

Clinical Pathology

Veterinary Diagnostic Services



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

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Contact Us

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Quality Control and Assurance

The Clinical Pathology laboratory performs daily internal quality control monitoring on all instrumentation. Bi-weekly monitoring of chemistry analytes is also performed by an external Quality Assurance program. The Clinical Pathology technologists and pathologists participate in a quarterly external proficiency program, the Veterinary Laboratory Association Quality Assurance Program (VLA).

Equipment maintenance is performed on a regular schedule along with yearly preventative maintenance.

Hematology

General Information

- Please provide a detailed history and clinical findings. This contributes to accurate hematologic interpretation.
- Whole blood in EDTA (purple top tube) is the required specimen for mammalian hematology. The **sample tube and slides** must be labelled with the animal and owner name.
- For non-mammalian: **birds and reptiles**, the preferred hematology specimen is whole blood in a lithium heparin (green top) tube.
- EDTA and lithium heparin tubes should be adequately filled. Tubes should be filled two-thirds full (the number of milliliters required will depend on the tube size) to provide a proper blood-to-anticoagulant ratio, this is especially important for tubes containing liquid EDTA. **For small volumes** (0.5 ml) use pediatric or Microtainer® tubes. **Under-filling** tubes can cause inaccurate results, such as:
 - an erroneously low hematocrit and red blood cell count (RBC)
 - inaccurate mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)
 - altered red blood cell and white blood cell morphology (i.e.: echinocytes)
- Gently mix blood well after collection and make blood smears. Cells in whole blood EDTA/heparin samples degrade rapidly after collection, **air dried blood smears should be made immediately** to preserve cellular morphology. **Smear evaluation is done on submitted smears**, provided quality is sufficient.
- Refrigerate the tube to delay further cell degradation. **Do not** freeze.
- **Do not** refrigerate smears. Ensure that labeled smears are dry and packaged in a slide holder or appropriate shipping container to prevent damage.

- If the blood sample has clotted, a second blood sample must be collected. Specimens that have clotted during blood draw can result in inaccurate cell counts (WBC, RBC and platelets).
- An EDTA blood sample, **three days or older cannot be accepted** for a CBC as cellular deterioration precludes accurate assessment and classification of erythrocytes and white cells. Age related changes includes lysis and disintegration of white cells, pyknotic nuclei, the appearance of toxic change and swelling and lysis of erythrocytes causing alterations in the red cell parameters. Platelets will clump, degranulate and lyse. Slides made at the time of blood draw can be evaluated.
- If you are submitting the samples with formalin-containing samples or cold packs, protect slide containers from moisture and formalin by placing them in separate sealed bags.

Complete Blood Count (CBC)

- The CBC includes total WBC count, RBC count, Hgb, Hct, MCV, MCH, MCHC, reticulocyte count (if anemic), automated platelet count, MPV, Plateletcrit (dogs only), total solids, manual leukocyte differential and smear evaluation of RBC and WBC morphology and parasite screen. Blood smears freshly made in-clinic are ideal for assessment. Fibrinogen is included with large animal CBC's.
- **Please note:** Ensure that blood smears made in-clinic are submitted concurrently with the EDTA whole blood sample, especially if there is to be any delay in shipping to the lab.
- If the submitted blood sample is clotted or severely hemolyzed (likely attributed to freezing) and a concurrent blood smear is submitted, we can provide a WBC differential, platelet estimate and red cell morphology and bill accordingly. A new sample could also be submitted.

Differential Only

- A manual leukocyte differential can be performed on a freshly made blood smear, but it is more informative if we are provided with the total white cell count as absolute numbers of leukocytes are more informative than relative numbers.

Preparation of Blood Smears

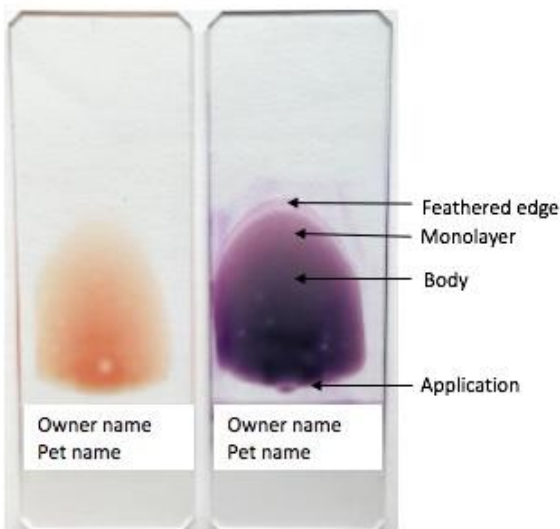
- Properly made blood smears are essential for reliable identification and evaluation of peripheral blood cells.
- Ensure the slides used for blood smears are clean and free from dirt or grease. Beveled edge slides with frosted ends for labeling are preferred.
- Write the **animal and owner name on the slide using a pencil.**
- **To make the slide** gently mix the anticoagulated whole blood well.
 - Fill a non-heparinized (plain) capillary hematocrit tube half way with well-mixed anticoagulated blood, place **1-2** small drops of blood approximately one cm from the frosted edge of the slide.
 - Using dominant hand, use the thumb and forefinger to hold the end of the spreader slide against the surface of the smear with the blood droplet at an angle of 30 to 45°.
 - Draw the spreader slide back to contact the blood and pause to allow the blood to spread and fill the angle between the two slides.
 - Push the spreader slide toward the end of the slide in one quick smooth motion.

