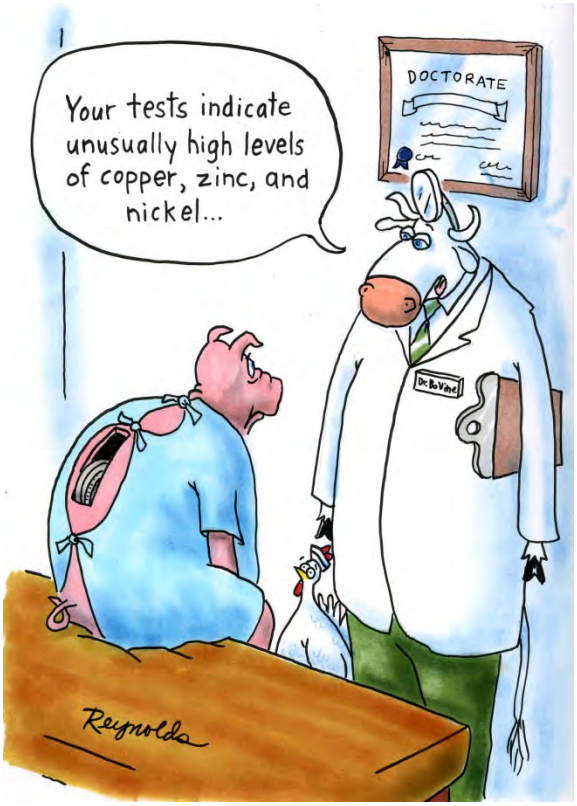
PART IV
PROTOCOL MANUAL

EVERYDAY GUIDE TO SAMPLE SUBMISSION
TEST PROTOCOLS
AND
REFERENCE VALUES



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Note: Some of the information in this protocol manual applies to Prairie Diagnostic Services Inc. (PDS) in particular, other information is general and relates to sample collection and test protocols.

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

Shipping of Samples

General Information on Sample Submission:

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 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 is 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) Take 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.

Note: If using Prairie Diagnostic Services Inc., refer to the most recent Test and Service Guide for additional sample submission information, fees, referred out testing, etc.

Prairie Diagnostic Services Inc.
52 Campus Drive
Saskatoon, SK S7N5B4
Phone: 306 966-7316
Fax: 306 966-2488
www.pdsinc.ca

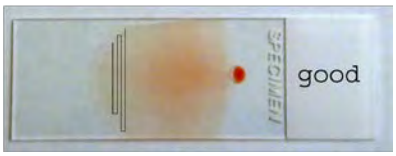
Shipping of Samples

1) 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).

PT/PTT/FDP

- Collect blood in blue top (sodium citrate) tubes.

- Appropriate volume is indicated on tubes 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; however, this is not necessary with PDS.

2) 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, we will either request more serum or do those tests that are prioritized by the submitter.

If hemolysis is >3+, you will be advised, as accuracy of some tests is compromised.

3) 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. Also, PDS technologists make cytospin smears of urine sediment if concerns are raised based on the wet mount examination. In this case, the clinician will be advised that a smear is available for cytological evaluation, if desired. There is an extra charge for urine cytology as it is not included in a routine U/A.

4) Cytology **Avoid contact with formalin; send 3-5 unstained slides, when possible.**

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 tissue 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 tissue 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 Smears

- Send air dried, unstained smears.

Bone Marrow Aspirates/Core (**See following pages for more detailed instructions**):

1) Sedate or anesthetize animal. Use aseptic technique.

2) If the animal is sedated only, inject local anesthetic deep into site as close as possible to periosteal surface of bone.

3) 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.

4) 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).

5) 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.)

6) Make smears using gentle "slide over slide" technique - no pressure on your part is required.

7) Air dry. Leave unstained.

8) Place any excess marrow into an EDTA tube.

9) 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

Materials Management, Veterinary Medical Centre, WCVM, will sell bone marrow biopsy needles to clinics. Call 966-7118 and provide an account for billing. Cost may vary. The bone marrow biopsy needle can also be sent by courier/bus to the clinic, at the clinic's expense. 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.

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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 adequacy 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

Figure 1

1. Shown here (left to right) are a Jamshidi $1\frac{7}{8}$-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 humerus 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

Bone marrow aspiration and core biopsy (cont'd)

Figure 2A

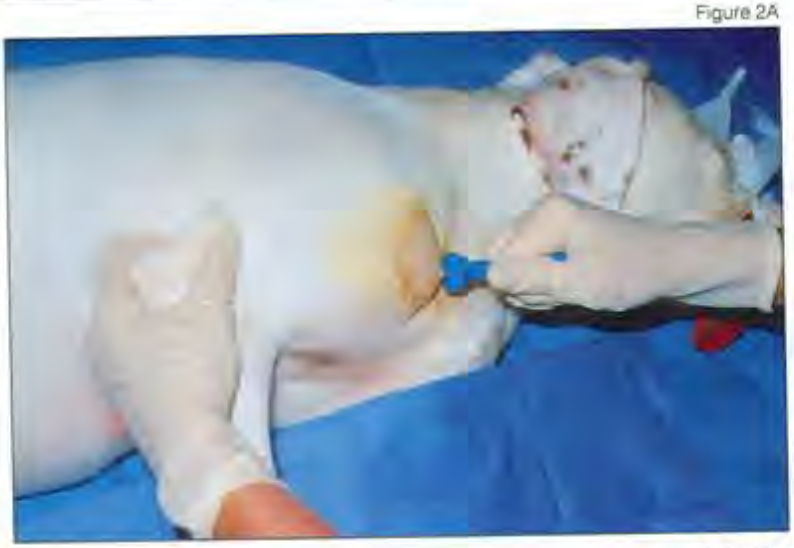
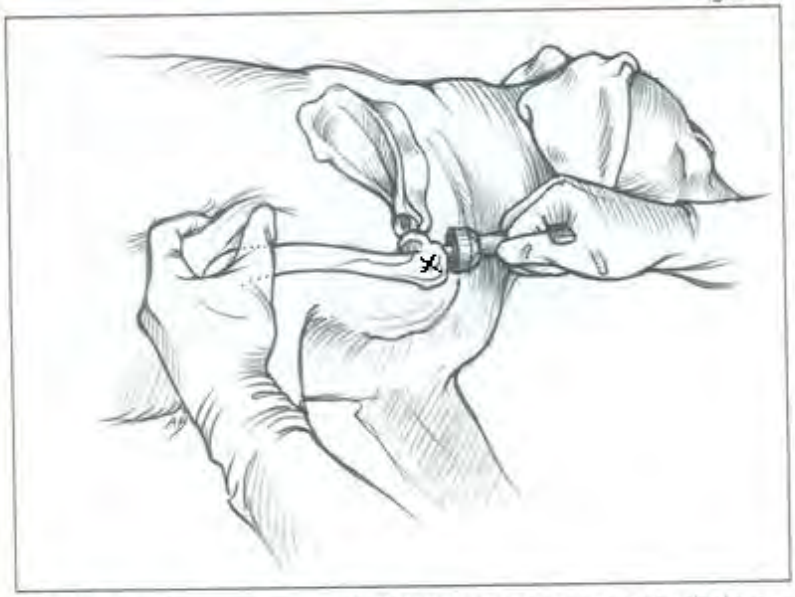


Figure 2B



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 scapulohumeral 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

