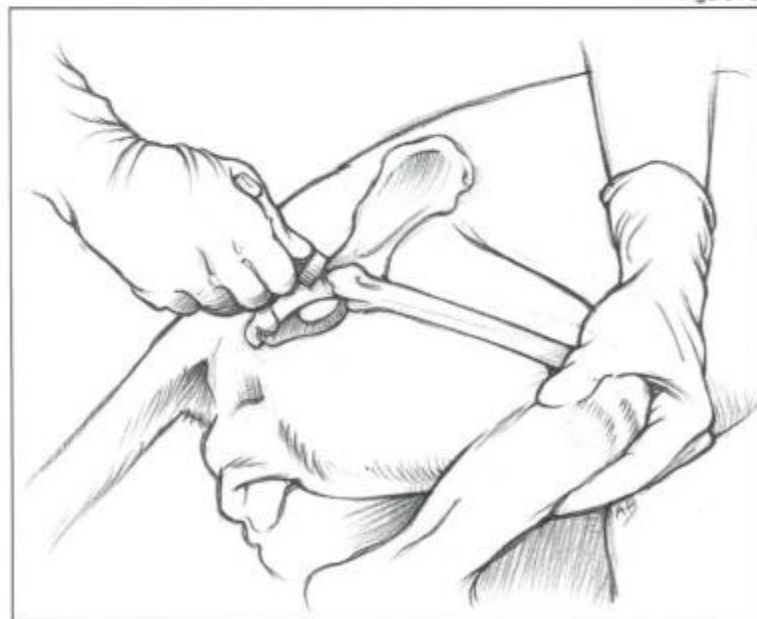


Figure 7B

7A & 7B. For a dorsal approach to the femur, place the biopsy needle into the trochanteric fossa and, with a firm rotating motion, direct the needle down the shaft toward the stifle.

mur can be collected using either the dorsal or lateral approach. For either approach, place the animal in lateral recumbency. Surgically prepare a small area (6 x 6 cm) over the proximal femur and the coxofemoral joint. Drape the patient.

For a dorsal approach to the femur, stabilize the femur by grasping the stifle. Using a No. 11 scalpel blade, make a small stab incision above the trochanteric fossa. Then insert the biopsy needle through the skin and fascia and into the fossa. Using a firm rotating motion, direct the needle down the shaft toward the stifle (*Figures 7A & 7B*). If the needle is properly placed, it should move in the same plane as the femur. Remove the stylet and aspirate as previously described.

For the lateral approach to the femur, which can be used in cats and small dogs, stabilize the femur by pressing the greater trochanter into the coxofemoral joint. Make a small incision over the lateral aspect of the proximal shaft of the femur (the approximate area of the third trochanter). Insert the biopsy needle through the muscle perpendicular to the femur. Using a firm rotating motion, advance the needle through the cortex and into the marrow cavity (*Figure 8*). Remove the stylet and aspirate. Prepare slides as discussed previously.

Aspirating marrow and collecting a core biopsy from the ilium

Aspirating bone marrow

To aspirate from the spine of the ilium, place the animal in either lateral or ventral recumbency. Sur-

DIAGNOSTIC MEDICINE

Bone marrow aspiration and core biopsy (cont'd)



Figure 8

8. To approach the proximal femur from the lateral aspect, stabilize the femur by applying firm pressure on the greater trochanter. Then direct the biopsy needle perpendicular to the proximal femur through the cortex and into the marrow cavity (approximately 1 cm).

gically prepare a 6- x 6-cm area over the dorsal wing of the ilium. Drape the patient.

Place a finger on both sides of the iliac spine to localize the site. Make a small stab incision directly over the widest portion of the cranial dorsal iliac spine. (In cats and small dogs, aspiration may be difficult at this site because of the narrow iliac spine. In these animals, bone marrow should be aspirated from the humerus or femur; core biopsies can also be taken from these sites.) Direct the needle perpendicular to the dorsal iliac spine and use a firm rotating motion to place the needle into the marrow cavity (Figures 9A & 9B). Remove the stylet and aspirate. Then prepare the slides for examination.

Collecting a core biopsy

Several sites have been used for collecting core biopsies, but the wing of the ilium is a common and easily accessible site. Alternatively, the site used for the aspiration biopsy may be used simultaneously to collect a core biopsy. However, a marrow aspiration cannot be performed by approaching the wing of the ilium laterally (the easiest approach for core biopsy) because of the narrow width of the marrow cavity at this site.

To collect a core biopsy from the wing of the ilium, place the animal in lateral recumbency and surgically prepare a 6- x 6-cm area over the lateral wing of the ilium. Drape the patient.

Using a No. 11 scalpel blade, make a small stab incision over the cranial aspect of the lateral wing of the ilium. With the stylet in place, direct the needle perpendicular to

DIAGNOSTIC MEDICINE

Bone marrow aspiration and core biopsy (cont'd)

Figure 9A



Figure 9B



9A & 9B. Firmly grasp the dorsal and ventral iliac spine and insert the biopsy needle perpendicular to the widest portion of the dorsal iliac spine. Advance the needle approximately 2 to 3 cm into the marrow cavity.

the ilium and through the middle gluteal muscle. With a rotating motion, push into the cortex of the ilium approximately 0.5 centimeter. Remove the stylet and advance the needle 1 cm into the marrow cavity, filling the bore of the needle with a core of marrow (*Figures 10A & 10B*). To dislodge the core, rock the needle back and forth several times and then withdraw the needle. This procedure can be repeated two or three times before withdrawing the biopsy needle from the animal.

Use the probe to push the core (or cores) back up through the needle bore and out the top of the biopsy instrument. Roll the core onto a slide for cytologic examination (*Figure 11*). Then place the core into a 10% formalin solution for histopathologic examination.

Conclusion

Bone marrow evaluation can be a valuable asset in evaluating patients with unexplained hemograms, fever of unknown origin, neoplastic diseases, hypercalcemia, and hyperproteinemia. Although cytologic interpretation of bone marrow aspirates requires experience, collecting samples is a relatively quick, inexpensive procedure. With minimal experience, you can become proficient at collecting adequate bone marrow and core biopsy samples.

SUGGESTED READING

1. Grindem, C.: Bone Marrow Biopsy and Evaluation. *Vet. Clin. N. Amer. Sm. Anim. Prac.* 19:689-696, 1989.
2. Lewis, H.B.; Rebar, A.H.: *Bone Marrow Evaluation in Veterinary Practice*. Reiston Purina, St. Louis, Mo., 1979.
3. Duncan, J.R.; Prasse, K.W.: *Veterinary Laboratory Medicine: Clinical Pathology*, 2nd Ed. Iowa State University Press, Ames, 1986; pp 3-72.

Continued

DIAGNOSTIC MEDICINE

Bone marrow aspiration and core biopsy (cont'd)

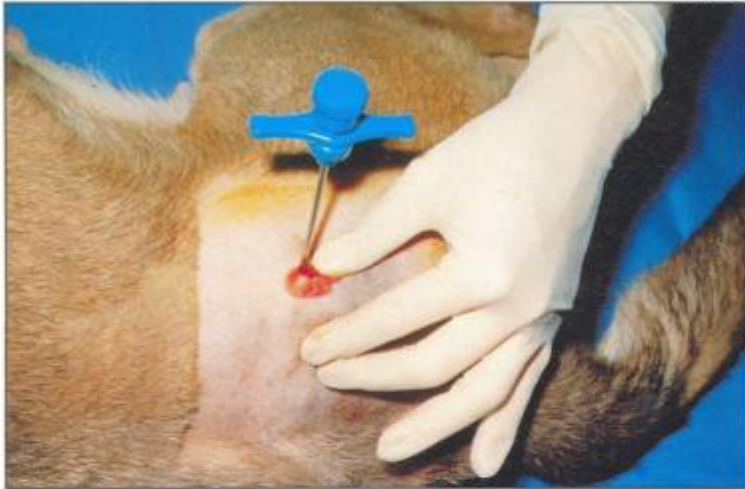


Figure 10A

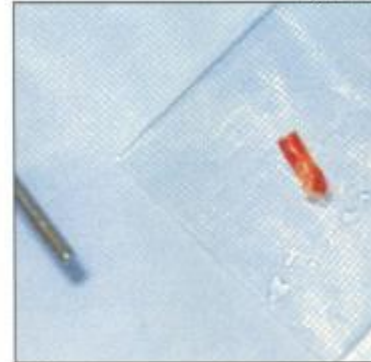


Figure 11

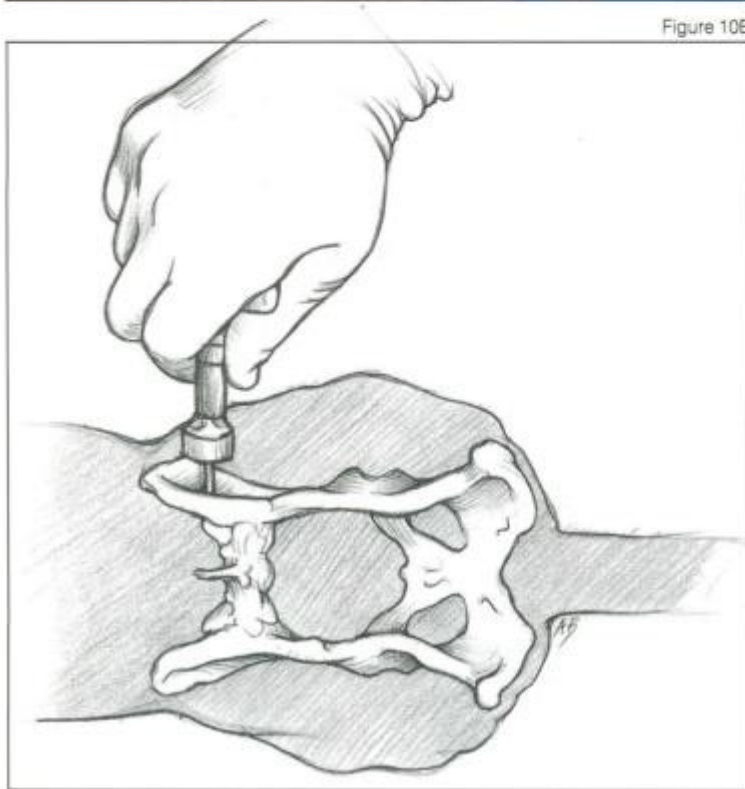


Figure 10B

10A & 10B. Once the core biopsy needle has been advanced through the cortex of the wing of the ilium, remove the stylet and advance the biopsy instrument approximately 1 cm into the marrow cavity, thus filling the bore of the needle with a core of marrow. **11.** Use the probe to push the core back up through the needle bore and out the top. Roll the core on a slide for cytologic examination. The core can then be placed into 10% formalin for histopathologic examination.

4. Benjamin, M.M.: *Outline of Veterinary Clinical Pathology*, 3rd Ed. Iowa State University Press, Ames, 1978; pp 15-24.

5. Jain, N.C.: *Schalm's Veterinary Hematology*, 4th Ed. Lea & Febiger, Philadelphia, Pa., 1986; pp 1-19.

6. Cowell, R.L.; Tyler, R.D.: *Diagnostic Cytology of the Dog and Cat*. American Veterinary Publications, Goleta, Calif., 1989; pp 99-120.

Hematology and Coagulation

Complete Blood Count (CBC)

Preferred tube – EDTA (purple top).

Heparin (green top) may be used, but often results in WBC clumping.

Fill tube to indicated line if possible - prevents artifactual sample dilution and morphological changes.

Make 2 blood smears, particularly if the sample will not arrive at the diagnostic laboratory within 8 hours of collection.

Laboratory staff welcome the opportunity to assist anyone who is unsure of proper slide-making technique.

Blood Grouping (Dogs and Cats)

Sample required: 0.5 ml EDTA blood

Canine - determination of DEA 1.1 positive or negative only
Feline - determination of Group A or B

Crossmatch

Canine/Feline/Equine

Notify lab of impending crossmatch. Early submission is preferred as the test takes at least 2 hrs.

Submit all samples at the same time rather than as they are collected.

Collect an EDTA and red top tube from recipient and each donor.

Volume required (recipient):

EDTA: 0.5 - 1 mL

Red Top: If ≤ 3 donors - 3 mL whole blood

If > 3 donors - 5 mL whole blood

Volume required (each donor):

EDTA: 0.5 - 1 mL

Red Top: 3 mL whole blood

PT/PTT

Your lab may require notification of impending coagulation submissions.

Blue top tubes (sodium citrate anticoagulant) are required.