- Allow the smears to air dry on a flat surface and place them in slide folders at room temperature until shipping.
- See the following video for more information: [youtube.com/watch?v=nbRUiWI2Qrs](https://www.youtube.com/watch?v=nbRUiWI2Qrs)

Characteristics of a Good Quality Blood Smear

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

Zones of a blood smear



Causes of Poor Quality Blood Smears

- Delay in making a smear can result in abnormal distribution of the leukocytes (typically accumulating at the feathered edge).
- Too large or too small of a blood drop.
- Spreader slide pushed across the horizontal slide in an abrupt or very slow manner can lead to either rupture or uneven distribution of the leukocytes in the blood film.
- Inappropriate angle of spreader slide
 - adjust the angle of the second slide to help compensate for very viscous (hemoconcentrated) or watery (anemic) blood samples
 - If the patient is anemic, increase the angle of the spreader slide.
 - If the patient is hemoconcentrated, decrease the angle of the spreader slide.
- Exposure to formalin, moisture or contaminants (fingerprints, water droplets, fibers or dried blood) on slide or spreader slide result in unusable slides.

Antibody/Antigen Testing

Canine and Feline Coombs' Test

- A Coombs' test is a direct antigen test (DAT) using a microtiter plate method that determines the presence of anti-erythrocytic antibodies, IgG, IgM and complement C3 in dogs and cats. The test aids in the diagnosis of immune mediated hemolytic anemia (IMHA) but is **not specific** for primary immune mediated disease processes, positive Coomb's test can also be seen with neoplasia and infectious diseases. A negative Coombs' test should not be used to rule out IMHA. False negative Coombs' test can occur due to a weak affinity antibody that is removed during washing, low numbers of adherent IgG, IgM or C3 on RBCs and marked anemia.
- The test should be interpreted in light of the CBC, red cell morphology (presence of spherocytes) and biochemistry panel.
- More information can be found in the ACVIM 2019 Consensus Statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. <https://doi.org/10.1111/jvim.15441>
- The test requires a minimum of 2 ml of EDTA whole blood.

Feline IDEXX SNAP® Combo FeLV Antigen / FIV Antibody Test

- Detects feline immunodeficiency virus (FIV) antibody and feline leukemia virus (FeLV).
- Requires a minimum of 1 ml of EDTA or heparin whole blood, plasma or serum.
- For additional information, please visit the [IDEXX website](#).

Canine IDEXX SNAP® 4Dx Plus Test

- Detects antibodies to *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Ehrlichia ewingii*, *Borrelia burgdoferi*, and antigen to *Dirofilaria immitis*. Detection of the antibodies suggests exposure to the organism, but not necessarily current disease. The tests should be interpreted in light of clinical signs, a concurrent CBC and if necessary further diagnostic testing.
- Requires a minimum of 1 ml of EDTA or heparin whole blood, plasma or serum.
- For additional information, please visit the [IDEXX website](#). Also see: k2publishing.ca/bayer_conference_report_2014.pdf

Urine Testing

Full urinalysis (urine dipstick and sediment examination)

- A full urinalysis includes a urine dipstick (chemistry), urine specific gravity and a microscopic sediment examination.
- A minimum of **2 ml** of fresh urine is required for a complete urinalysis.
- The sample must be labelled with the animal and owner name.

- Urine should be collected in sterile medical containers and tightly sealed. Red or yellow top no-additive plastic or glass tubes are acceptable, as are sterile urine containers.
 - Samples submitted in a syringe with a needle attached **will not be accepted**.
 - Refrain from submitting urine in the following containers:
 - non-sterile glass or plastic containers, e.g., food jars
 - capped syringes
 - red-top plastic blood collection tubes with clot activator; the crystalline clot activator in these plastic tubes interferes with the microscopic sediment examination
- Clearly indicate the method of collection: cystocentesis, free catch, catheter or litter box.
- Refrigerate within one hour of collection. Prevent sunlight or UV exposure and keep refrigerated until shipping.
- Samples should be submitted the same day (preferably on ice) or within 24 hours to prevent deterioration of the sample.
- When requesting both urinalysis and urine culture; submit one urine for Microbiology in a **sterile** container and one urine for Clinical Pathology.

Urine Cytology

- Request a urine cytology only if a prior urinalysis has **marked** cellularity or abnormal cells on a fresh sample. **Or** if there is a bladder mass and the sample has been obtained by traumatic catheterization.
- Urine must be **fresh and submitted the same day** unless prior arrangements have been made, as the cells deteriorate quickly. Please also submit a freshly-made unstained smear, using the blood smear technique along with the urine in a red or yellow top no-additive plastic or glass urine tube.