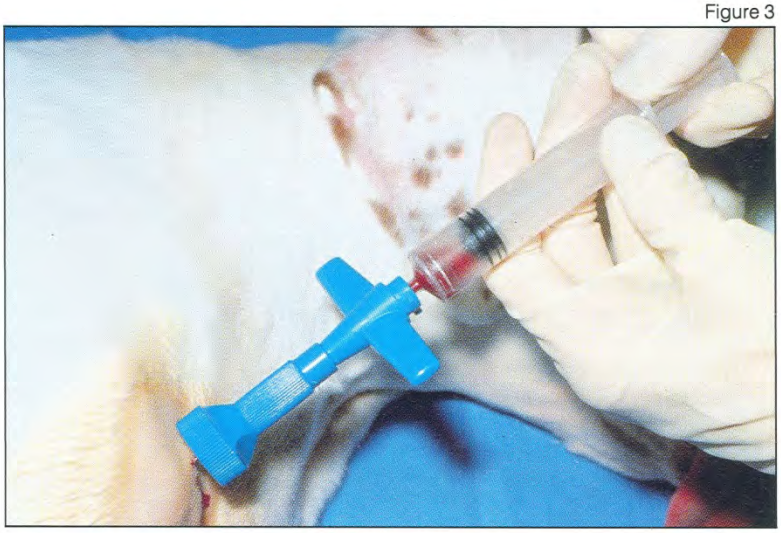
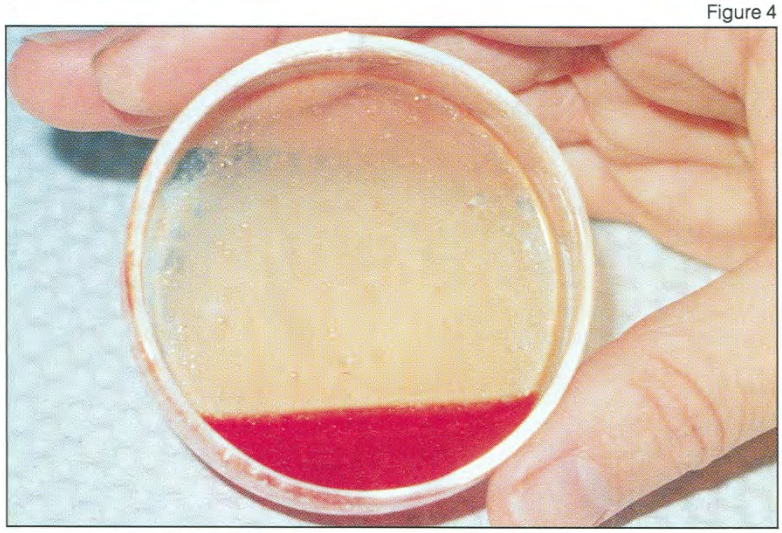


Figure 4



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 never recommend the vertical pull-apart method as suction tends to rupture cells.

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.

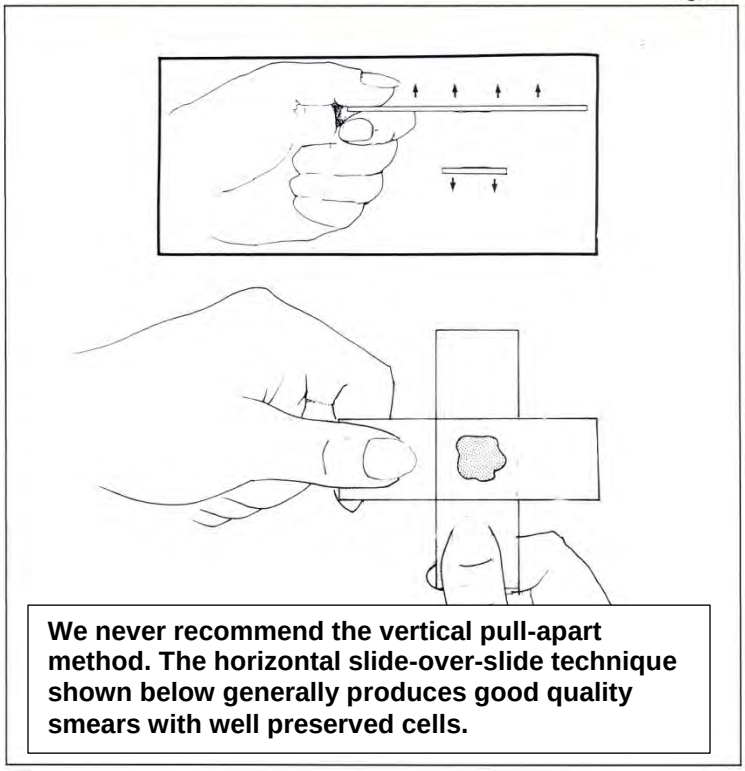
Aspirates should routinely be prepared using both the vertical and horizontal pull-apart techniques. Often the clinical signs and lab findings that prompt bone marrow examination cannot help pinpoint whether the disease is neoplastic or non-neoplastic.

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

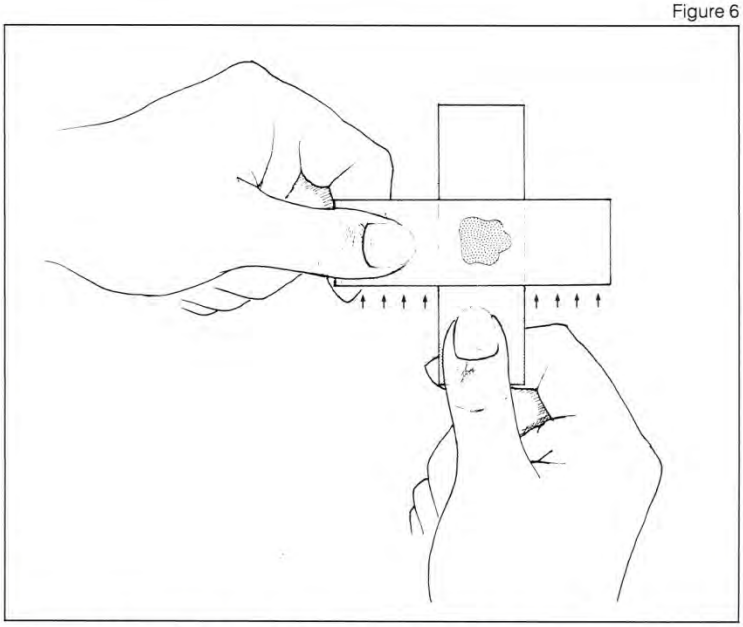
Bone marrow from the proximal fe-

Figure 5



We never recommend the vertical pull-apart method. The horizontal slide-over-slide technique shown below generally produces good quality smears with well preserved cells.

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.

Figure 7A

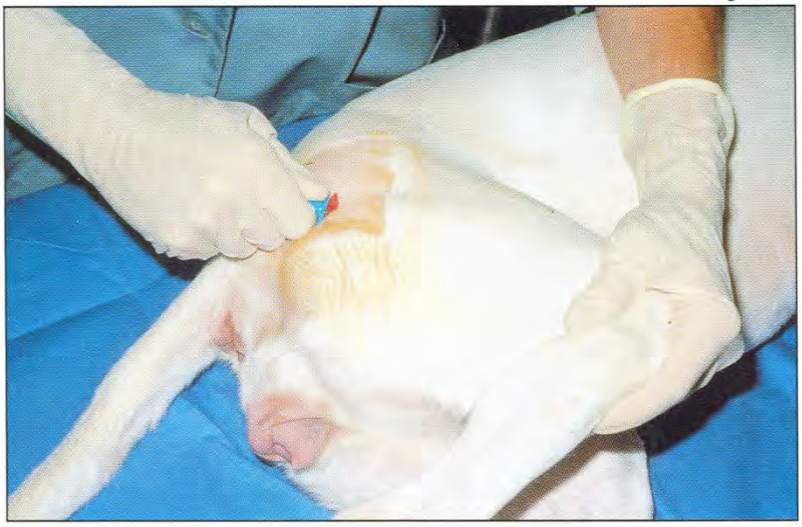
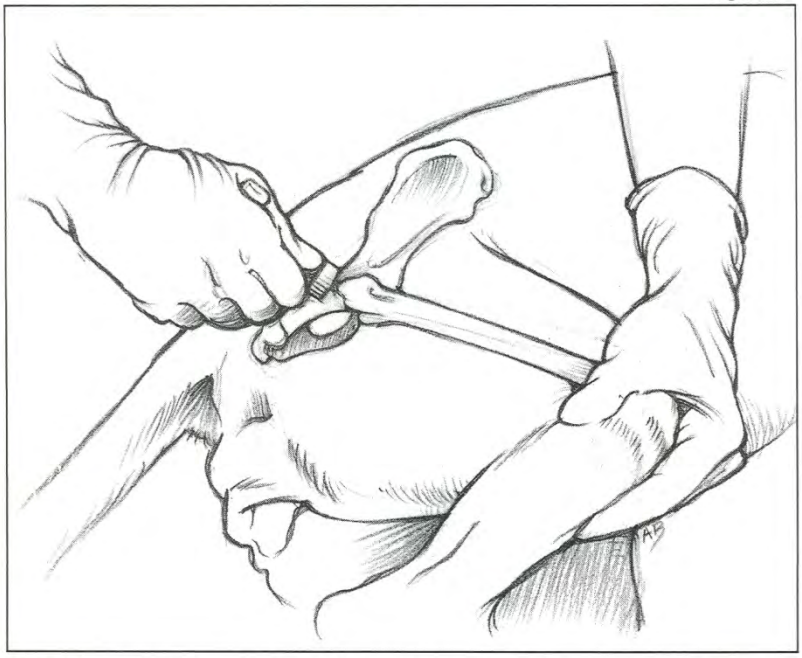


Figure 7B



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.