Required volume is stated on the tubes, and is critical. The ratio of anticoagulant to whole blood must be 1:9.

A non-hemolyzed, non-lipemic sample is preferred. Needle removal prior to expulsion of blood into tube is recommended as this helps to avoid hemolysis. Mix gently.

A "normal" species control **MAY** be required - please check with lab (usually not required for canine, feline, equine species)

Submit sample to lab ASAP post collection; if there is to be a delay, separate plasma from cells using a plastic pipette, transfer plasma into a plastic tube and ship frozen.

FDPs (Fibrin Degradation Products)

Performed on same sample as PT/PTT (blue top/Na citrate tube).

von Willebrand Factor

Collect appropriate amount of blood into sodium citrate tube (blue top). Centrifuge and transfer plasma into a plastic tube or syringe made leakproof (1 mL plasma required).

Von Willebrand Factor is very labile, so sample must be processed immediately.

Hemolysis and lipemia **MUST** be avoided - a hemolyzed sample requires recollection. The lab will notify you if hemolysis is present, so please do not allow the patient to leave, if at all possible, before this has been determined. Avoid lipemia by fasting the patient for 12 hours.

Not all diagnostic laboratories perform this test. Your sample may be shipped to a referral laboratory. Check with your laboratory first.

Platelet Aggregation

Check with your laboratory to determine if and where this test can be performed.

Serum Chemistry

Ammonia Tolerance Test

Check with your laboratory to determine if and where this test can be performed. For instance, this test is offered through Prairie Diagnostic Services Inc. (PDS), however the time-sensitive nature and special handling procedures preclude the test from being offered outside the Veterinary Medical Centre of the Western College of Veterinary Medicine.

Principle: Ammonia (predominantly ammonium, NH_4^+) is produced in the intestinal tract, mostly by bacterial breakdown of proteins and amino acids. Ammonia then enters the portal circulation and is used for urea and protein synthesis by hepatocytes. Fasting blood ammonia and the ammonia tolerance test assess hepatic function. This test, unlike bile acids, is not affected by hyperbilirubinemia, and can be performed in the presence of cholestasis. However, this is a specialized test that is not performed by all reference laboratories and proper sample handling procedures are crucial and time-sensitive.

Procedure:

1. Fast patient for 12 hr.
2. Collect 1 mL of heparinized blood (green top tube). Avoid hemolysis or blood clots. Label “pre”. IMMEDIATELY wrap sample in paper towel and place on ice pack. Submit to the laboratory, along with submission form.
3. If the patient has CNS signs, wait for the “pre” result prior to administration of ammonium chloride. The challenge test is **NOT** indicated if fasting hyperammonemia exists and an additional ammonium load could exacerbate hepatic encephalopathy.
4. Ammonium chloride (100 mg/kg, but not in excess of 3 grams total) – is mixed with 20-50 mL of water.
5. Administer ammonium chloride via stomach tube.
6. Collect 1 mL of heparinized blood (green top tube) 30 minutes after ammonium chloride administration. Avoid hemolysis or blood clots. Label “post”. IMMEDIATELY wrap sample in paper towel and place on ice pack. Submit to the laboratory, where the pre and post plasma samples are frozen prior to transport to a referral laboratory.

Reference Cutoff Values:

	Pre ($\mu\text{mol/L}$)	Post ($\mu\text{mol/L}$)
All species	<80	<80

Interpretation:

Fasting and/or post-challenge hyperammonemia could be due to hepatic insufficiency, acquired or congenital portovascular abnormalities, or a congenital or acquired urea cycle enzyme deficiency (rare). These conditions result in decreased ammonia clearance from the portal blood. Clinically significant hyperammonemia can also occur for nonhepatic reasons including: increased ammonia production (e.g. urea toxicity in cattle and bacterial infections such as clostridial enterocolitis in horses) or increased ammonia intake (e.g. ammoniated forage toxicity in cattle).

Bile Acids

Principle: Bile acids are synthesized by hepatocytes, secreted into bile, and released into the small intestine where they aid in the digestion and absorption of fat and fat-soluble nutrients. Normally, almost all bile acids are reabsorbed from the small intestine into the portal circulation, taken up by hepatocytes, and re-secreted into the biliary tract. In some species, with or without a gall bladder, bile is continuously secreted into the gut (e.g. horses, cattle, and certain birds), whereas in others (e.g. dogs and cats), bile is stored and concentrated in the gall bladder and typically secreted only after food is ingested. Postprandial serum bile acids (SBA) concentration is usually higher than fasted concentrations in animals that do not continuously secrete bile into the small intestine. In species that do continuously secrete bile into the small intestine, fasting and postprandial sampling is unwarranted thus a single/random sample is used. Although a fasted SBA may be preferred for many birds, recommended fasting times vary and published RI frequently do not specify whether fasted or not. Ideally, an avian specialist should be consulted prior to testing.

Procedure:

1. Fast patient for 12 hr (see exceptions above).
2. Collect 1 mL blood (red top tube). Label “pre or random”. **Avoid hemolysis.**
3. Feed animal: (≥ 2 teaspoons [≥ 10 g] for cats and small dogs; ≥ 2 tablespoons [≥ 30 g] for larger dogs). Some protocols recommend feeding canned growth diets because a higher fat content may more reliably stimulate gallbladder contraction, but guidelines vary.
4. Collect 1 mL blood (red top tube) 2 hr post ingestion. Label “postprandial”. **Avoid hemolysis.**

Reference Cutoff Values:

	<u>Pre</u> ($\mu\text{mol/L}$)	<u>Post</u> ($\mu\text{mol/L}$)
Canine	<10	<20
Feline	<10	<20
	<u>Random</u> ($\mu\text{mol/L}$)	
Equine	<15	
Bovine:		
beef	<126	
dairy	<88	
6 mo dairy heifers	<64	
Avian:		
many species	<70	
Amazon parrots	<144	
	(highly variable depending on the species; check with laboratory)	

Interpretation:

- Elevated SBA concentration indicates impaired hepatic function, abnormal enterohepatic/portal circulation, and/or cholestasis.
- Fasted SBA concentration is higher than the postprandial concentration up to 20% of the time in dogs. This may occur if the gallbladder spontaneously contracts during the fasting period (interprandial contraction) or because of other gall bladder or intestinal variables. The postprandial SBA concentration is not considered to be of diagnostic value when this occurs.
- SBA testing is usually not done in hyperbilirubinemic patients as it cannot be interpreted as a test of hepatic function under these circumstances.
- Anorexia alone can result in SBA concentration of up to 20 $\mu\text{mol/L}$ in horses.

Reference:

Thrall, Weiser, Allison, Campbell. Veterinary Hematology and Clinical Chemistry, 2nd ed. Wiley-Blackwell, 2012.

Blood Gases (Available only to clinics situated in close proximity to the diagnostic laboratory)

(Many blood gas instruments also provide Na, K, Cl, free calcium, glucose, lactate, and HgB)

- 1) Collection technique must minimize or eliminate exposure to air.
- 2) Venous or arterial puncture must be clean, to avoid fibrin strands, platelet clumps, and/or clotting. Blood should be as rapidly flowing as possible. 3 mL of blood must be aspirated into the syringe without delay. Analysis of blood volumes less than 1 ml are discouraged.
- 3) Commercially available dry heparinized syringes are preferred for blood gas analysis.
- 4) Following acquisition of blood sample, remove needle and eliminate air bubble from sample. Cap syringe.
- 5) Thoroughly mix heparin with blood immediately following sampling by inversion or rolling to ensure adequate and complete anticoagulation.
 - Include temperature of patient and whether venous or arterial sample.
 - Submit to lab ASAP. If there will be delay, place on ice/ice pack; the sample will be stable for 1 hour.

Calcium – Free (“ionized”) (Available only to clinics situated in close proximity to the diagnostic laboratory)

Background: The concentration of free calcium ions is a more clinically relevant measurement than that of total calcium, since calcium that is not bound (free) is biologically active.

In plasma (or serum), if the total calcium is approximately 2.40 mmol/L, plasma proteins (mostly albumin) bind approximately 0.9 mmol/L. Another 0.25 mmol/L is complexed with various anions (bicarbonate being the most important). The remaining 1.25 mmol/L of calcium is the free (sometimes referred to as “ionized” calcium, though all body calcium is ionized).

Free calcium is important in the transmission of nerve impulses, muscle contraction, enzymatic conversions, coagulation, and many other physiological activities.

Physiologically there is a close relationship between free calcium and pH. Free calcium increases with a decrease in pH, because the calcium binding capacity of the plasma proteins decreases with pH decrease. Hence, free calcium and pH should be measured simultaneously.

The calculated free calcium at pH 7.4 reflects the above relationship between free calcium, pH, and plasma proteins. This value may be of assistance in differentiating between changes in free calcium due to pH change, and changes due to other causes.

Submission:

**Collect ANAEROBICALLY - otherwise CO₂ is released which increases the

pH**. See blood gas collection method. Serum is not an appropriate sample.

Cortisol and Endogenous Adrenocorticotrophic Hormone (ACTH)

Cortisol General Information

- Stable in most body fluids.
- Most often evaluated in serum (red top tube), infrequently, in urine.
- Do **NOT** use serum separator tubes (SST).
- If testing will be delayed, separate serum from cells and freeze.
- Factors potentially affecting cortisol concentration or its measurement include: stress; ketoconazole and topical or systemic glucocorticoids (suppress cortisol concentration); glucocorticoids (not including dexamethasone) that cross-react with the cortisol assay (these may spuriously increase cortisol concentration).
- Serum cortisol has the best interpretive value when the baseline concentration is compared to an evocative test result (e.g. adrenocorticotrophic hormone [ACTH] stimulation test or low dose dexamethasone suppression test [LDDST]).

Canine Serum Cortisol Reference Intervals (RI) and Cutoff Concentrations:

Sample	Cortisol Concentration (nmol/L)	Interpretation
Baseline:	<20–270	
Post ACTH stim (1hr):	230-570 >660 ≤55 No/little change from baseline	Not hyperadrenocorticism or stress or hypoadrenocorticism Hyperadrenocorticism or stress Hypoadrenocorticism Iatrogenic hyperadrenocorticism Hypoadrenocorticism Critical illness-related corticosteroid insufficiency (CIRCI)
Post LDDST (8hr):	<40 >40	Not hyperadrenocorticism or stress Hyperadrenocorticism or stress

Hyperadrenocorticism (HAC)

- Naturally-occurring hyperadrenocorticism (HAC) may be due to pituitary-dependent hyperadrenocorticism (PDH) or functional adrenal tumor (FAT) in dogs, although PDH accounts for the majority of cases.
- The LDDST is the preferred screening test for diagnosing naturally-occurring HAC in dogs due to greater sensitivity and specificity.¹
- In iatrogenic canine HAC, ACTH stimulated cortisol concentrations vary (these could be below detection; low – 20-138 nmol/L; or low normal) **but typically show little change from baseline concentrations.**

Hypoadrenocorticism

- In naturally-occurring canine hypoadrenocorticism, baseline and ACTH stimulated cortisol concentrations are ≤55 nmol/L, though most commonly <28 nmol/L.¹ A single baseline cortisol of >55 nmol/L may help exclude a diagnosis of hypoadrenocorticism in the dog.²
- Most cases of hypoadrenocorticism in the dog result from primary adrenal gland failure.² ACTH stimulation test results for most dogs with hypoadrenocorticism are usually distinct compared to normal dogs.¹
- Equivocal post ACTH cortisol concentrations (56 to 229 nmol/L) in dogs suspected to have hypoadrenocorticism can occur for the following reasons:^{1,2}

Medication (patient history):

- Prior glucocorticoid administration (adrenocortical atrophy, spuriously high measurement of endogenous cortisol in a patient with hypoadrenocorticism).
- Prior treatment with mitotane, trilostane, or ketoconazole.

ACTH Administered (test variables):

- Failure of ACTH product or protocol used (potency, dosage, administration, sample collection).

Patient Variables:

- Dogs with FAT that secrete sex hormones; these are rare and patients usually exhibit clinical signs of HAC.
- Dogs in the early stages of primary hypoadrenocorticism.
- Some dogs affected with secondary hypoadrenocorticism.

□ Some dogs affected with critical illness-related corticosteroid insufficiency (CIRCI) but their pre-
ACTH cortisol is usually above the RI for baseline cortisol.