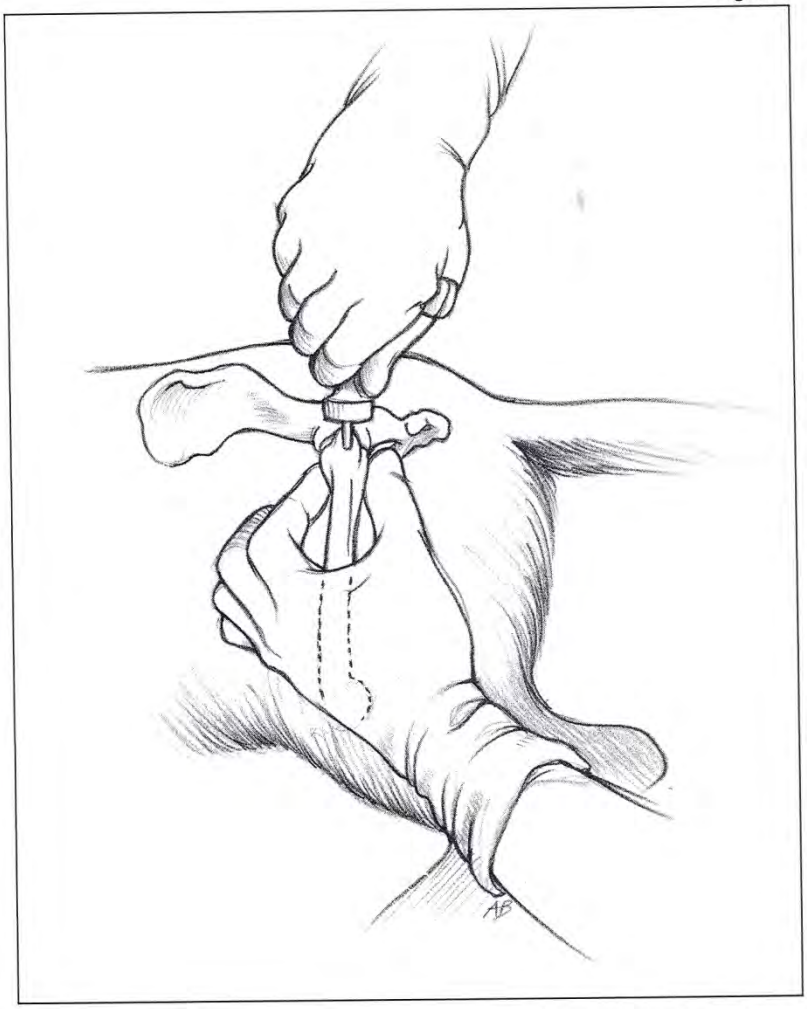
For the lateral approach to the femur, which can be used in cats and small dogs, stabilize the femur by pressing the greater trochanter into the coxofemoral joint. Make a small incision over the lateral aspect of the proximal shaft of the femur (the approximate area of the third trochanter). Insert the 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-

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

Bone marrow aspiration and core biopsy (cont'd)

Figure 9A

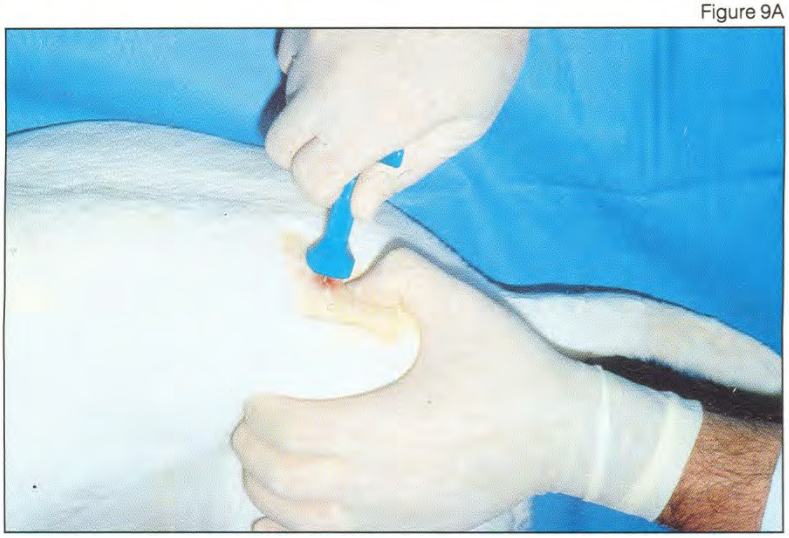
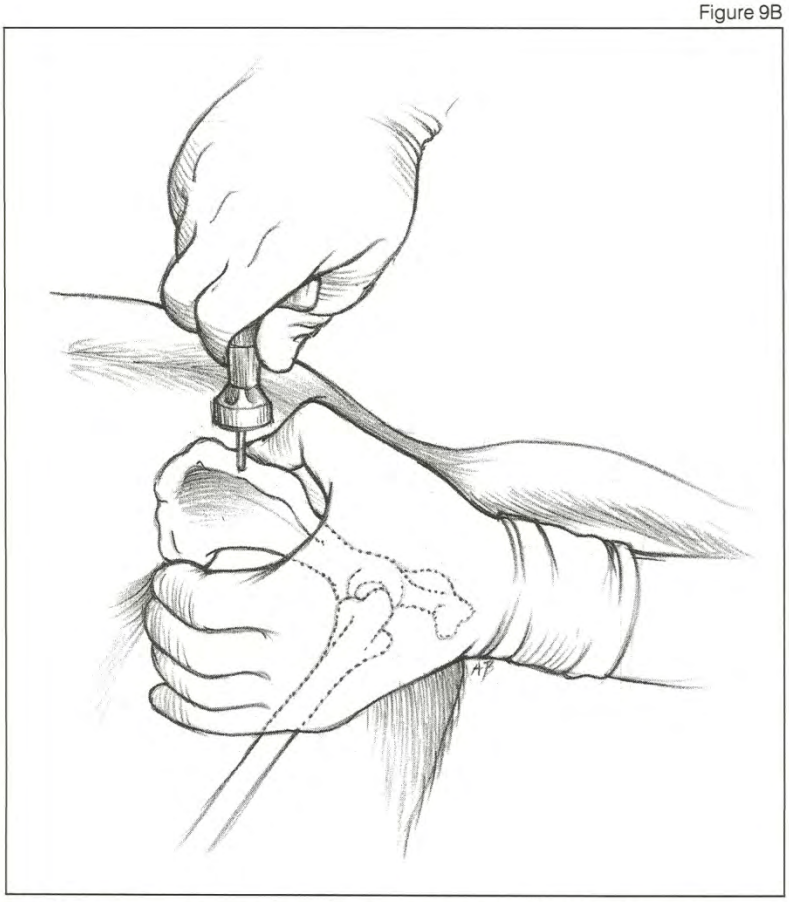


Figure 9B



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*:669-696; 1989.

2. Lewis, H.B.; Rebar, A.H.: *Bone Marrow Evaluation In Veterinary Practice*. Ralston 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

Bone marrow aspiration and core biopsy (cont'd)

Figure 10A

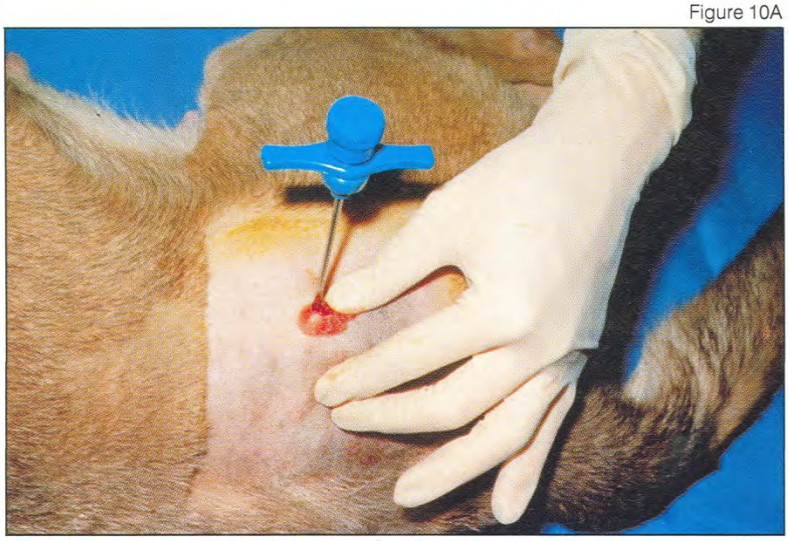


Figure 10B

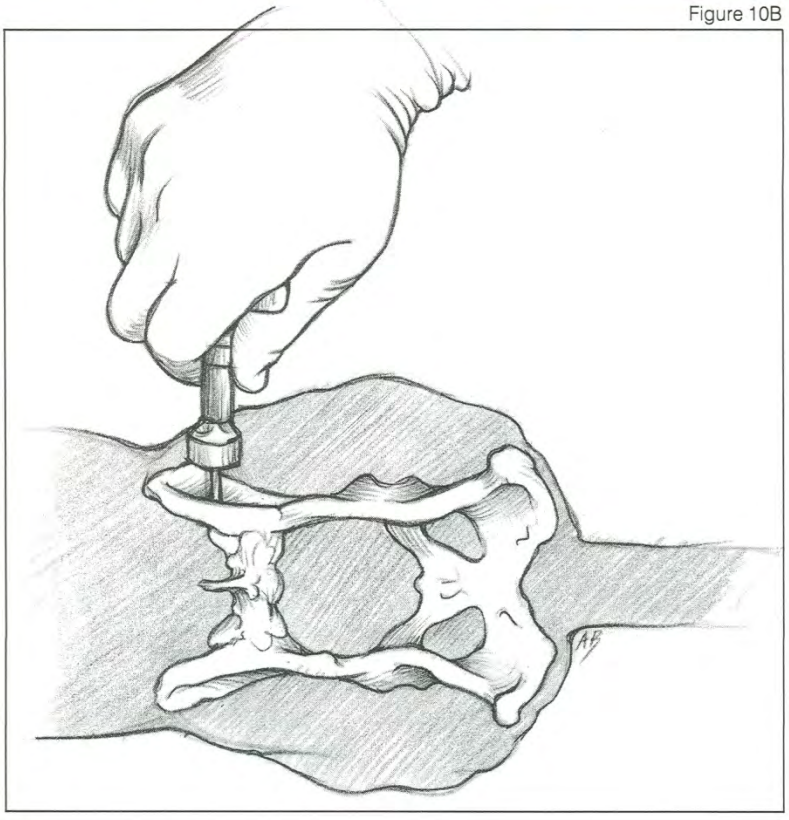
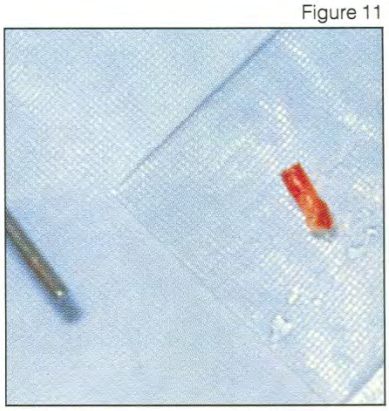


Figure 11



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.

1) HEMATOLOGY AND COAGULATION

CBC - Complete Blood Count

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.
M**ake 2 blood smears**, particularly if the sample will not arrive 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

Notes: Canine -> determination of DEA 1.1 +/- 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**):		Volume required (**each donor**):	
EDTA:	0.5 - 1 mL	EDTA:	0.5 - 1 mL
Red Top:	If ≤ 3 donors - 3 mL whole blood	Red Top:	3 mL whole blood
	If > 3 donors - 5 mL whole blood		

PT/PTT

Your lab may require notification of impending coagulation submissions.
Blue top tubes (sodium citrate anticoagulant) are required. These are available from PDS on request.
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 (not required for canine, feline, equine species for PDS)
Bring 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).

Results will be interpreted by the clinical pathologist according to established reference intervals.

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.

Samples are sent to IDEXX, Veterinary Laboratory Services, Markham, Ontario.
Ph:1-800-667-3411.

Platelet Aggregation

Not currently available at PDS.

Ammonia Tolerance Test

This test is only available to the Veterinary Medical Centre (VMC), WCVM. Analysis is done at the Royal University Hospital.

If animal has severe CNS signs, wait for the "pre" result prior to administration of ammonium chloride.

1) Ammonium chloride (100 mg/kg) – is required. This can be weighed/prepared by the VMC Pharmacy or the PDS Clinical Pathology Laboratory.

2) Fast patient for 12 hours.

3) Collect 1 mL of heparinized (green top tube) sample. Avoid hemolysis. Label 'PRE". IMMEDIATELY wrap sample in paper towel and place on ice pack. Submit to the laboratory, along with submission form.

4) Administer ammonium chloride via stomach tube. If vomiting occurs, notify lab.

5) Collect "post" sample (1 mL heparinized sample) 30 minutes after ammonium chloride administration.

   Post sample should also be IMMEDIATELY wrapped in paper towel and placed on ice pack. Submit to the laboratory. The lab freezes the pre and post samples prior to transport to RUH.

6) VMC arranges transport to RUH. PDS Clinical Pathology staff will package the samples and provide directions to the chemistry laboratory at RUH. Signed document of sample receipt is to be returned to PDS Clinical Pathology Laboratory.

Reference Interval:

	Pre (umol/L)	Post (umol/L)
All species	<80	<80

Bile Acids

1) Fast patient for 12 hours (not required for horses or ruminants).

2) Collect 1 mL blood (red top tube; green top – heparin, also acceptable). Label “pre”.

3) Feed animal: Dogs P/D Cats C/D

(Note: minimum of 2 teaspoons for animals < 10 lbs; 2 tablespoons for large dogs)

4) Collect 1 mL blood (red top/green top tube) 2 hrs post ingestion. Label “post”.

Avoid Hemolysis

Reference Values:

	Fasting (µmol/L)	Postprandial (µmol/L)	Hepatic Biopsy Recommended
Canine	<10	<20	>30
Feline	<10	<20	>30
Equine	<20 (fasting or postprandial)		
Bovine: beef	<126 (fasting or postprandial)		
dairy	<88		
6 mo dairy heifers	<64		
Avian: many species	<70		
Amazon parrots	<144		

(highly variable depending on the species; check with laboratory)

Blood Gases

(Includes Na, K, Cl, free calcium, glucose, lactate, 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) Approximately 20 - 50 IU of heparin per mL of blood is recommended.

    a) This amount is closely approximated if heparin is used to fill the syringe dead space; do not include needle dead space - i.e. use a 25 ga needle to draw heparin into syringe, completely express heparin back into vial, remove needle and replace with new needle for blood collection.

    b) Commercially available dry heparinized syringes do not require additional liquid heparin.

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.

6) The amount of heparin should be reduced for reduced volumes of blood. Errors in blood gas and electrolyte determination due to dilution and ion binding can occur when samples contain excessive heparin.