Recommendations when equivocal results are obtained:

Repeat ACTH stimulation testing 1-4 weeks later to help exclude test variables or to identify dogs in early stages of primary hypoadrenocorticism. Measuring endogenous ACTH prior to ACTH stimulation testing may help with interpretation (e.g. dogs with secondary hypoadrenocorticism may be identified).

Feline Serum Cortisol Reference Intervals (RI) and Cutoff Concentrations:

Sample	Cortisol Concentration (nmol/L)	Interpretation
Baseline:	<320	
Post ACTH stim (1hr):	166-414 >524 ≤55 No/little change from baseline	Not hyperadrenocorticism or stress or hypoadrenocorticism Hyperadrenocorticism or stress Hypoadrenocorticism Iatrogenic hyperadrenocorticism Hypoadrenocorticism Critical illness-related corticosteroid insufficiency (CIRCI)
Post LDDST (8hr):	<40 >40	Not hyperadrenocorticism or stress Hyperadrenocorticism or stress

Hyperadrenocorticism

- Naturally-occurring HAC is uncommon in cats but, similar to the disease in dogs, may be due to PDH or FAT, with PDH accounting for the majority of cases.¹
- The LDDST is the preferred screening test for diagnosing naturally-occurring HAC in cats, although a combination of tests may be required.¹
- The ACTH stimulation test is **NOT** recommended for the diagnosis of naturally-occurring HAC in cats because it lacks sensitivity, but it is currently the best test to confirm iatrogenic HAC, a rare condition in cats.¹
- In iatrogenic feline HAC, ACTH stimulated cortisol concentrations vary (these could be below detection; low – 20-138 nmol/L; or low normal) **but typically show little change from baseline concentrations.**

Hypoadrenocorticism

- Naturally-occurring hypoadrenocorticism is rare in cats. When it does occur, it is generally due to primary adrenocortical disease. In addition to an inadequate response with the ACTH stimulation test, affected cats would also be expected to have markedly elevated ACTH concentrations, consistent with primary adrenocortical failure.¹

Note: Much of the information provided in the corresponding section for dogs, applies or may apply to cats. The low incidence of adrenocortical disease in cats and subsequent relative lack of research in this area, limit the availability of reference material on this subject.

ACTH Stimulation Test

Use:

To differentiate between normal adrenal function and HAC (Cushing's) in dogs; to detect iatrogenic HAC in dogs and cats; and to differentiate between normal adrenal function and hypoadrenocorticism (Addison's) in dogs and cats. This test can also be used to monitor response to therapy (e.g. trilostane or mitotane) in patients receiving treatment for HAC.

Method:

1. Collect at least 1 mL of blood in a red top tube. Label "pre".
2. Dogs: 5 ug/kg cosyntropin (Cortrosyn®) IV to a maximum of 250 ug (1 vial) per dog. Collect at least 1 mL of blood in a red top tube 1 hr post injection. Label "1hr post".
For monitoring trilostane or mitotane therapy in dogs: a dose of **1 ug/kg** Cortrosyn® IV may be used, instead of 5 ug/kg.³ The ACTH stimulation test should be initiated 4-6 hr post pill (treatment).

- Cats: 125 ug cosyntropin (Cortrosyn®) IV (1/2 vial) per cat. Collect at least 1 mL of blood in a red top tube at 1 hr post injection. Label “1hr post”.

Monitoring Treatment for Canine Hyperadrenocorticism

Treatment	Test	Cortisol Concentration	Interpretation
trilostane	Post ACTH stim (1hr):	30-150 nmol/L*	Ideal response ¹
mitotane	Post ACTH stim (1hr):	30-150 nmol/L**	Ideal response ¹

* Up to 240 nmol/L is acceptable if clinical signs are controlled

**Up to 220 nmol/L is acceptable if clinical signs are controlled

- When treating HAC, correction of clinical signs is very important (e.g. return to “normal” water consumption of <66 mL/kg/day, and correction of appetite and activity level). There should also be no adverse effects from the therapy itself (e.g. gastrointestinal signs, lethargy, weakness).

Low Dose Dexamethasone Suppression Test (LDDST)

Use:

To differentiate between normal adrenal function and HAC. The test is performed on patients that have clinical, physical, and laboratory findings suggestive of HAC. If HAC is confirmed, the LDDST may also help differentiate between the two causes: PDH and FAT.

Method:

- Collect at least 1 mL of blood in a red top tube. Label “pre”.
- Inject 0.01 mg/kg dexamethasone IV for dogs (0.1 mg/kg for cats).
- Collect at least 1 mL of blood in a red top tube 4 and 8 hr post injection –label as “4 hr post” and “8 hr post”, respectively.

LOW DOSE DEXAMETHASONE SUPPRESSION TEST (LDDST): canine and feline		
Cortisol concentration		Interpretation
4 hr post	8 hr post	
	<40 nmol/L	Not hyperadrenocorticism or stress
	>40 nmol/L	Hyperadrenocorticism or stress
<50% of baseline <40 nmol/L	<50% of baseline	Pituitary-dependent hyperadrenocorticism (PDH)
>50% of baseline >40 nmol/L	>50% of baseline	Functional adrenal tumor or PDH

Further Explanation:

The LDDST may also help differentiate between PDH and FAT. Suppression supports PDH and is defined as any of the following cortisol concentrations:

- <50% of baseline at 4 hr
- <40 nmol/L at 4 hr
- <50% of baseline at 8 hr¹

Lack of suppression, as defined above, could be due to FAT or PDH.

In **cats**, the following are more sensitive in indicating suppression consistent with PDH:

- <50% of baseline at 4 hr
- <50% of baseline at 8 hr¹

High Dose Dexamethasone Suppression Test (HDDST)

Use:

To help differentiate between PDH and FAT in a patient that already has an established diagnosis of HAC and has not yet received any therapy.

Method:

1. Collect at least 1 mL of blood in a red top tube. Label “pre”.
2. Inject 0.1 mg/kg dexamethasone IV for dogs (1 mg/kg for cats).
3. Collect 1 ml of blood in a red top tube at 4 and 8 hr post injection - label as “4 hr post” and “8 hr post”, respectively.

HIGH DOSE DEXAMETHASONE SUPPRESSION TEST (HDDST): canine and feline		
Cortisol concentration		Interpretation
4 hr post	8 hr post	
<50% of baseline <40 nmol/L	<50% of baseline <40 nmol/L	Pituitary-dependent hyperadrenocorticism (PDH)
>50% of baseline >40 nmol/L	>50% of baseline >40 nmol/L	Functional adrenal tumor or PDH

Further Explanation:

Cortisol concentrations at 4 or 8 hrs post injection should suppress with PDH. Suppression is defined as any of the following:

- <50% of baseline at 4 hr
- <40 nmol/L at 4 hr
- <50% of baseline at 8 hr
- <40 nmol/L at 8 hr

Cortisol concentrations are not suppressed in patients with adrenal tumors. Suppression with the HDDST confirms PDH, however lack of suppression may indicate either PDH or FAT, as 25% of dogs with PDH do not suppress with the HDDST. This percentage is even higher in cats.

Endogenous ACTH

As indicated above, a significant proportion of dogs and cats with PDH do not suppress with the HDDST. In these cases, endogenous ACTH concentration may assist in differentiating PDH and FAT. Plasma ACTH concentration should be high with PDH and low with FAT.

Endogenous ACTH concentration is also used to diagnose adrenal insufficiency caused by pituitary disease (secondary hypoadrenocorticism). These patients have low-normal to low endogenous ACTH concentrations, and signs referable to glucocorticoid lack but not mineralocorticoid lack. As about 10% of dogs with primary hypoadrenocorticism (caused by adrenocortical pathology) have serum electrolytes within RI, endogenous ACTH can also be useful to differentiate between primary disease (high ACTH concentrations) and secondary disease (low-normal to low ACTH concentrations). The patients with primary hypoadrenocorticism and unaffected electrolyte concentrations (also called atypical Addison’s disease) may eventually develop electrolyte abnormalities and require mineralocorticoid supplementation. Therefore, distinguishing between primary and secondary disease by measuring endogenous ACTH concentration at the time of diagnosis signals the need to closely monitor electrolytes in those dogs with primary disease.

Method:

1. Collect 1 mL of blood in a purple top tube (EDTA).
2. Centrifuge immediately after collection.
3. Transfer plasma to a plastic tube (most BD blood collection tubes are plastic) and freeze immediately.
4. Ship sample to the laboratory on ice packs, requesting ACTH concentration.
(Samples with marked lipemia and/or hemolysis may not be suitable for analysis. Fasting may be advisable.)

Interpretation:

Endogenous ACTH RI provided by the reference laboratory should be used when interpreting the test results.

Urine Cortisol to Creatinine Ratio (UCCR)

Reference (cutoff) Value: (urine should be collected in a non-stressful environment)

Dogs: $\leq 10 \times 10^{-6}$

Cats: $< 36 \times 10^{-6}$

Interpretation:

A UCCR above the cutoff value can be found in dogs and cats with HAC, but also in patients with nonadrenal disorders, and in healthy stressed dogs and cats. Additional screening testing (usually the LDDST) must be done to further evaluate for HAC. A UCCR below the cutoff value (particularly a very low ratio) is strong evidence that HAC is **NOT** present, because the test has a high negative predictive value. A UCCR less than but closer to the cutoff value may warrant repeating or follow up with additional screening testing.

(Note: Another test to distinguish PDH from FAT in dogs and cats is dexamethasone suppression of the UCCR.^{1,4} This test involves multiple collections of urine for UCCR and multiple administrations of oral dexamethasone [0.1 mg/kg per dose for both dogs and cats]. The procedures may be completed in the home environment, with submission of the pre- and post-dexamethasone urine samples for UCCR analysis. This test may be more widely used in the future, particularly for cats.⁴)

References:

¹ Feldman, Nelson, Reusch, Scott-Moncrieff eds, 4th ed. Canine and Feline Endocrinology, Elsevier, 2015.

² Gold, Langlois, Refsal. Evaluation of basal serum or plasma cortisol concentrations for the diagnosis of hypoadrenocorticism in dogs. J Vet Intern Med 2016;30:1798-1805.

³ Aldridge, Behrend, Kemppainen, *et al.* Comparison of 2 doses for ACTH stimulation testing in dogs suspected of or treated for hyperadrenocorticism. J Vet Intern Med 2016;30:1637-1641.

⁴ Rand. Clinical Endocrinology of Companion Animals, Wiley-Blackwell, 2013.

Equine Pituitary Pars Intermedia Dysfunction (Equine Cushing's)

A pituitary pars intermedia dysfunction (PPID) working group (Equine Endocrinology Group, EEG) has established recommendations for the diagnosis and treatment of PPID and these are regularly updated on their website (<http://sites.tufts.edu/equineendogroup>). The following laboratory tests may aid in confirming a diagnosis of PPID in horses and ponies suspected of having the disease: dexamethasone suppression test (measuring cortisol); baseline endogenous ACTH concentration; thyrotropin releasing hormone (TRH) stimulation test (measuring ACTH). Occasionally, more than one test may be required to diagnose PPID.

Dexamethasone Suppression Test (DST)

The DST is increasingly being replaced by other tests described below for the diagnosis of PPID. Clinicians may choose to avoid the DST in a patient with a predisposition to laminitis. Also, the alternative tests below may allow for detection at an earlier stage of development of the disease so that, with appropriate treatment, many effects of PPID may be averted.

Method:

1. Collect at least 1 mL of blood at approximately 5 pm in red top tube - label this baseline sample "pre". Do not use serum separator tubes (SST).
2. Inject dexamethasone (40 ug/kg) IM.
3. Collect at least 1 mL of blood at approximately noon the following day in red top tube - label "19 hr post".
4. Submit the paired serum samples chilled, requesting cortisol concentrations.

Interpretation:

Normal adrenal function:

Pituitary pars intermedia dysfunction

19 hr cortisol:

≤ 28 nmol/L

> 28 nmol/L

Endogenous ACTH Concentration

Endogenous ACTH concentration can be used to diagnose PPID and it can also be used to monitor response to therapy for PPID. Baseline ACTH concentrations are variable and seasonal (higher in the fall than in winter through to summer).

Method:

1. Collect 1 mL of blood in a purple top tube (EDTA).
2. Centrifuge immediately after collection.
3. Transfer plasma to a plastic tube and freeze immediately.
4. Ship sample to the laboratory on ice packs, requesting ACTH concentration.
(Samples with marked lipemia and/or hemolysis may not be suitable for analysis.)