- 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.

- Available only to local clients.

Calcium – Free ("Ionized")

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 CO_2 is released which increases the pH.

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

Available only to local clients.

Cortisols - General Information

- Very stable in most body fluids.
- Can be analyzed in serum (red top tubes), or urine.
- If testing will be delayed, separate serum from cells and freeze.
- Label all tubes with identification and collection time.
- Cortisols are of most use when comparing baseline to stimulated (ACTH Stimulation Test) or suppressed (Dexamethasone Suppression Test) values.

Cortisols - Reference Values

Canine:	Baseline cortisol:	<20–270	nmol/L	
	Post ACTH stim:	230-570	nmol/L	Normal
		>660	nmol/L	**Hyper**adrenocorticism (HAC)
		570-660	nmol/L	Equivocal (for HAC)
		<55	nmol/L	**Hypo**adrenocorticism
	Post LDDST (8hr):	<20	nmol/L	Normal
		20-40	nmol/L	Equivocal (for HAC)
		>40	nmol/L	**Hyper**adrenocorticism

(see additional information regarding tests and interpretations below)

In iatrogenic canine hyper/hypoadrenocorticism, stimulated values vary, but typically show little change from baseline values. In natural canine hypoadrenocorticism, baseline and stimulated values are <55 nmol/L.[3]

Feline:	Baseline cortisol:	<320	nmol/L	

Monitoring of Treatment for Canine Hyperadrenocorticism:

Trilostane therapy:

Post ACTH stim:	41.4-152	nmol/L	Ideal response[1]
	50-200	nmol/L	Ideal response[2]

op-DDD (Lysodren®) therapy:

Post ACTH stim:	27.6-138	nmol/L	Ideal response[3]

[1] Vaughan, Feldman *et al*. Evaluation of twice daily, low dose trilostane treatment administered orally in dogs with naturally occurring hyperadrenocorticism, JAVMA 232:9, 2008.
[2] Ettinger & Feldman eds, 7th ed. Textbook of Veterinary Internal Medicine, 2010.
[3] Feldman & Nelson, eds, 3rd ed. Canine and Feline Endocrinology and Reproduction, 2004

● When treating hyperadrenocorticism, clinical indicators of response to therapy are also important (e.g. return to “normal” water consumption of < 66 mL/kg/day, and correction of appetite and activity level).

Cortisols - ACTH Stimulation Test

Use:

To differentiate between normal adrenal function and hyperadrenocorticism (Cushing's) in dogs or it can be used 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 (eg. trilostane or Lysodren) for a previously diagnosed hyperadrenocorticism patient. The target ranges vary depending on the medication being used.

Method:

1. Collect at least 1 mL of blood in a red top tube [do not use serum separator tubes (SST)]. Label "pre".
2. Inject 1 vial Cortrosyn (0.25 mg synthetic ACTH) IM for DOGS, ½ vial for CATS. Due to the increased cost of Cortrosyn, scientists have determined that it can be reconstituted and stored frozen at -20C in plastic syringes up to 6 months. A smaller amount of Cortrosyn can be used in smaller dogs (low dose ACTH stimulation test): 50ug/10kg body weight IV. The WCVM VMC Pharmacy sells Cortrosyn as a vial (.25 mg or 250 ug) or in a syringe (50 or 62.5 ug). If more than 2 syringes are needed, it is recommended to use an entire vial.
3. Collect at least 1 mL of blood in a plain red top tube 1 hour post injection. Label "post". For cats, post ACTH samples are collected at 30 and 60 minutes.
4. If using ACTH Gel (porcine) use 2.2 IU/kg IM for dogs and collect "post" sample at 2 hours.

Cortisols - Low Dose Dexamethasone Suppression Test - (LDDST):

Use:

To differentiate between normal adrenal function and hyperadrenocorticism (HAC). The test is typically performed on dogs that have clinical signs, physical exam findings and/or laboratory findings that already allow suspicion of HAC. This test is infrequently used in the few suspected cases of feline HAC and the dosage is listed in brackets below. Dexamethasone will not be measured as cortisol by the test method and one-time doses should not interfere with test results. If HAC is confirmed, the LDDST may also help differentiate between the two causes: pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent disease caused by a functional adrenal tumor (FAT).

Method:

1. Collect at least 1 mL of blood sample in a red top tube [do not use serum separator tubes (SST)]. Label "pre".
2. Inject 0.01 mg/kg dexamethasone IV for dogs (0.1 mg/kg for cats).
3. Collect at least 1 mL of blood in a red top tube 3 (some texts list 4 hours, which is fine) and 8 hours post injection –label as "3 or 4 hr post" and "8 hour post", respectively.

Interpretation of LDDST: (canine)

	3 or 4 hours		8 hours
Normal canine adrenal function:	<20 nmol/L		<20 nmol/L
Hyperadrenocorticism (HAC)			>40 nmol/L
PDH	<50% of baseline	**OR**	<50% of baseline
or			
PDH	<40 nmol/L		>40 nmol/L

Differentiating PDH and FAT based on LDDST:

An "escape" pattern is sometimes seen in dogs with PDH. That is, there is suppression at 3 or 4 hours which disappears at 8 hours. Once HAC has been confirmed (8 hour post LDDST cortisol >40 nmol/L), the LDDST may help differentiate between PDH and FAT. Suppression supports PDH and is defined as any of the following: <50% of baseline at 4 or 8 hours, <40 nmol/L at 4 hours. (Canine and Feline Endocrinology and Reproduction by Feldman & Nelson, 3rd ed.).

Feline values not available (see Canine and Feline Endocrinology and Reproduction by Feldman & Nelson, 3rd ed.)

Cortisol - High Dose Dexamethasone Suppression Test - HDDST:

Use:

To help differentiate between pituitary dependent hyperadrenocorticism (PDH) and a functional adrenal tumor (FAT) in a canine 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 [do not use serum separator tubes (SST)]. 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 3 or 4 and 8 hours post injection - label as "3 or 4 hr post" and "8 hr post", respectively.

Interpretation: (canine)

	3 or 4 hours		8 hours
PDH	<50% of baseline	AND/**OR**	<50% of baseline
or			
PDH	<40 nmol/L	**OR**	<40 nmol/L

Differentiating PDH and FAT based on HDDST:

Cortisol levels at 4 or 8 hrs post injection should suppress to < 50% of baseline or values should be <40 nmol/L in PDH. Cortisol levels 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 PDH dogs do not suppress on the HDDST.

Cortisols - Ultra High Dose Dexamethasone Suppression Test (UHDDST)

Use:
Same as HDDST.

May capture rare pituitary tumor that doesn't suppress with HDDST.

Method:
Same as HDDST but inject 1.0 mg/kg (dogs) dexamethasone IV.

Interpretation:
Same as HDDST.

Urine Cortisol: Creatinine Ratio (UCCR)

Reference (cut-off) Value: dogs $\leq 10 \times 10^{-6}$ (based on collection of urine sample at home in a non-stressful environment).

Interpretation:
A UCCR above the cut-off value can be found in dogs with hyperadrenocorticism, in many dogs with nonadrenal disorders, and in healthy stressed dogs. Additional screening testing (LDDST or ACTH response test) must be done to further evaluate for HAC. A ratio within reference limits is strong evidence that hyperadrenocorticism is not present.

Equine Dexamethasone Suppression Test

Use:

To differentiate normal adrenal function from hyperadrenocorticism.

Method:

1. Collect at least 1 mL of blood between 4 and 6 pm in red top tube [do not use serum separator tubes (SST)] - label this baseline sample "pre".
2. Inject dexamethasone (40 ug/kg) IM.
3. Collect at least 1 mL of blood at noon the following day (19 hour) in red top tube [do not use serum separator tubes (SST)] - label "19 hour post".

Interpretation:

	19 hours:
Normal adrenal function:	≤28 nmol/L
HAC	>28 nmol/L

Cortisol levels at 19 hours post injection should suppress to ≤ 28 nmol/L in horses with normal adrenal function.

Cortisol levels >28 nmol/L at 19 hours post injection are consistent with hyperadrenocorticism.

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:

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

* 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 –> High Pi, low Ca++.

High fat diet –> Low Pi, high Ca++.

Seasonal diets –> affect FE of Na+.

- On all grass diet –> FE of Na+ increased in summer.
- On all grass diet –> FE of K+ increased in summer.
- On all hay diet –> FE of Cl- decreased in spring.

*Pi = Inorganic phosphorous

Hypothyroidism in Dogs and Hyperthyroidism in Cats:

Sample required: 1 mL red top tube. Do not use SST tubes.

Background: Hypothyroidism is the most common endocrine disorder in **dogs**. Diagnosis of hypothyroidism using a resting serum thyroxine (T4) value is often not possible due to suppression of serum T4 concentration in non-thyroidal illness. However, a low resting T4 in a dog that is not clinically ill and has findings consistent with hypothyroidism may be sufficient evidence to diagnose and treat hypothyroidism. Hyperthyroidism is the most common endocrine disorder in older **cats** and is usually easily diagnosed based on a high resting T4 level.

Principle: The majority of hypothyroid dogs have primary disease (originating within the thyroid glands) resulting in lower than normal serum T4, and higher than normal endogenous TSH concentrations. Secondary hypothyroidism occurs in less than 5% of hypothyroid dogs and is the result of impaired production of TSH by the anterior pituitary gland. Dogs with secondary hypothyroidism have low serum T4 and TSH concentrations. Hyperthyroid cats usually have nodular hyperplasia or adenoma(s) of the thyroid gland(s).

Reference Values

Test	Canine (nmol/L)	Feline (nmol/L)
Baseline T4	12 - 40	13 - 50
Baseline T3	<.6 - 1.2	<.6 - 1.1
Endogenous TSH	0.03 - 0.58 ng/mL	NA

Most hypothyroid dogs have a low resting T4 and high endogenous TSH level (> 0.58 ng/mL). Baseline T4 may also be low with non-thyroidal illness (euthyroid sick syndrome).
Hyperthyroidism is common in older cats and is usually easily diagnosed based on a high resting T4 level. Under certain circumstances, baseline T4 may be within reference limits in hyperthyroid cats and the T3 Suppression Test may be useful in making the diagnosis (see below). T3 determination is part of the T3 Suppression Test. T3 is rarely measured under other circumstances where it is of minimal diagnostic value.

Hypothyroid Therapy Monitoring

Measure trough levels (just prior to dose) and peak levels (4-6 hrs post pill).
PLEASE indicate in history if dog is receiving medication, dosage schedule, and time of sample collection relative to last treatment.

T3 Suppression Test - Cats

Background: In cats with mild or early hyperthyroidism, the serum/plasma T4 and T3 may fluctuate in and out of the reference interval. Concurrent serious illness can cause decreases in T4. If clinical signs support hyperthyroidism, but T4 level is within the reference interval, a T3 suppression test may be indicated.

Collect 1 mL blood (red top tube). Do not use SST tubes. Label “pre”. Freeze plasma or serum if submission is delayed.

Administer oral T3 (Liothyronine) 25 µg 3 x/day for 2 days. On the 3rd morning, give 25 µg (1 tablet).

Collect 1 mL blood (red top tube) 2 - 4 hrs after last tablet. Label “post”.

Request both T3 AND T4.

Results:

	T4 (nmol/L)
Normal cat	<20
Hyperthyroid cat	>20

T3 will be elevated; this value is used only to ensure that the Liothyronine was administered successfully.

GI Function Tests (All are referred out by PDS)

1) Trypsin-Like Immunoreactivity (TLI): Pancreatic Function Test (Canine, Feline)

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; otherwise, no sooner than 3 hours 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.
- Unnecessary to send samples frozen or chilled.

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.

2) Fecal Alpha-1 Protease Inhibitor (for dogs only).

- 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.
- $Alpha_1$-Protease Inhibitor is not heat stable and cooling in transit is required if samples are shipped via overnight carrier.

3) Serum Cobalamin (Vitamin B12) (Canine, Feline).

(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

Values 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.

4) Folate: Absorbed in proximal intestine only.

Control Ranges: Canine 7.0 - 39.0 nmol/L
Feline 27.0 – 46.0 nmol/L

Values 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.

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

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

Ph: 979 862-2861
Fax: 979 862-2864
E.mail: gilab@CVM.tamu.edu
www.cvm.tamu.edu/gilab

5) Pancreatic Lipase Immunoreactivity (PLI) – canine, feline

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/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:
Provide a quantitative assessment of urinary protein excretion by comparing urine protein to urine creatinine in a single urine sample. The UPC is a close estimation of 24 hour urinary protein excretion, however the relationship between UPC and 24 hour protein excretion is not constant.

Indications:
1. Determine significance of a positive protein reaction from a urine dipstick test result
2. Early detection of chronic kidney disease (CKD) – proteinuria may be present prior to azotemia
3. Prognostic indicator in CKD
4. IRIS (International Renal Interest Society) CKD clinical substaging and determining need for therapeutic intervention.[1]

Interpretation of UPC:

Healthy dogs and cats < 0.5

Once localized to the kidneys, **Persistent renal proteinuria** refers to proteinuria defined in both dogs and cats as either UPC ≥ 0.5 or microalbuminuria present with repeated testing on 3 or more occasions, 2 or more weeks apart.[2] The following guidelines have been suggested with regards to persistent renal proteinuria to localize the disease process:

Dogs: UPC ≥ 2.0 Glomerular renal disease
Cats: UPC ≥ 1.0 High index of suspicion for presence of glomerular renal disease

***Please see references for additional clinical information regarding proteinuria.**

Method/Calculations
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 regarding glomerular protein loss provided there is no evidence of urinary tract inflammation or hemorrhage.
3. Urine creatinine and protein (measured on the chemistry analyzer) are converted to the same units (g/L) by using the following calculation:

$$\text{Urine protein to creatinine ratio (UPC)} = \frac{\text{Urine protein (g/L)} \times 88.4 \times 100}{\text{Urine creatinine (µmol/L)}}$$

References:

1. IRIS (International Renal Interest Society) – http://www.iris-kidney.com/
2. 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.

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 stone to laboratory.

Routine testing involves a 3-4 week turn-around time.

There is no charge for the analysis of canine and feline samples.

(Urolith Submission Form – next page)

UNIVERSITY of GUELPH
LABORATORY SERVICES

CANADIAN VETERINARY UROLITH CENTRE
UROLITH SUBMISSION

This $50.00 urolith analysis provided compliments of: MEDI-CAL ROYAL CANIN VETERINARY DIET

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:** cvuc@uoguelph.ca

NOTE: SUBMITTING CLINICS ARE RESPONSIBLE FOR SHIPPING CHARGES.

PLEASE SUBMIT DRY UROLITHS IN A CLEAN PLASTIC VIAL (Urethral plugs: ½ in urine, ½ 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 ☐

37-09

4) CYTOLOGY

CSF Analysis (Canine, Feline)

(NOTE: A degree of variability exists in the literature with respect to reference values for CSF cytology – values provided under* Normal category were updated Jan/09 based on recent literature*)

1) Normal

- Nucleated cells: < 5 x 10^6/L

- Protein:	Any site:	Cervical:	Lumbar:
Canine		<0.25 g/L	<0.45 g/L
Feline		<0.25 g/L	<0.45 g/L
Equine	<1.05 g/L		
Bovine	<0.663 g/L		
Llama	<0.668 g/L		
Ferret	<0.68 g/L		

- Differential: Mostly mononuclear -> mature lymphocytes monocytes (cytocentrifuged for concentration)

2) Mild Mononuclear Inflammation

- Nucleated cells: <30 x 10^6/L
- Protein: >0 .25 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 x 10^6/L (neutrophils - bacterial encephalitis and meningitis), but usually <50 x 10 6/L (mononuclear - viral encephalitis and meningitis, listeriosis)
- Protein: >0.25 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 hrs)

5) Contamination (blood)

- RBCs increased, platelets

Peritoneal Fluid (horses)