Interpretation:

Use seasonal reference intervals (RI) provided by the reference laboratory to interpret endogenous ACTH results. Horses with PPID are expected to have ACTH concentrations above the RI. Horses receiving treatment (generally pergolide) for PPID are ideally monitored twice yearly, in the spring and fall, with a goal of maintaining endogenous ACTH concentration within the RI.

TRH Stimulation Test

Method:

1. Collect 1 mL of blood in a purple top tube (EDTA). Mark as “pre”.
2. Inject 1 mg of TRH IV. (TRH available in Canada; contact Veterinary Medical Centre, Western College of Veterinary Medicine pharmacy)
3. Collect 1 mL of blood in a purple top tube (EDTA) exactly 10 minutes following TRH administration. Mark as “post”.
4. Centrifuge both samples immediately after collection.
5. Transfer plasma to plastic tubes (marked as “pre” and “post”) and freeze immediately.
5. Ship samples to the laboratory on ice packs, requesting ACTH concentrations.
(Samples with marked lipemia and/or hemolysis may not be suitable for analysis.)

Interpretation:

Horses with PPID have a marked increase in plasma ACTH concentration in response to TRH (interpret results relative to the RI provided by the reference laboratory performing the test).

Equine PPID and Insulin Dysregulation

PPID may be accompanied by insulin dysregulation (ID) which is also a component of equine metabolic syndrome (EMS). Conversely, horses with EMS may be predisposed to PPID. Horses with ID are particularly susceptible to laminitis. It is recommended that horses >10 yr with EMS be monitored for PPID. ID refers to increased insulin response to oral sugars or consumed feeds, fasting hyperinsulinemia, and tissue insulin resistance. The tests used for the diagnosis and monitoring of ID are: fasting insulin concentration, the oral sugar/glucose test (measuring postprandial insulin and glucose), and the insulin tolerance test (measuring glucose). See the EEG website for test protocols and expected results (<http://sites.tufts.edu/equineendogroup>).

Electrolytes - Fractional Excretion

The Fractional Excretion (FE) reflects the kidney's ability to maintain homeostasis.

Normally, the FE of any dietary element absorbed by the intestine and excreted primarily by the kidney, should increase if the dietary intake increases. Likewise, if GFR is decreased, FE will be increased; intake is constant, so less is required to maintain homeostasis. This involves filtration, reabsorption, and secretion.

FE is affected by dietary intake, serum concentrations, hormones, and concentrations of other ions (Na^+ , Cl^-)

Calculation:

$$\text{FE} = \frac{(\text{urine electrolyte} \times (\text{plasma creatinine}) \times 100}{(\text{plasma electrolyte}) \times (\text{urine creatinine})}$$

Measurements are done on urine and plasma collected at the same time.

In large animals, FE of Na^+ is used rather than the water deprivation test to identify tubular disease/dysfunction.

Na^+ excretion $> 1\%$ is suggestive of primary tubular disease. (HOWEVER, if the animal is off feed/water, FE of Na^+ can increase without tubular disease.)

Na^+ excretion of $< 1\%$ is usually equated with prerenal azotemia or acute glomerulonephritis.

HOWEVER, it is possible to have reduced FE of Na^+ excretion ($< 1\%$) with acute renal failure in the presence of sepsis, liver cirrhosis and Na^+ retention condition. Healthy horses can have FE of Na^+ of 0.01 - 0.91%. Horses with prerenal azotemia can have FE of Na^+ 0.5 - 1.0%. Some authors suggest that because of overlap in FE of Na^+ values between normal, prerenal, and renal azotemia, diagnosis of renal disease should NOT be made unless FE Na^+ is $> 3\%$.

Cl^- clearance correlates well with Na^+ , so is not normally determined.

FE of K^+ :

The equine kidney is the major route of K^+ excretion due to the herbivore diet being high in K^+ . The kidney adjusts to intake change very slowly – normal plasma K^+ levels are maintained in the face of tissue/body deficits. In horses, FE of K^+ is strongly correlated with GFR and creatinine clearance. Therefore, in renal disease with normal K^+ , a very low FE of K^+ may indicate a need for K^+ supplementation.

FE of Ca^{++} and FE of Pi^* are difficult to use in horses due to the normally high secretion and crystallization in urine.

Ruminants:

FE of Na^+ is frequently used to differentiate prerenal and renal azotemia.

FE of Na^+ and FE of Cl^- are closely related.

Dietary intake affects FE, therefore it is suggested that FE be run on several normal cows in the herd at the same time as the affected animal.

Dietary intake affects the FE of several electrolytes. In herds with a high Na^+ intake, FE of Na^+ has been up to 1.97%.

High grain diet \rightarrow High Pi, low Ca^{++} .

High fat diet \rightarrow Low Pi, high Ca^{++} .

Seasonal diets \rightarrow affect FE of Na^+ .

- On all grass diet \rightarrow FE of Na^+ increased in summer.
- On all grass diet \rightarrow FE of K^+ increased in summer.
- On all hay diet \rightarrow FE of Cl^- decreased in spring.

*Pi = Inorganic phosphorous

Thyroid Tests

Thyroid Disease in Dogs and Cats

Sample required: 1 mL red top tube. Do not use serum separator tubes (SST).

Hypothyroidism in Dogs

Hypothyroidism is the most common endocrine disorder in dogs. Most hypothyroid dogs have primary disease (originating within the thyroid gland) resulting in a low baseline thyroxine (T4) and free T4 concentration and high endogenous TSH concentration. Secondary hypothyroidism occurs in less than 5% of hypothyroid dogs and results from impaired production of TSH by the anterior pituitary gland. Dogs with secondary hypothyroidism have a low baseline T4 concentration and low endogenous TSH concentration. Diagnosis of hypothyroidism using baseline T4 concentration alone is often not possible due to suppression of serum T4 concentration when nonthyroidal illness is present (euthyroid sick syndrome). However, a low baseline T4 concentration in a dog that is not clinically ill and has findings consistent with hypothyroidism may be sufficient evidence to diagnose and treat hypothyroidism.

Hyperthyroidism in Cats

Hyperthyroidism is the most common endocrine disorder in older cats and is usually due to the presence of nodular hyperplasia or adenoma(s) of the thyroid gland. The diagnosis is usually easily confirmed based on a high baseline T4 concentration. Under certain circumstances, baseline T4 concentration may be within the RI in cats suspected to have hyperthyroidism. Possible explanations are: concurrent nonthyroidal illness; episodic T4 secretion; or other factors. Under these circumstances, additional diagnostic options are:

- free T4 (expected to be high)
- repeat T4 in a few weeks (expected to be high)
- T3 suppression test (expected to be inadequate suppression; described below)
- endogenous TSH (expected to be low/undetectable using canine TSH assay; see below)

Reference Intervals

<u>Test</u>	<u>Canine</u>	<u>Feline</u>
Baseline T4	12-40 nmol/L	13-50 nmol/L
Baseline T3	<0.6-1.2 nmol/L	<0.6-1.1 nmol/L
Endogenous TSH	0.03-0.58 ng/mL	{<0.03-0.15 ng/mL ¹ <0.03-0.3 ng/mL ² }

(Note that while RI for baseline T3 are provided, T3 is seldom measured in dogs or cats as it is currently considered to be of limited diagnostic value.)

T3 Suppression Test for Cats with Suspected Hyperthyroidism

Procedure:

1. Collect 1 mL blood (red top tube). Label “pre”. Freeze plasma or serum if submission is delayed.
2. Administer oral T3 (liothyronine) 25 µg 3 x/day for 2 days. On the 3rd morning, give 25 µg (1 tablet).
3. Collect 1 mL blood (red top tube) 2 - 4 hrs after last tablet. Label “post”.
4. Request T3 and T4 for both pre and post samples.

<u>Results:</u> (all units are nmol/L)	<u>Pre-T3</u>	<u>Pre-T4</u>	<u>Post-T3</u>	<u>Post-T4</u>
Normal	<0.6-1.1	13-50	>1.1	<20
Hyperthyroid	<0.6-1.1	13-50	>1.1	>20

(T3 is measured to ensure that liothyronine successfully administered; Post-T4 concentration of 20 nmol/L is nondiagnostic.)

Endogenous TSH to Help Confirm Hypothyroidism and Suspected Hyperthyroidism in Cats

Although nonthyroidal illness and treatment for hyperthyroidism most commonly explain low T4 concentration in cats, naturally-occurring hypothyroidism does occur, but is rare. Adult-onset and congenital hypothyroidism have been documented in cats. Both forms are generally primary (due to thyroid pathology), with congenital hypothyroidism being reported more frequently than adult-onset. Congenital hypothyroidism results in decreased growth rate and disproportionate dwarfism. Serum T4 concentration is low and endogenous TSH concentration is high in these patients. The canine TSH assay (chemiluminescent enzyme immunoassay - Immulite) has been used to measure TSH concentration in cats.^{1,2}

In addition to aiding in the diagnosis of primary hypothyroidism in cats, the canine TSH assay may be useful when feline hyperthyroidism is suspected, but cannot be confirmed by baseline T4 concentration. In this case, T4 concentration is often high normal in a patient with early hyperthyroidism or concurrent illness, with or without overt clinical signs of hyperthyroidism. Endogenous TSH concentration is expected to be below the RI/undetectable in this situation; however, a low proportion of euthyroid cats may also have undetectable TSH concentrations. This underscores the need for a feline-specific endogenous TSH assay capable of distinguishing low normal to below RI TSH concentration in cats. Also endogenous TSH should not be evaluated in isolation without the aid of additional tests of thyroid function (e.g. T4, free T4, T3 suppression).

Hypothyroid Therapy Monitoring in Dogs³

PLEASE indicate in history if dog is receiving medication, dosage schedule, and time of sample collection relative to last treatment. However, the ideal time to obtain a sample for monitoring purposes is 4-6 hr post-pill, regardless of the treatment regime.

T4:

Peak T4 concentration (4-6 hrs post-pill) should be in the upper half of or just above the RI (i.e. 39-77 nmol/L). Also, all serum T4 concentrations should be >19 nmol/L regardless of time interval between pill administration and sampling. A dosage decrease should be considered whenever T4 concentration is >77 nmol/L and/or when clinical signs of hyperthyroidism are present.

TSH:

Measurement of TSH concentration may be useful in dogs in which the TSH concentration is above the RI at the time of diagnosis. TSH concentration when measured 4-6 hrs after adequate thyroid hormone supplementation, should be within the RI.

¹Wakeling. Use of thyroid stimulating hormone (TSH) in cats. CVJ 2010;Vol.51:33-34.

²Peterson, Guterl, Nichols, Rishniw. Evaluation of serum thyroid-stimulating hormone concentration as a diagnostic test for hyperthyroidism in cats. J Vet Intern Med 2015;29:1327-1334.

(Note: variations in TSH RI^{1,2} may relate to normal healthy cat populations used by the different researchers.)

³Feldman, Nelson, Reusch, Scott-Moncrieff. Canine and Feline Endocrinology, 4th ed. Elsevier, 2015.

GI Function Tests

Trypsin-Like Immunoreactivity (TLI)

Background:

Radioimmunoassay of TLI in a single fasting serum sample is a sensitive and specific test for the identification of exocrine pancreatic insufficiency in dogs and cats.

Submission:

An overnight fast is preferable; at a minimum, 3 hr after eating as animals with EPI may exhibit a slight transient rise in serum TLI in response to a meal.

3 mL red top tube. Absolute minimum is 1 mL, but does not allow for repeats. Freezing/chilling not required.

TLI results are reported as ng/ml = µg/L.

Results:

Canine	5.0 - 35 µg/L	Reference Interval
	<2.0	Diagnostic for EPI.
	2.0 - 3.5	Sometimes associated with clinical signs of EPI. Repeat in 1 mo using 12-18 hr fast.
	3.5 - 5.0	RARELY associated with signs of EPI. May reflect subclinical pancreatic disease (e.g. chronic pancreatitis).
Feline	12 - 82 µg/L	Reference Interval
	≤8.0	Diagnostic for EPI.
	8.0 – 12.0	Equivocal. Repeat in 1 mo.
	>100	Acute pancreatitis OR decreased excretion due to impaired renal perfusion/function.

Fecal Alpha-1 Protease Inhibitor

- Test for protein-losing enteropathy - dogs only.
- 1 g each of 3 fecal specimens from 3 different bowel movements.
- Submit in special pre-weighed tubes only (tubes must be ordered from GI lab prior to sampling).
- Freeze each sample as it is collected.
- Alpha1-Protease Inhibitor is not heat stable and cooling in transit is required if samples are shipped via overnight carrier.