(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 x 10^9/L (usually $\leq$4.0 x 10^9/L)
   - Protein: (usually) $\leq$15 g/L
   - Differential: approximately 50% neutrophils, 50% mononuclears

2) Non-Septic Exudate

   - Nucleated cells: >10 x 10^9/L
   - Protein: > 23 g/L
   - Differential: Neutrophils > macrophages

3) Septic Exudate

   - Nucleated cells: > 10 x 10^9/L
   - Protein: > 34 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 x 10^9/L (low cellularity)
- Protein: $\leq$ 25 g/L
- Differential: - Macrophages, mesothelial cells, non-degenerative neutrophils
- Causes: - Increased capillary hydrostatic pressure
  - Decreased plasma oncotic pressure - albumin <10 g/L
  - Lymphatic obstruction

2) Modified Transudate

- Nucleated cells: $\leq$ 7 x 10^9/L **and** Protein > 25 g/L

or

- Nucleated cells: > 1.5 x 10^9/L **and** Protein $\leq$ 30 g/L

or

- Nucleated cells: > 1.5 - 7 x 10^9/L **and** Protein 25 - 30 g/L
- Differential: - Macrophages, mesothelial cells, non-degenerate neutrophils
- Examples: - Cardiomyopathy
  - Congestive heart failure
  - Chylous effusions
  - Neoplasm (lymphosarcoma, carcinoma)

3) Exudate

- Nucleated cells: > 7.0 x 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

5) 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

6) Chylous Effusion - Many Etiologies

- Milky appearance (presence of chylomicrons)
- Nucleated cells: 1.4 - 20 x 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 typically falls into the exudate range; however, sometimes 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 - 23 x 10^9/L
- > Protein 35-80 g/L (av = 60), 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 smudge 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

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 < 3×10^9/L
  - Horse < 0.5×10^9/L
  - Cow < 1×10^9/L
- RBC: rare
- Differential: Mononuclears > 90% (<10% neutrophils)

2) Degenerative Joint Disease

- Nucleated cells: < 5×10^9/L
- Viscosity normal
- Increased quantity of fluid
- Differential: Mononuclears >60%, increased macrophages, synovial lining cells
- Causes: Degenerative disease, osteoarthritis, trauma (blood components)

3) Non-Septic Inflammation - Immune Mediated

- Nucleated cells: 5 - 50 x 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 x 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

A P P E N D I X

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	$x\ 10^{12}$/L	$10^6/mm^3$
MCV	1	fL	um^3
MCH	1	pg	pg
MCHC	10	g/L	g/dl
Platelets	0.001	$x\ 10^9$/L	mm^3
Fibrinogen	10	g/L	g/dl
WBC	0.001	10^9/L	mm^3

Consult laboratory for other conversion factors.

HEMATOLOGY - GENERIC REFERENCE VALUES

PARAMETER	UNITS	CANINE	FELINE	BOVINE	EQUINE	PORCINE	OVINE
H7							
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	16.6-220	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 (mature pigs)	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 - 1
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

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HEMATOLOGY GENERIC REFERENCE VALUES (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				<7	<6		
Protein Fibrinogen Ratio	Cattle	<10 - - - Absolute hyperfibrinogenemia 10 - 15 - Dehydration, hyperfibrinogenemia or botn >15 - - - Normal					
							<15 15-20 >20 Equine
PT	seconds	7.5-9.9	7.0-11.0	12.8-19.6	7-11.0	9.5-12.0	

PTT	seconds	9.6 - 13.8	9.5 - 17.8	14.9-38.9	27.7 - 49.6	13 - 21	---
FDP Titre		< 10 mg / L all species					
FDP	< 10 mg/L	- normal					
	10 - 40 mg/L	- slightly increased, usually associated with hematomas, inflammatory disease, renal failure, liver failure, vasculitis, thrombosis					
	> 40 mg/L	- usually, only associated with disseminated intravascular coagulation					

Regenerative Response to Anemia

Degree of Regeneration	Canine % Retics	Canine Absolute Value	Feline % Retics	Feline Absolute Value
None	<1	0.06 x 10^{12}/L	0.5	< 0.015 x 10^{12}/L
Slight	4	0.15 x 10^{12}/L	2	0.05 x 10^{12}/L
Moderate	10	0.30 x 10^{12}/L	4	0.10 x 10^{12}/L
Marked	>25	0.50 x 10^{12}/L	>5	0.20 x 10^{12}/L

RPI - [Reticulocyte Production Index] - Dogs only
1 = Normal
>1 = Regeneration
>3 = Massive Regeneration (associated with hemolysis or hemorrhage).

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CHEMISTRY

GENERIC REFERENCE VALUES

	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	2.80-3.54	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	umol/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	---	2.5-5.50	1.20-4.60	1.50-4.00	---	---
Total Bilirubin	umol/L	0-30	0-17	4-102	0-17	0-4	0-3
Direct Bilirubin	umol/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	---
Gamma GT	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	0.60-1.50	0.60-1.50	0.54-1.22
Calculated Osmolality	mmol/Kg	274-306	280-320	280-320	280-320	280-320	280-307

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CHEMISTRY REFERENCE VALUES (Continued)

Blood Gases	UNITS	BOVINE	CANINE	EQUINE	FELINE	PORCINE	OVINE
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

*pO_2 Venous - Jugular 65.0 mmHg Cephalic 58.0
*pCO_2 Arterial - Usually 5-6 mmHg lower than venous

Ionized Calcium

	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 - REFERENCE VALUES

	UNITS	BOVINE	CANINE	EQUINE	FELINE	PORCINE	OVINE
Protein Electrophoresis (agarose gel)							
Albumin	g/L	24-36	24-40	29-38	26-44	19-24	28-34
Glob: alpha 1	g/L	7-12	2-4	7-13	13-18	9-12	2-6
Glob: alpha 2	g/L	---	4-9	7-13	---	---	3-7
Globulin: beta	g/L	6-12	13-17	4-12	10-15	8-11	3-7
Globulin: gamma	g/L	16-32	4-8	9-15	12-27	3-7	7-13

Iron	umol/L	over 14.5	all species
TIBC	umol/L	44.8 - 62.7	all species

		Canine	Feline
Cortisols Baseline	nmol/L	20-270	<320

Endo, TSH	ng/ml	0.03-0.58 (Canine - no Feline values)					
T3	nmol/L	--	< 0.6 -1.2	*	0.10-1.03	--	--
T4	nmol/L	--	12-40	*	13-50	--	--
T3 Suppression	nmol/L		----		T4 <20		

Ammonia	umol/L	all species <80

		CANINE	FELINE	EQUINE*
Bile Acids umol	Fasting	<10	<10	Random <9.0
	Postprandial	<20	<20	*values may be higher when **OFF** feed

Osmolality (measured)	mmol/kg	285-315	285-315	285-315	285-315	285-315	285-315
CSF Protein	g/L	0.16-0.33	<0.25	0.29-0.72	<0.25	0.24-0.40	0.08-0.70

* check with pathologist

CHEMISTRY REFERENCE VALUES (Continued)

Protein Electrophoresis:

		Llama
Albumin	g/L	32 - 44
alpha-1	g/L	0.4 - 2.0
alpha-2	g/L	2.4 - 5.2
beta	g/L	6.8 - 16.4
gamma	g/L	3.9 - 14.7
alb:glob		0.80 - 2.00

*Alk Phos levels and phosphorus levels for goat:
- a number of the normal animals used in this normal value study were less than one year of age.