Cobalamin (Vitamin B12)

(Pancreatic function must be assessed before these results can be interpreted as they may be abnormal in EPI.)

Cobalamin: Absorbed in distal small intestine (last 25% only).

Reference Intervals: Canine 150 - 700 pmol/L
 Feline 600 – 1800 pmol/L

Concentrations below the reference interval are seen in patients with EPI, bacterial overgrowth in the upper small intestine, or disease affecting the distal small intestine. There is no known significance of values exceeding the control range.

Folate

Reference Intervals: Canine 7.0 - 39.0 nmol/L
 Feline 27.0 – 46.0 nmol/L

Concentrations above the reference interval are consistent with EPI or bacterial overgrowth in the upper small intestine. Values below the reference interval are consistent with disease affecting the proximal small intestine. (Absorbed in proximal intestine only.)

Note: Dietary deficiency of Cobalamin and Folate is highly improbable and even starvation of several weeks does not cause serum Cobalamin and Folate to become subnormal in dogs. Subnormal serum concentrations, therefore, reflect a state of chronic malabsorption; however, not all intestinal diseases causing malabsorption of vitamins are sufficiently severe or long-standing to deplete the body stores of each vitamin. Therefore, not all dogs and cats with intestinal diseases have abnormal results. Conversely, not all dogs with intestinal disease have intestinal morphological abnormalities.

Canine samples are sent to IDEXX Veterinary Laboratory Services, Markham, Ontario 1-800-667-3411, However, check with your diagnostic laboratory to determine the most convenient referral laboratory for your geographic location.

Feline samples are sent to Dr. David Williams, GI Lab, College of Veterinary Medicine, College Station, Texas.

Phone: 979 862-2861
Fax: 979 862-2864
Email: gilab@CVM.tamu.edu
www.cvm.tamu.edu/gilab

Pancreatic Lipase Immunoreactivity (PLI)

Background: PLI is a highly sensitive and specific test for pancreatitis in **dogs** and **cats**. This test is a species- specific immunoassay that measures the concentration of the pancreatic lipase protein.

Submission: 0.5 ml (dogs); 1.0 ml (cats) serum (red top tube); 12 hour fasting sample. Ship on ice.

Interpretation: Interpretation will be provided by the laboratory to which the sample is submitted.

Urine Chemistry

Urine GGT to Creatinine Ratio

Detects renal tubular damage due to drug (antibiotic) toxicity in horses and dogs. Require: 1 ml urine in a urine tube or red top vacutainer.

Reference Intervals:

Foals: 28.22 +/- .27

Urine Protein to Creatinine Ratio (UPC)

Purpose:

The UPC on a random urine sample provides a quantitative assessment of proteinuria by evaluating urine protein relative to its creatinine concentration. Prerenal and postrenal causes of proteinuria should be ruled out (by thorough assessment of clinical, historical, and physical findings; and completion of a minimum data base, defined as: CBC, biochemical panel, urinalysis – including urine sediment examination) before proceeding with the UPC to be assured that protein loss is renal in origin (i.e. due to renal pathology). The degree of proteinuria facilitates determination of the source of protein loss with the highest UPCs being associated with primary glomerular disease.

Indications:

1. Determine significance of a positive protein reaction from a urine reagent test strip
2. Detect primary glomerular disease (glomerulonephritis or amyloidosis)
3. Detect early chronic kidney disease (CKD) – proteinuria may be present prior to azotemia
4. Serve as prognostic indicator in CKD
5. Substage CKD according to International Renal Interest Society (IRIS) guidelines
6. Determine the need for action (e.g. monitoring, therapeutic intervention, diagnostic evaluation) in CKD proteinuric patients and non-CKD, non-azotemic, proteinuric patients

Guidelines for Interpreting Renal Proteinuria in CKD and Non-CKD Cats and Dogs:

UPC (CKD staging)		Interpretation	UPC (non-CKD patients)
Cats	Dogs		Cats and Dogs
<0.2	<0.2	Not proteinuric	<0.5
0.2 - 0.4	0.2 - 0.5*	Equivocal proteinuria	0.5 - 1.0*
>0.4 - <2.0**	>0.5 - <2.0**	Proteinuric (tubulointerstitial or early glomerular)	>1.0 - <2.0***
≥2.0	≥2.0	Proteinuric (likely glomerular)	>2.0**

* Monitoring recommended

** Therapeutic intervention recommended for azotemic CKD patients and for non-CKD patients

*** Diagnostic evaluation warranted

- IRIS provides information regarding substaging of CKD according to proteinuria on their website and this information is updated regularly.
- The UPC should be measured in all CKD cases, provided there is no evidence of prerenal or postrenal proteinuria.
- Patients (CKD and non-CKD) with equivocal proteinuria should be re-evaluated within 2 months and re-classified accordingly.
- Persistent proteinuria in CKD patients is based on UPC testing on 3 or more occasions, 2 or more weeks apart.

Persistent proteinuria in CKD patients (UPC >0.4 in cats and >0.5 in dogs) is consistent with tubulointerstitial or early glomerular disease. A UPC of ≥ 2.0 is strongly suggestive of glomerular disease in both CKD and non-CKD patients.

Method:

1. Collect a random urine sample (cystocentesis or midstream catch).
2. A complete urinalysis must be done prior to a UPC. The UPC can only be interpreted with confidence when there is no evidence of urinary tract inflammation, infection, and/or hemorrhage.

References:

1. Lees et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM forum consensus statement (Small Animal). J Vet Intern Med, 2005;19:377-385.
2. Harley and Langston. Proteinuria in dogs and cats. CVJ, 2012;53:631-638.
3. IRIS (International Renal Interest Society) – <http://www.iris-kidney.com/>

Urolith (Calculus) Analysis

X-ray crystallography method.
Provides a quantitative analysis of mineral composition.

Analyzed at the University of Guelph.

Submission:

Complete Urolith Analysis Request (see following page) as well as a laboratory requisition.

Submit urolith to laboratory.

Routine testing involves a 3-4 week turn-around time.
There is no charge for the analysis of canine and feline samples; however, shipping/handling charges normally apply.

Urolith Submission Form (next page)

This \$50.00 urolith analysis provided
compliments of:



FOR OFFICE USE ONLY
LSD Lab No. _____
Resubmission on this animal? Yes
Previous no. _____

Mail or Courier to:

Canadian Veterinary Urolith Centre
University of Guelph, Laboratory Services Division,
95 Stone Rd. W.,
Guelph, ON N1H 8J7 Phone: 519-823-1268 ext. 57234 or 57454 Fax: 519-767-6240 EMAIL: crucc@uoguelph.ca

NOTE: SUBMITTING CLINICS ARE RESPONSIBLE FOR SHIPPING CHARGES.

PLEASE SUBMIT DRY UROLITHS IN A CLEAN PLASTIC VIAL (Urethral plugs: 1/2 in urine, 1/2 in clean plastic vial)

Clinic Name	Owner
Address City	Address
Province Postal Code	City
Veterinarian	Province Postal code
Phone () Fax ()	Phone () Fax ()
Email	Email

ANIMAL INFORMATION: PLEASE FILL IN THE BLANK OR CHECK WHERE APPROPRIATE:

Specimen submitted: calculus urethral plug sediment Animal's name: _____
 Source of calculus: (check all applicable) renal pelvis ureter bladder urethra other _____
 Species: canine feline Breed: _____ Sex: male female neuter/spay
 Age: _____ mo yr Body condition: thin normal overweight Weight: _____ kg
 Environment of animal: outdoor indoor both Did the owner move or board this animal in the last 3 months? yes no
 Duration of problem: _____ wk mo # of OTHER animals on premises: cats _____ dogs _____ other: _____

LIST CLINICAL PROBLEMS:

1. _____	2. _____
----------	----------

RELEVANT HISTORY:

Was a urine sample obtained? Yes No If yes: AM or PM
 Approximate hours since last meal: 0-2 2-6 6-10 10-14 14+
 Was the urine obtained: free flow catheter cystocentesis
 Were crystals present? yes no
 Specify type(s): struvite calcium oxalate phosphate urate other _____
 Were any of the following seen in the urine? WBCs RBCs bacteria
 What was the urine pH? _____
 What was the urine specific gravity? 1.0.....
 Was the urine cultured? yes no If yes, was it sterile? yes no isolates: _____

Were antibiotics given? yes no
 Was blood taken? yes no If yes, was there hypercalcemia? yes no

DIET **DRY** **CANNED**
 What diet was fed prior to urolith diagnosis?
 How long has this diet been fed? _____ mo. yr _____ mo. yr
 Amount fed _____ % _____ %
 Feeding ad lib meal fed ad lib meal fed
 If meal fed, #of meals? _____
 Were treats fed? yes no Were table foods fed? yes no
 At the time of urolith diagnosis, was the animal receiving: steroids vitamin C
 Type of cat litter: clumping clay recycled newspaper NoSorb other
 Length of time on this type of litter < 3 months > 3 months
 Does the cat also urinate outside? yes no If yes: in the Summer Spring Fall Winter

FOR PREVIOUS UROLITHS ONLY:

Previous uroliths? yes no unknown If yes, date of detection _____
 Composition: struvite calcium oxalate phosphate urate other _____
 Source: (check all applicable) renal pelvis ureter bladder urethra other _____
 Date previous urolith voided or removed: _____

Thank you for completing this questionnaire. The information provided will be used for ongoing research into urolith prevention.

Preferred language of correspondence: English French

Cytology

CSE Analysis

(NOTE: A degree of variability exists in the literature with respect to reference intervals for CSF cytology. Information provided under "Normal" below, was updated Jan/09 based on recent literature. Differentials may be species-related.)

1) Normal

- Nucleated cells: $< 5 \times 10^6/L$
- Protein:

	<u>Any site:</u>	<u>Cervical:</u>	<u>Lumbar:</u>
Canine		$<0.25 \text{ g/L}$	$<0.45 \text{ g/L}$
Feline		$<0.25 \text{ g/L}$	$<0.45 \text{ g/L}$
Equine	$<1.05 \text{ g/L}$		
Bovine	$<0.663 \text{ g/L}$		
Llama	$<0.668 \text{ g/L}$		
Ferret	$<0.68 \text{ g/L}$		
- Differential: Mostly mononuclear -> mature lymphocytes monocytes (cytocentrifuged for concentration)

2) Mild Mononuclear Inflammation

- Nucleated cells: $<30 \times 10^6/L$
- Protein: $>0.25 \text{ g/L}$
- Differential: Mostly mononuclear
- Causes: Tumors, mild fungal infection, non-neoplastic degenerative disease (disc herniation, degenerative myelopathy, discospondylitis): contrast medium -> post myelogram sampling

3) Inflammatory

- Nucleated cells: May be $>100 \times 10^6/L$ (neutrophils - bacterial encephalitis and meningitis), but usually $<50 \times 10^6/L$ (mononuclear - viral encephalitis and meningitis, Listeriosis)
- Protein: $>0.25 \text{ g/L}$
- Mycotic, protozoal agents, GME -> mixed inflammatory reaction
- Parasitic disease, Cryptococcosis, Protothecosis -> eosinophils

4) Hemorrhage

- Color: yellow -> red -> brown; may clear on centrifugation
- Intact RBCs
- Protein increased
- RBCs increased, erythrophagocytosis (if $> 2-3 \text{ hrs}$)

5) Contamination (blood)

- RBCs increased, platelets

Fluid Effusions (Equine Peritoneal Fluid)

(NOTE: A degree of variability exists in the literature with respect to reference values for equine peritoneal fluid cytology)

1) Normal to Modified Transudate

- Nucleated cells: $0.5 - 9.0 \times 10^9/L$ (usually $\leq 4.0 \times 10^9/L$)
- Protein: (usually) $\leq 15 \text{ g/L}$
- Differential: approximately 50% neutrophils, 50% mononuclears

2) Non-Septic Exudate

- Nucleated cells: $> 10 \times 10^9/L$
- Protein: $> 23 \text{ g/L}$
- Differential: Neutrophils $>$ macrophages

3) Septic Exudate

- Nucleated cells: $> 10 \times 10^9/L$
- Protein: $> 34 \text{ g/L}$
- Differential: Degenerate neutrophils, bacteria

4) Hemorrhage (see next section #5)

Fluid Effusions (Thoracic, Peritoneal [Except Horses], Pericardial)

(NOTE: A degree of variability exists in the literature with respect to reference values for fluid effusions.)