** - very young age - related values

CHEMISTRY
GENERIC REFERENCE VAULES

	UNITS	FOALS <24 HOURS (n = 16)	FOALS <3 MONTHS (n = 26)	GOATS (n = 22)	LLAMA	MUSKOX
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	25 - 33	22 - 38	21 - 31	14 - 36	14 - 32
Anion Gap	mmol/L	10 - 2	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	umol/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	umol/L	18 - 122	<70	<5	<5	<1
Direct Bilirubin	umol/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	

REFERENCE VALUES

Avian Hematology and Chemistry

	African Grey Parrot	Amazon Parrot	Blue-Headed Parrot	Budgerigar	Parakeet
WBC (x 10^3)	5 - 11	6 - 11	4 - 11	3 - 8	4.5 - 9.5
Differential					
- Heterophils %	45 - 75	30 - 75	40 - 70	45 - 70	40 - 75
- Lymphocytes %	20 - 50	20 - 65	20 - 50	20 - 45	20 - 60
- Monocytes %	0 - 3	0 - 3	0 - 2	0 - 5	0 - 3
- Eosinophils %	0 - 2	0 - 1	0 - 1	0 - 1	0 - 1
- Basophils %	0 - 5	0 - 5	0 - 5	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
RBC (x 10^{12}/L)	2.4 - 4.5	2.5 - 4.5	2.4 - 4.1	2.5 - 4.5	---
Total Protein (g/L)	30 - 50	30 - 50	26 - 50	25 - 45	25 - 45
Glucose (nmol/L)	10.6 - 19.4	12.2 - 19.4	10.0 - 16.6	11.1 - 22.2	11.1 - 19.4
Calcium (nmol/L)	2.00 - 3.24	2.00 - 3.24	2.5 - 3.74	---	---
ALT (U/L)	100 - 350	130 - 350	150 - 350	150 - 350	150 - 400
LDH (U/L)	150 - 450	160 - 240	200 - 550	150 - 450	150 - 450
Creatinine (umol/L)	9 - 35	9 - 35	9 - 26	9 - 35	9 - 35
Uric Acid (umol/L)	240 - 600	120 - 600	240 - 710	240 - 830	240 - 710
Potassium (mmol/L)	2.6 - 4.2	3.0 - 4.5	3.0 - 4.5	---	---
Sodium (mmol/L)	134 - 152	136 - 152	130 - 150	---	---
T4 (nmol/L)	4 - 26	1 - 13	3 - 14	32 - 57	3 - 31

REFERENCE VALUES

Avian Hematology and Chemistry

	Cockatiels	Cockatoos	Conures	Domestic Ducks	Grand Electus Parrots
WBC (x 10^3)	5 - 10	5 - 11	4 - 11	4.5 - 13	6 - 11
Differential					
- Heterophils %	40 - 70	45 - 75	40 - 75	30 - 70	40 - 75
- Lymphocytes %	25 - 55	20 - 50	20 - 50	20 - 65	20 - 50
- Monocytes %	0 - 2	0 - 4	0 - 3	0 - 3	0 - 2
- Eosinophils %	0 - 2	0 - 2	0 - 3	0 - 4	0 - 1
- Basophils %	0 - 6	0 - 5	0 - 5	0 - 5	0 - 5
HCT (L/L)	0.45-0.57	0.40-0.55	0.42-0.55	0.30-0.43	0.45-0.55
RBC (x 10^{12}/L)	2.5 - 4.7	2.2 - 4.5	2.5 - 4.5	2.3 - 4.5	2.5 - 4.0
Total Protein (g/L)	22 - 50	25 - 50	25 - 45	25 - 60	30 - 50
Glucose (nmol/L)	11.1 - 25.0	11.0 - 19.4	11.1 - 19.4	8.3 - 16.6	9.9 - 20.0
Calcium (nmol/L)	2.12 - 3.24	2.00 - 3.24	2.00 - 3.74	2.50 - 4.49	2.25 - 3.99
ALT (U/L)	100 - 350	150 - 350	125 - 350	5 - 100	150 - 350
LDH (U/L)	125 - 450	225 - 650	125 - 420	150 - 800	200 - 400
Creatinine (umol/L)	9 - 35	9 - 35	9 - 44	9 - 44	9 - 35
Uric Acid (umol/L)	210 - 650	210 - 650	150 - 625	120 - 710	180 - 600
Potassium (mmol/L)	2.5 - 4.5	2.5 - 4.5	3.4 - 5.0	3.0 - 4.5	---
Sodium (mmol/L)	132 - 150	131 - 157	134 - 148	130 - 155	---
T4 (nmol/L)	9 - 31	10 - 57	3 - 12	10 - 43	6 - 13

REFERENCE VALUES

Avian Hematology and Chemistry

	Finches	Lovebirds	Macaw	Mynah Bird	Phillipine Blue-Naped Parrot	Toucan
WBC (x 10^3)	3 - 8	3 - 8	6 - 13.5	6 - 11	4.5 - 11.5	4 - 10
Differential						
- Heterophils %	20 - 65	40 - 75	45 - 70	25 - 65	35 - 70	35 - 65
- Lymphocytes %	20 - 65	20 - 55	20 - 50	20 - 50	20 - 60	25 - 50
- Monocytes %	0 - 1	0 - 2	0 - 3	0 - 3	0 - 5	0 - 4
- Eosinophils %	0 - 1	0 - 1	0 - 2	0 - 3	0 - 0	0 - 4
- Basophils %	0 - 5	0 - 6	0 - 5	0 - 7	0 - 5	0 - 5
HCT (L/L)	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)	2.5 - 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)	30 - 50	22 - 51	30 - 50	23 - 45	30 - 50	30 - 50
Glucose (nmol/L)	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 (nmol/L)	---	2.25-3.74	2.25-3.24	2.25-3.24	2.50-3.99	2.50-3.74
ALT (U/L)	150 - 350	100 - 350	100 - 280	130 - 350	130 - 350	130 - 330
LDH (U/L)	---	100 - 350	75 - 425	600-1000	130 - 425	200 - 400
Creatinine (umol/L)	---	9 - 35	9 - 44	9 - 53	9 - 35	9 - 35
Uric Acid (umol/L)	240 - 710	180 - 650	150 - 680	240 - 600	240 - 600	240 - 830
Potassium (mmol/L)	---	2.5 - 3.5	2.5 - 4.5	3.0 - 5.1	---	---
Sodium (mmol/L)	---	137 - 150	136 - 155	136 - 152	---	---
T4 (nmol/L)	---	3 - 24	13 - 52	6 - 12	4 - 13	6 - 41

*value given for blue and gold Macaw. T4 for others varies as to species.

Prairie Diagnostic Services -- Clinical Pathology

Hematology Reference Intervals - ADVIA 2120 - Dec 3/2013

TEST	CANINE Guelph	FELINE Guelph	EQUINE Guelph	BOVINE Cornell
WBC	4.9 - 15.4	4.2 - 13.0	5.1 - 11.0	5.9 - 14.0
RBC	5.8 - 8.5	6.2 - 10.6	6.9 - 10.7	5.0 - 7.2
Hgb	133 - 197	93 - 153	112 - 169	87 - 124
HCT (MCV x RBC)	0.39 - 0.56	0.28 - 0.49	0.28 - 0.44	0.25 - 0.33
MCV	62 - 72	39 - 52	42 - 53	38 - 51
MCH (Hgb/RBC)	21 - 25	13 - 17	14 - 18	14 - 19
MCHC (Hgb/HCT)	330 - 360	300 - 344	328 - 364	340 - 380
RDW	11 - 14	14 - 17	16 - 20	15.0 - 19.4
Total Solids	56 -74	58 - 82	59 - 73	69 - 87
Fibrinogen	NA	NA	1 - 6	1 - 7
Segs - %	40 - 80	34 - 80	43 - 77	15 - 53
Segs - Absolute	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	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	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	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	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	rare	0.0 - 0.2	0.0 - 0.12	0.0 - 0.1
NRBC				
Platelet	200 - 900	300 - 700	100 - 600	100 - 800
MPV	7 - 14	8 - 21	6 - 11	5.7 - 8.0

1. For WBC, RBC, Hgb, HCT, MCV, MCH, MCHC, MPV
   a. Canine, Feline, Equine - Guelph Reference Intervals adopted.
   b. Bovine - Cornell Reference Intervals adopted.
2. For Platelets - no change from previous reference intervals.
3. Total Protein, Fibrinogen - no change from previous reference intervals.
4. Differential Reference Intervals for Canine, Feline and Bovine - no change from previous reference intervals.
5. New Differential Reference Intervals for Equines.

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