- 1) Transudate
 - Nucleated cells: $\leq 1.5 \times 10^9/L$ (low cellularity)
 - Protein: ≤ 25 g/L
 - Differential: - Macrophages, mesothelial cells, non-degenerativeneutrophils
 - Causes:
 - Increased capillary hydrostatic pressure
 - Decreased plasma oncotic pressure - albumin <10 g/L
 - Lymphatic obstruction

- 2) Modified Transudate (any fluid between a transudate and an exudate)
 - Nucleated cells: $\leq 7 \times 10^9/L$ **and** Protein > 25 g/L
or
 - Nucleated cells: $> 1.5 \times 10^9/L$ **and** Protein ≤ 30 g/L
or
 - Nucleated cells: $> 1.5 - 7 \times 10^9/L$ **and** Protein 25 - 30 g/L
 - Differential: - Macrophages, mesothelial cells, non-degenerativeneutrophils
 - Examples:
 - Cardiomyopathy
 - Congestive heart failure
 - Chylous effusions
 - Neoplasm (lymphosarcoma, carcinoma)

- 3) Exudate
 - Nucleated cells: $> 7.0 \times 10^9/L$ (high cellularity)
 - Protein: >30 g/L
 - Differential:
 - Non-septic: Non-degenerate neutrophils, macrophages
 - Septic: Degenerate neutrophils, macrophages (may see bacteria in neutrophils as well as extracellularly)
 - Causes of non-septic exudates:
 - Chronic chylothorax or bile leakage (if no bacterial infection present in biliary tract - especially gall bladder)
 - Longstanding modified transudate
 - Neoplasm
 - Non-microbial irritants
 - Causes of septic exudates:
 - Nocardia (acid fast positive)
 - Actinomyces (acid fast negative)
 - Pleomorphic filamentous beaded rods (Gram positive)
 - Other bacteria

4) Hemorrhagic

- Protein: 35 g/L
- Recent hemorrhage:
 - intact RBCs, platelets (clumps), leukocytes, similar morphology and distribution as peripheral blood
- Longstanding or resolving hemorrhage:
 - hypersegmented neutrophils, absence of platelets, macrophages containing RBCs (erythrophagia) and hemosiderin

5) Chylous Effusion - Many Etiologies

- Milky appearance (presence of chylomicrons)
- Nucleated cells: $1.4 - 20 \times 10^9/L$
- Protein inaccurately elevated by refractometry due to triglycerides
- Acute - modified transudate
- Chronic - non-septic exudate

Note: Feline Infectious Peritonitis Virus produces a non-septic effusion which classically falls into the exudate range; however, often the nucleated cell count is not sufficiently high to “fit” in this category. Although the pathogenesis of fluid accumulation is inflammatory due to vasculitis, there may be a tremendous outpouring of high protein fluid which dilutes out the nucleated cell numbers. Therefore, the characteristics of FIP fluid are:

- > Nucleated cells: $0.2 - 20 \times 10^9/L$
- > Protein 40-90 g/L (average = 65), highly proteinaceous, stippled background
- > Differential: non-degenerate neutrophils, macrophages +/- small lymphocytes and plasma cells

Lymph Nodes

- 1) Normal
 - Predominantly (80%) small to medium lymphocytes, some ruptured cells.
 - +/- Lymphoblasts, plasma cells, macrophages, neutrophils
- 2) Reactive (immune-stimulated)
 - Mainly (>70%) small to medium lymphocytes
 - Large lymphocytes and blasts increased
 - Frequent plasma cells - (eccentric nuclei, clear Golgi zone)
 - Few mitotic figures
- 3) Lymphadenitis
 - Predominance of inflammatory cells: neutrophils, macrophages, eosinophils
 - +/- Organisms, degenerate or non-degenerate neutrophils
 - +/- Necrosis (karyolysis) and hemorrhage
- 4) Lymphosarcoma
 - Predominance of lymphoblasts - monomorphic, uniform population, large nuclei, dispersed chromatin, prominent nucleoli, basophilic cytoplasm
 - Increased mitotic figures
 - Decreased proportion of small/medium lymphocytes (<40%)
 - Abnormal nuclear contours
 - Increased free cytoplasmic fragments
- 5) Metastatic Neoplasia
 - Presence of cells not normally found in lymph nodes
 - Common tumors: Carcinomas, mast cell tumors, malignant melanomas

Synovial Fluid

- 1) Normal
 - Nucleated cells:

Dog	$< 3 \times 10^9/L$
Horse	$< 0.5 \times 10^9/L$
Cow	$< 1 \times 10^9/L$
 - RBC: rare
 - Differential: Mononuclears $> 90\%$ ($< 10\%$ neutrophils)
- 2) Degenerative Joint Disease
 - Nucleated cells: $< 5 \times 10^9/L$
 - Viscosity normal to slightly decreased
 - Increased quantity of fluid
 - Differential: Mononuclears $> 60\%$, increased macrophages, synovial lining cells (may be in strands or clumps)
 - Causes: Degenerative disease, osteoarthritis, trauma (blood components)
- 3) Non-Septic Inflammation - Immune Mediated
 - Nucleated cells: $5 - 50 \times 10^9/L$
 - +/- Reduced viscosity
 - Differential: Non-degenerate neutrophils $> 40\%$, +/- LE cells
 - Causes: Rheumatoid arthritis, lupus erythematosus, chronic infectious diseases
- 4) Septic Inflammation
 - Nucleated cells: $25 - 250 \times 10^9/L$ (higher with bacterial infections)
 - Viscosity -> reduced
 - Mucin clot -> poor quality
 - Differential: Degenerate or non-degenerate neutrophils (75 - 90%)
- 5) Hemorrhage
 - Many RBCs; differential similar to that of peripheral blood

HEMATOLOGY - GENERIC REFERENCE INTERVALS

PARAMETER	UNITS	CANINE	FELINE	BOVINE	EQUINE	PORCINE	OVINE
WBC	x 10 ⁹ /L	6.0 - 17.1	5.5 - 19.5	4.0 - 12.0	5.5 - 12.5	11.0 - 22.0	4.0 - 12.0
RBC	x 10 ¹² /L	5.5 - 8.5	5.0 - 10.0	5.0 - 10.0	6.5 - 12.5	5.0 - 8.0	8.0 - 16.0
Hemoglobin	g / L	120 - 180	80 - 150	80 - 150	110 - 190	100 - 160	80 - 160
HCT	L / L	0.37 - 0.55	0.24 - 0.45	0.24 - 0.46	0.32 - 0.52	0.32 - 0.50	0.24 - 0.50
MCV	fL	60 - 77	39 - 55	40 - 60	34 - 58	50 - 68	23 - 48
MCH	pg	19.5 - 24.5	13 - 17	11 - 17	12.5 - 20.5	16.6 - 22.0	9.0 - 12.0
MCHC	g / L	320 - 360	300 - 360	300 - 360	310 - 370	300 - 340	310 - 380
RDW	%	11.0 - 14.0	14.2 - 20.0	16.7 - 23.3	18.0 - 24.6		
Platelets	x 10 ⁹ /L	200 - 900	300 - 700	100 - 800	100 - 600	250 - 850	250 - 750

Differential

Segs	x 10 ⁹ /L	3.6 - 11.5	2.5 - 12.5	0.6 - 4.0	2.7 - 6.7	3.08 - 10.4	0.7 - 6.0
	%	60 - 70	35 - 75	15 - 45	30 - 65	28 - 47	10 - 50

Bands	x 10 ⁹ /L	0.0 - 0.3	0.0 - 0.3	0.0 - 0.12	0.0 - 0.1	0.0 - 0.88	rare
	%	0 - 3	0 - 3	0 - 2	0 - 2	0 - 4	rare

Eos	x 10 ⁹ /L	0.01 - 1.25	0.0 - 1.5	0.0 - 2.4	0.0 - 0.925	0.055 - 2.42	0.0 - 0.1
	%	2 - 10	2 - 12	2 - 20	0 - 4	0 - 11	0 - 10

Basos	x 10 ⁹ /L	rare	rare	0.0 - 0.2	0.0 - 0.17	0.0 - 0.44	0.0 - 0.3
	%	rare	rare	0 - 2	0 - 3	0 - 2	0 - 3

Lympho	x 10 ⁹ /L	1.0 - 4.8	1.5 - 7.0	2.5 - 7.5	1.5 - 5.5	4.29 - 13.6	2.0 - 9.0
	%	12 - 30	20 - 55	45 - 75	25 - 70	39 - 62	40 - 75

Monos	x 10 ⁹ /L	0.15 - 1.35	0.0 - 0.85	0.025 - 0.84	0.0 - 0.8	0.22 - 2.2	0.0 - 0.75
	%	3 - 10	1 - 4	2 - 7	0 - 7	2 - 10	0 - 6

HEMATOLOGY - GENERIC REFERENCE INTERVALS (continued)

PARAMETER	UNITS	CANINE	FELINE	BOVINE	EQUINE	PORCINE	OVINE
Plasma Protein	g/L	51 - 72	56 - 76	57 - 81	60 - 77	34 - 60	60 - 79
Plasma Fibrinogen				1 - 7	1 - 6	1 - 4	
Protein Fibrinogen Ratio	Cattle	{ < 10 10 - 15 > 15	Absolute hyperfibrinogenemia Dehydration, hyperfibrinogenemia or both Normal or dehydration			< 15 15 - 20 > 20	Horses
PT	Seconds	6.1 - 7.8*	8.4 - 9.6*	12.8 - 19.6**	7.0 - 11.0**	9.5 - 12**	
PTT	Seconds	8.0 - 11.5*	11.8 - 17.6*	14.9 - 38.9**	27.7 - 49.6**	NA	

FDP Titre	< 5 ug/mL all species		
FDP***	< 5	ug/mL	Normal
	> / = 5 < 20	ug/mL	Slightly increased, usually associated with hematomas, inflammatory diseases, renal failure, liver failure, vasculitis, thrombosis.
	> / = 20	ug/mL	Usually only associated with disseminated intravascular coagulation and fibrinolysis.

* = PDS Reference Intervals established using STAGO STA Satellite Coagulation Analyzer

** = PDS Reference Intervals established using MLA Electra 750A Coagulation Analyzer

*** = Values based on STAGO FDP Plasma Kit

Grading degrees of anemia according to hematocrit (Hct) in common domestic species.

	Canine	Feline	Equine	Bovine
Hct (L/L)	0.39 - 0.56	0.28 - 0.49	0.28 - 0.44	0.25 - 0.33
Mild	0.30 - 0.38	0.22 - 0.27	0.22 - 0.27	0.20 - 0.24
Moderate	0.20 - 0.29	0.15 - 0.21	0.15 - 0.21	0.14 - 0.19
Severe	< 0.20	< 0.15	< 0.15	< 0.14

Note: Approximations only as normal hematocrit for patient is usually not known.

Grading the regenerative response to anemia in dogs and cats.

Dog			Cat		
Degree of Regeneration	Reticulocytes %	Absolute Aggregate Reticulocytes (x10 ⁹ /L)	Degree of Regeneration	Reticulocytes %	Absolute Aggregate Reticulocytes (x10 ⁹ /L)
None	≤ 1	< 60	None	≤ 0.5	< 45
Mild	> 1 - 4	> 70 - 280	Mild	> 0.5 - 3	> 45 - 240
Moderate	> 4 - 10	> 280 - 700	Moderate	> 3 - 8	> 240 - 640
Marked	> 10	> 700	Marked	> 8	> 640

Note: Absolute aggregate reticulocytes calculated using an RBC count of 7x10¹²/L for dogs and 8x10¹²/L for cats.
Terminology used for degree of regeneration is subjective and varies according to the literature and other sources.

RPI - Reticulocyte Production Index - Dogs only

- 1 = Normal
- > 1 = Regeneration
- > 3 = Massive Regeneration (associated with hemolysis or hemorrhage).

CHEMISTRY - GENERIC REFERENCE INTERVALS

PARAMETER	UNITS	BOVINE	CANINE	EQUINE	FELINE	PORCINE	OVINE
Sodium	mmol/L	140 - 152	144 - 157	138 - 148	150 - 160	146 - 150	143 - 151
Potassium	mmol/L	3.6 - 5.4	3.6 - 6.0	3.2 - 5.0	4.0 - 5.8	4.7 - 7.1	4.2 - 5.8
Chloride	mmol/L	100 - 119	115-126	101 - 110	118 - 128	105 - 113	108 - 116
Bicarb	mmol/L	20 - 32	17 - 29	20 - 32	14 - 26	20 - 32	23-33
Anion Gap	mmol/L	14 - 26	14 - 26	10 - 25	13 - 26	10 - 25	12 - 24
Calcium	mmol/L	2.00 - 2.67	2.21 - 3.00	2.8 - 3.54	2.23 - 2.80	2.74 - 2.82	2.30 - 2.86
Phosphorus	mmol/L	1.08 - 2.76	0.82 - 1.87	0.85 - 1.45	1.03 - 1.92	1.30 - 3.55	1.01 - 2.44
Magnesium	mmol/L	0.60 - 1.28	0.70 - 1.16	0.60 - 1.20	0.74 - 1.12	0.81 - 1.25	0.95 - 1.26
Urea	mmol/L	< 7.5	3.0 - 10.5	3.5 - 7.0	5.0 - 11.0	3.0 - 8.5	6.9 - 14.0
Creatinine	µmol/L	67 - 175	60 - 140	110 - 170	90 - 180	90 - 240	60 - 105
Glucose	mmol/L	1.8 - 3.8	3.3 - 5.6	3.6 - 5.6	3.3 - 5.6	3.6 - 5.3	2.71 - 3.9
Cholesterol	mmol/L	-	2.5 - 5.5	1.2 - 4.6	1.5 - 4.0	-	-
Total Bilirubin	µmol/L	0 - 30	0 - 17	4 - 102	0 - 17	0 - 4	0 - 3
Direct Bilirubin	µmol/L	0 - 3	0 - 4	0 - 7	0 - 4	0 - 4	0 - 5
Alk Phos	U/L	50 - 375	12 - 106	95 - 250	10 - 35	110 - 340	37 - 229
CALP	U/L	-	0 - 16	-	-	-	-

CK	U/L	< 350	< 300	< 500	< 300	< 500	< 300
AST	U/L	46 - 118	20 - 50	197 - 429	20 - 50	25 - 57	48 - 156
ALT	U/L	-	5 - 69	10 - 23	13 - 55	34 - 58	-
GGT	U/L	< 31	< 8	< 25	< 5	< 25	< 76
SDH	U/L	4.0 - 48.0	0.0 - 25.0	0.0 - 15.0	0.0 - 12.0	0.0 - 8.5	< 50
Amylase	U/L	-	360 - 1100	-	490 - 1000	-	-
Lipase	U/L	-	180 - 460	-	0 - 122	-	-
Serum Protein	g/L	66 - 86	51 - 72	60 - 77	66 - 77	34 - 60	64 - 81
Albumin	g/L	28 - 45	29 - 38	31 - 43	22 - 38	26 - 45	32 - 42
Alb:Glob		0.66 - 1.30	0.60 - 1.50	0.60 - 1.50	0.6 - 1.50	0.60 - 1.50	0.54 - 1.22
Calculated Osmolality	mmol/Kg	274 - 306	280 - 320	280 - 320	280 - 320	280 - 320	280 - 307

Blood Gases

pH		7.35 - 7.50	7.31 - 7.42	7.20 - 7.55	7.24 - 7.40	7.30 - 7.50	7.32 - 7.50
pCO ₂ venous	mmHg	34 - 45	35 - 45	38 - 46	29 - 42	35 - 45	35 - 45
pO ₂ arterial	mmHg	80 - 110	80 - 110	80 - 110	80 - 110	80 - 110	80 - 110
HCO ₃	mmol / L	24 + 4	24 + 4	24 + 4	24 + 4	24 + 4	24 + 4
Base Excess		0 ± 3	0 ± 3	0 ± 3	0 ± 3	0 ± 3	0 ± 3

* pO₂

Venous - Jugular 65.0 mmHg Cephalic 58.0

* pCO₂

Arterial - Usually 5 - 6 mmHg lower than venous.

Free Calcium

UNITS EQUINE BOVINE CANINE FELINE PORCINE PIGLETS

Ca ++	mmol / L	1.31 - 1.79	1.08 - 1.32	1.27 - 1.51	1.27 - 1.47	1.30 - 1.50	1.23 - 1.47
pH		7.34 - 7.50	7.36 - 7.52	7.24 - 7.52	7.20 - 7.44	7.26 - 7.56	7.33 - 7.59
Ca ++	mmol / L at pH 7.40	1.32 - 1.80	1.10 - 1.34	1.28 - 1.48	1.22 - 1.40	1.30 - 1.50	1.29 - 1.47

CHEMISTRY-GENERIC REFERENCE INTERVALS

PARAMETER	UNITS	FOALS	FOALS	GOATS	LLAMA	MUSKOX
		< 24 HOURS (n = 16)	< 3 MONTHS (n=26)	(n=22)		
Sodium	mmol/L	132 - 148	133 - 143	141 - 157	152 - 162	137 - 147
Potassium	mmol/L	3.2 - 4.8	3.5 - 5.1	4.2 - 6.6	3.7 - 7.8	4.3 - 6.1
Chloride	mmol/L	92 - 106	93 - 103	102 - 116	110 - 128	95 - 113
Bicarb	mmol/L	25 - 33	22 - 38	21 - 31	14 - 36	14 - 32
Anion Gap	mmol/L	10 - 21	5 - 21	14 - 26	9 - 26	12 - 32
Calcium	mmol/L	2.56 - 3.16	2.76 - 3.44	2.20 - 2.72	2.09 - 2.78	2.24 - 2.82
Phosphorus	mmol/L	0.81 - 2.33	1.51 - 2.55	0.68 - 3.28	1.21 - 2.77	1.22 - 3.26
Magnesium	mmol/L	0.76 - 1.36	0.70 - 1.14	0.97 - 1.41	0.85 - 1.34	0.87 - 1.43
Urea	mmol/L	3.5 - 10.6	1.6 - 7.6	2.5 - 7.3	4.1 - 10.5	7.5 - 22.7
Creatinine	µmol/L	* 96 - 248	82 - 142	47 - 103	119 - 294	137 - 429
Glucose	mmol/L	4.1 - 14.1	5.2 - 11.2	2.3 - 4.7	2.8 - 6.0	2.9 - 5.5
Total Bilirubin	µmol/L	18 - 122	< 70	< 5	< 5	< 1
Direct Bilirubin	µmol/L				< 2	
Alk Phos	U/L	* 1551 - 4159	* 644 - 2366	99 - 761	29 - 139	< 1517
CK	U/L	< 1200	< 450	< 500	< 300	< 600
AST	U/L	12 - 308	69 - 357	55 - 157	110 - 220	40 - 108
GGT	U/L	< 70	< 50	17 - 77	< 74	43 - 135
TP	g/L	37 - 71	52 - 72	54 - 84	51 - 73	60 - 80
Albumin	g/L	24 - 34	22 - 34	28 - 40	see next page	27 - 41
Alb:Glob	-		0.47 - 1.31	0.57 - 1.25	see next page	0.62 - 1.32
Cholesterol	mmol/L				< 3.50	
SDH	U/L				0 - 32.0	
ALT	U/L				< 10.0	
Amylase	U/L				816 - 2932	
Lipase	U/L				< 129.0	

* - age-related values.

**CHEMISTRY – GENERIC REFERENCE
INTERVALS**

UNITS BOVINE CANINE EQUINE FELINE PORCINE OVINE LLAMA

Protein Electrophoresis

Albumin	g/L	24 - 36	24 - 40	29 - 38	26 - 44	19 - 24	28 - 34	32 - 44
Glob: alpha 1	g/L	7 - 12	2 - 4	7 - 13	13 - 18	9 - 12	2 - 6	0.4 - 2.0
Glob: alpha 2	g/L	-	4 - 9	7 - 13	-	-	3 - 7	2.4 - 5.2
Globulin: beta	g/L	6 - 12	13 - 17	4 - 12	10 - 15	8 - 11	3 - 7	6.8 - 16.4
Globulin: gamma	g/L	16 - 32	4 - 8	9 - 15	12 - 27	3 - 7	7 - 13	3.9 - 14.7
alb:glob								0.80 - 2.0

Osmolality
(measured)

mmol/kg	285 - 315	285 - 315	285 - 315	285 - 315	285 - 315	285 - 315
---------	-----------	-----------	-----------	-----------	-----------	-----------

Iron

µmol/L	over 14.5 all species					
--------	-----------------------	--	--	--	--	--

TIBC

µmol/L	44.8 - 62.7 all species					
--------	-------------------------	--	--	--	--	--

Cortisols

	CANINE			FELINE		
--	---------------	--	--	---------------	--	--

Baseline

nmol/L	<20 - 270			<320		
--------	-----------	--	--	------	--	--

Endo, TSH

ng/mL	0.03 - 0.58			<0.03-0.15 or <0.03-0.3 (see thyroid section)		
-------	-------------	--	--	---	--	--

T3

nmol/L	<0.6 - 1.2			<0.6 - 1.1		
--------	------------	--	--	------------	--	--

T4

nmol/L	12 - 40			13 - 50		
--------	---------	--	--	---------	--	--

T3 Suppression

nmol/L	-			T4 <20		
--------	---	--	--	--------	--	--

Ammonia

µmol/L	all species < 80					
--------	------------------	--	--	--	--	--

Bile Acids

	CANINE		FELINE		EQUINE	
Fasting	< 10		< 10		Random < 15.0	
Postprandial	< 20		< 20		Values may be higher when OFF feed	

µmol

REFERENCE INTERVALS - AVIAN HEMATOLOGY AND CHEMISTRY

PARAMETER	African Grey Parrot	Amazon Parrot	Blue-Headed Parrot	Budgerigar	Parakeet	Cockatiel	Cockatoo	Conure
WBC (x10 ⁹)	5 - 11	6 - 11	4 - 11	3 - 8	4.5 - 9.5	5 - 10	5 - 11	4 - 11
Differential								
Heterophils %	45 - 75	30 - 75	40 - 70	45 - 70	40 - 75	40 - 70	45 - 75	40 - 75
Lymphocytes %	20 - 50	20 - 65	20 - 50	20 - 45	20 - 60	25 - 55	20 - 50	20 - 50
Monocytes %	0 - 3	0 - 3	0 - 2	0 - 5	0 - 3	0 - 2	0 - 4	0 - 3
Eosinophils %	0 - 2	0 - 1	0 - 1	0 - 1	0 - 1	0 - 2	0 - 2	0 - 3
Basophils %	0 - 5	0 - 5	0 - 5	0 - 5	0 - 5	0 - 6	0 - 5	0 - 5
HCT	L / L 0.43 - 0.55	0.45 - 0.55	0.44 - 0.60	0.45 - 0.57	0.46 - 0.58	0.45 - 0.57	0.40 - 0.55	0.42 - 0.55
RBC	x 10 ¹² /L 2.4 - 4.5	2.5 - 4.5	2.4 - 4.1	2.5 - 4.5	-	2.5 - 4.7	2.2 - 4.5	2.5 - 4.5
Total Protein	g / L 30 - 50	30 - 50	26 - 50	25 - 45	25 - 45	22 - 50	25 - 50	25 - 45
Glucose	mmol / L 10.6 - 19.4	12.2 - 19.4	10.0 - 16.6	11.1 - 22.2	11.1 - 19.4	11.1 - 25.0	11.0 - 19.4	11.1 - 19.4
Calcium	mmol / L 2.0 - 3.24	2.00 - 3.24	2.5 - 3.74	-	-	2.12 - 3.24	2.00 - 3.24	2.00 - 3.74
ALT	U / L 100 - 350	130 - 350	150 - 350	150 - 350	150 - 400	100 - 350	150 - 350	125 - 350
LDH	U / L 150 - 450	160 - 240	200 - 550	150 - 450	150 - 450	125 - 450	225 - 650	125 - 420
Creatinine	μmol / L 9 - 35	9 - 35	9 - 26	9 - 35	9 - 35	9 - 35	9 - 35	9 - 44
Uric Acid	μmol / L 240 - 600	120 - 600	240 - 710	240 - 830	240 - 710	210 - 650	210 - 650	150 - 625
Potassium	mmol / L 2.6 - 4.2	3.0 - 4.5	3.0 - 4.5	-	-	2.5 - 4.5	2.5 - 4.5	3.4 - 5.0
Sodium	mmol / L 134 - 152	136 - 152	130 - 150	-	-	132 - 150	131 - 157	134 - 148
T4	nmol / L 4 - 26	1 - 13	3 - 14	32 - 57	3 - 31	9 - 31	10 - 57	3 - 12

REFERENCE INTERVALS - AVIAN HEMATOLOGY AND CHEMISTRY

PARAMETER		Domestic	Grand	Blue and			Phillipine		
		Duck	Electus Parrot	Finch	Lovebird	Gold Macaw	Mynah Bird	Blue-Parrot	Toucan
WBC (x10 ⁹)		4.5 - 13	6 - 11	3 - 8	3 - 8	6 - 13.5	6 - 11	4.5 - 11.5	4 - 10
Differential									
Heterophils %		30 - 70	40 - 75	20 - 65	40 - 75	45 - 70	25 - 65	35 - 70	35 - 65
Lymphocytes %		20 - 55	20 - 50	20 - 65	20 - 55	20 - 50	20 - 50	20 - 60	25 - 50
Monocytes %		0 - 3	0 - 2	0 - 1	0 - 2	0 - 3	0 - 3	0 - 5	0 - 4
Eosinophils %		0 - 4	0 - 1	0 - 1	0 - 1	0 - 2	0 - 3	0	0 - 4
Basophils %		0 - 5	0 - 5	0 - 5	0 - 6	0 - 5	0 - 7	0 - 5	0 - 5
HCT	L / L	0.30 - 0.43	0.45 - 0.55	0.45 - 0.62	0.44 - 0.57	0.45 - 0.55	0.44 - 0.55	0.45 - 0.55	0.45 - 0.60
RBC	x 10 ¹² /L	2.3 - 4.5	2.5 - 4.0	2.5 - 4.6	3.0 - 5.1	2.5 - 4.5	2.4 - 4.0	2.4 - 5.0	2.5 - 4.5
Total Protein	g / L	25 - 60	30 - 50	30 - 50	22 - 51	30 - 50	23 - 45	30 - 50	30 - 50
Glucose	mmol / L	8.3 - 16.6	9.9 - 20.0	11.1 - 25.0	11.1 - 22.2	11.1 - 19.4	10.6 - 19.4	10.6 - 19.4	12.2 - 19.4
Calcium	mmol / L	2.5 - 4.49	2.25 - 3.99	-	2.25 - 3.74	2.25 - 3.24	2.25 - 3.24	2.50 - 3.99	2.50 - 3.74
ALT	U / L	5 - 100	150 - 350	150 - 350	100 - 350	100 - 280	130 - 350	130 - 350	130 - 330
LDH	U / L	150 - 800	200 - 400	-	100 - 350	75 - 425	600 - 1000	130 - 425	200 - 400
Creatinine	µmol / L	9 - 44	9 - 35	-	9 - 35	9 - 44	9 - 53	9 - 35	9 - 35
Uric Acid	µmol / L	120 - 710	180 - 600	240 - 710	180 - 650	150 - 680	240 - 600	240 - 600	240 - 830
Potassium	mmol / L	3.0 - 4.5	-	-	2.5 - 3.5	2.5 - 4.5	3.0 - 5.1	-	-
Sodium	mmol / L	130 - 155	-	-	137 - 150	136 - 155	136 - 152	-	-
T4	nmol / L	10 - 43	6 - 13	-	3 - 24	13 - 52	6 - 12	4 - 13	6 - 41



Hematology Reference Intervals - ADVIA 2120i

TEST	Units	CANINE Guelph	FELINE Guelph	EQUINE Guelph	BOVINE Cornell
WBC	x 10 ⁹ /L	4.9 - 15.4	4.2 - 13.0	5.1 - 11.0	5.9 - 14.0
RBC	x 10 ¹² /L	5.8 - 8.5	6.2 - 10.6	6.9 - 10.7	5.0 - 7.2
Hgb	g/L	133 - 197	93 - 153	112 - 169	87 - 124
HCT (MCV x RBC)	L/L	0.39 - 0.56	0.28 - 0.49	0.28 - 0.44	0.25 - 0.33
MCV	fL	62 - 72	39 - 52	42 - 53	38 - 51
MCH (Hgb/RBC)	pg	21 - 25	13 - 17	14 - 18	14 - 19
MCHC (Hgb/HCT)	g/L	330 - 360	300 - 344	328 - 364	340 - 380
RDW	%	11 - 14	14 - 17	16 - 20	15.0 - 19.4
Total Solids	g/L	56 - 74	58 - 82	59 - 73	69 - 87
Fibrinogen	g/L	NA	NA	1 - 6	1 - 7
Segs - %	%	40 - 80	34 - 80	43 - 77	15 - 53
Segs - Absolute	x 10 ⁹ /L	3.0 - 10.0	2.1 - 15.0	1.78 - 8.02	0.95 - 3.8
Bands - %	%	0 - 1	0 - 2	0 - 0	0 - 1
Bands - Absolute	x 10 ⁹ /L	0.0 - 0.1	0.0 - 0.2	0.0 - 0.0	0.0 - 0.1
Lymphs - %	%	15 - 50	11 - 57	18 - 52	32 - 76
Lymphs - Absolute	x 10 ⁹ /L	1.2 - 5.0	1.0 - 6.9	1.4 - 4.08	1.9 - 8.7
Monos - %	%	2 - 10	1 - 6	0 - 4	2 - 9
Monos - Absolute	x 10 ⁹ /L	0.08 - 1.0	0.0 - 0.6	0.0 - 0.42	0.1 - 0.8
Eos - %	%	0 - 10	0 - 14	0 - 7	1 - 19
Eos - Absolute	x 10 ⁹ /L	0.0 - 1.1	0.1 - 1.5	0.0 - 0.66	0.0 - 1.8
Baso - %	%	rare	0 - 2	0 - 2	0 - 2
Baso - Absolute	x 10 ⁹ /L	rare	0.0 - 0.2	0.0 - 0.12	0.0 - 0.1
NRBC	/100 WBC				
Platelet	x 10 ⁹ /L	117-418	93-514	83-270	252-724
MPV	fL	7 - 14	8 - 21	6 - 11	5.7 - 8.0

- For WBC, RBC, Hgb, HCT, MCV, MCH, MCHC, MPV, Platelets (September 2019).
 - Canine, Feline, Equine - Guelph Reference Intervals adopted.
 - Bovine - Cornell Reference Intervals adopted.
- Total Protein, Fibrinogen – Developed by PDS (April 2002).
- Differential Reference Intervals for Canine, Feline and Bovine – Developed by PDS (April 2002).
- Differential Reference Intervals for Equines – Developed by PDS (December 2013).



Prairie Diagnostic Services – Clinical Pathology

Chemistry Reference Intervals November 30, 2012

TEST	UNITS	CANINE 1 - 6 yrs n=57	FELINE 1 - 9 yrs n=87	EQUINE 3 - 15 yrs n=19	BOVINE (DAIRY) 2 - 7 yrs n=108	BOVINE (BEEF) 2 - 7 yrs n=23	OVINE n=53
Sodium	mmol/L	140 - 153	147 - 160	132 - 142	138 - 148	135 - 143	137 - 152
Potassium	mmol/L	3.8 - 5.6	3.9-5.5	3.5 - 5.0	3.7 - 5.3	4.1 - 5.3	3.9 - 5.7
Na/K		28 - 38					
Chloride	mmol/L	105 - 120	111 - 125	92 - 103	91 - 104	91 - 104	97 - 111
Bicarb	mmol/L	15 - 25	11 - 22	26 - 35	17 - 33	18 - 24	17 - 29
Anion Gap	mmol/L	12 - 26	15 - 30	13 - 21	17 - 29	20 - 28	17 - 33
Calcium	mmol/L	1.91 - 3.03	2.26 - 2.86	2.39 - 3.80	2.21 - 2.61	2.26 - 2.74	2.43 - 3.23
Phosphorus	mmol/L	0.63 - 2.41	1.08 - 2.21	0.53 - 1.19	1.45 - 2.59	1.35 - 2.55	1.06 - 2.62
Magnesium	mmol/L	0.70 - 1.16	0.74 - 1.12	0.66 - 1.20	0.81 - 1.13	0.91 - 1.17	0.77 - 1.17
Urea	mmol/L	3.5 - 11.4	6.0 - 11.4	4.1 - 14.7	3.5 - 10.3	3.7 - 8.7	4.5 - 12.1
Creatinine	umol/L	41 - 121	78 - 178	52 - 126	49 - 95	30 - 126	55 - 107
Amylase	U/L	343 - 1375	400 - 1807				
Lipase	U/L	25 - 353	12 - 32				
Glucose	mmol/L	3.1 - 6.3	3.5 - 8.1	4.1 - 5.5	1.6 - 4.4	2.0 - 3.2	2.9 - 8.5
Cholesterol	mmol/L	2.70 - 5.94	1.62 - 4.32				
Triglycerides	mmol/L	0.07 - 1.35	0.26 - 1.07				
T. Bili	umol/L	1 - 4	0 - 3	2 - 41	1 - 5	1 - 3	1 - 11
D. Bili	umol/L	0 - 2	0 - 1	1 - 7	0 - 3		0 - 3
I. Bili	umol/L	0 - 2.5	0 - 1.5	3.9 - 32.8			
ALP	U/L	9 - 90	11 - 56				
GGT	U/L	0 - 8	0 - 6	8 - 33	12 - 39	4 - 26	9 - 61
ALT	U/L	19 - 59	22 - 90				
GLDH	U/L	0 - 7	1 - 5	0 - 5	7 - 36		
SDH	U/L	0 - 4	0 - 6	2 - 7	5 - 30	6 - 34	5 - 29
AST	U/L			6 - 347	42 - 131	62 - 150	62 - 260
CK	U/L	51 - 418	75 - 471	88 - 439	64 - 344	0 - 490	31 - 347
T. Protein	g/L	55 - 71	53 - 84	60 - 74	68 - 87	66 - 84	61 - 81
Albumin	g/L	32 - 42	28 - 43	27 - 36	32 - 38	31 - 37	33 - 39
Globulin	g/L	20 - 34	23 - 45	26 - 41	32 - 52		
A/G Ratio		1.06 - 1.82	0.77 - 1.64	0.80 - 1.30	0.55 - 1.19	0.64 - 1.08	0.70 - 1.38
Bile Acids	umol/L	0 - 10	0 - 10				
Fructosamine	umol/L	180 - 350	219 - 347				
NEFA	mmol/L				0.10 - 0.37		
BHB	mmol/L				0.32 - 1.30		

Canine, Feline, Equine Reference Intervals established using the COBAS C311 Chemistry Analyzer

Bovine & Ovine Reference Intervals established using the HITACHI 912 Chemistry Analyzer

Canine Fructosamine - Reference Intervals are from the IDEXX

Lipase, Feline Fructosamine, NEFA & BHB - Reference Intervals are from the Animal Health Lab in Guelph

Supersedes: None

APPENDIX

TABLES FOR SI CONVERSION

**TO CONVERT SI UNITS TO CONVENTIONAL UNITS, DIVIDE BY THE CONVERSION FACTOR.

**TO CONVERT CONVENTIONAL UNITS TO SI UNITS, MULTIPLY BY THE THE CONVERSION FACTOR

TEST	FACTOR	SI UNITS	CONVENTIONAL UNITS
Sodium	1	mmol/L	mEq/L
Potassium	1	mmol/L	mEq/L
Chloride	1	mmol/L	mEq/L
Carbon Dioxide	1	mmol/L	mEq/L
Calcium	0.2495	mmol/L	mg/dl
Phosphorus	0.3229	mmol/L	mg/dl
Magnesium	0.4114	mmol/L	mg/dl
Urea	0.357	mmol/L	mg/dl BUN
Creatinine	88.1	μmol/L	mg/dl
Glucose	0.05551	mmol/L	mg/dl
Cholesterol	0.02586	mmol/L	mg/dl
T and D Bili	17.1	μmol/L	mg/dl
T.Protein	10	g/L	g/dl
Albumin	10	g/L	g/dl
Osmolality	1	mmol/kg	mOsm/kg
Iron	0.1791	μmol/L	μg/dl
TIBC	0.1791	μmol/L	μg/dl
Cortisol	27.59	nmol/L	μg/dl
T3	0.01536	nmol/L	ng/dl
T4	12.87	nmol/L	μg/dl
Digoxin	1.281	nmol/L	ng/dl
Phenobarbital	43.06	μmol/L	mg/dl
Uric Acid	59.48	μmol/L	mg/dl
Hemoglobin	10	g/L	g/dl
PCV	0.010	L/L	%
RBC	1	$\times 10^{12}/L$	$10^6/mm^3$
MCV	1	fL	μm^3
MCH	1	pg	pg
MCHC	10	g/L	g/dl
Platelets	0.001	$\times 10^9/L$	mm^3
Fibrinogen	10	g/L	g/dl
WBC	0.001	$10^9/L$	mm^3

Consult laboratory for other conversion factors.