Urine Protein:Creatinine Ratio

- A UPC ratio should only be performed after a complete urinalysis has been performed to ensure the sediment is quiet; red cells, white cells, casts and bacteria can contribute protein in the sample and may make accurate interpretation of results difficult.
- Interpretation of results depends on integration of the serum biochemistry, urinalysis, CBC results and clinical signs. Causes of prerenal protein loss such as: endocrine diseases, fever, exercise and hypertension should be ruled out, as well as post renal causes such as infection and inflammation.
- Patients with **equivocal** proteinuria regardless of the presence or absence of azotemia should be re-evaluated within 2 months and re-classified accordingly.
- Persistent proteinuria in patients with CKD 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.
- IRIS (International Renal Interest Society) provides updated information regarding substaging and restaging of kidney disease according to proteinuria and hypertension on their website: iris-kidney.com
- A minimum of 2 ml of fresh urine is required for determination of UPC ratio.

UPC ratio	Canine	Feline
Non-proteinuric	< 0.2	< 0.2
Questionable	0.2-0.5	0.2-0.4
Proteinuric	> 0.5	> 0.4

Urine Electrolytes

- Urine electrolyte testing of Na, K, Cl, can be performed on urine samples. Measurement of urine electrolytes allows calculation of fractional excretion (FE) of a particular electrolyte compared to serum creatinine and the particular electrolyte, it reflects the kidneys ability to maintain homeostasis. A minimum of 1 ml of urine and 1 ml of serum is required. Urine and serum should be collected at the same time. The patient **should not** have had diuretics or fluids for accurate interpretation.
- $FE = \frac{(\text{urine electrolyte}) \times (\text{serum creatinine}) \times 100}{(\text{serum electrolyte}) \times (\text{urine creatinine})}$

Cytology

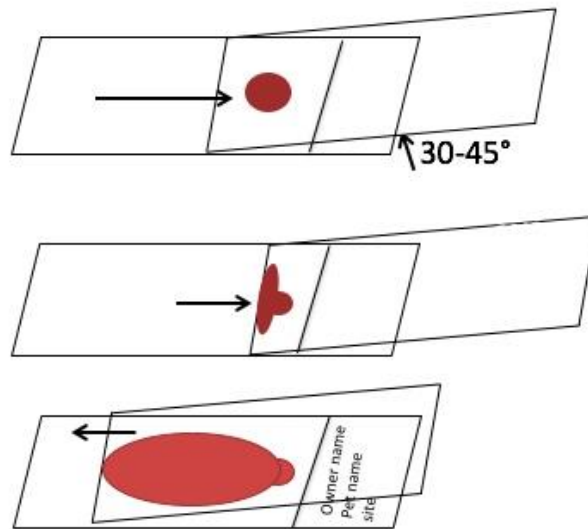
- A detailed history and clinical findings are important for accurate cytological interpretation.
- The following **must** be included in a cytology submission:
 - patient signalment, history and clinical signs
 - gross description of site or mass with specific anatomic location, size, shape and consistency
 - any previous results or case submission numbers, radiographic or ultrasound findings
- Label **all smears** with the animal, owner name and site aspirated (**samples can be rejected if not labeled**).
- Submit fresh, air-dried, unstained smears or in-clinic stained smears. **Do not** heat fix slides.
- Keep all slides at room temperature and protected from moisture, contamination, formalin fumes and extreme temperatures.
- Make and submit multiple slides (up to four slides per site).

Smear Preparation Guidelines

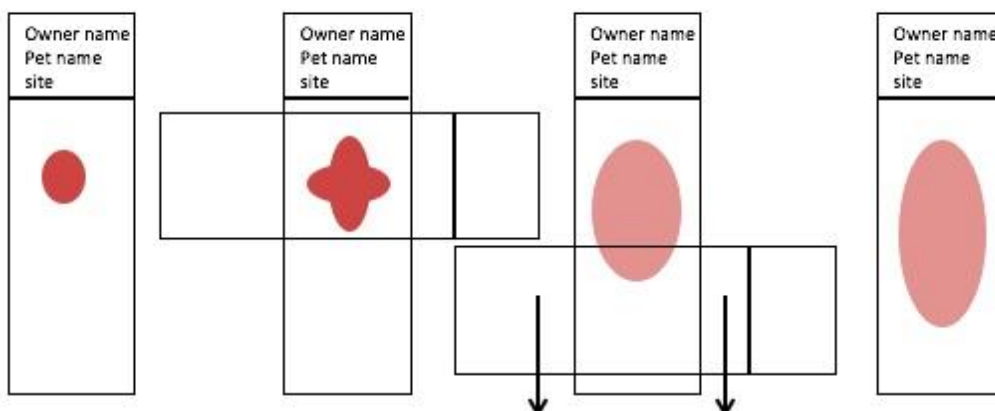
- Samples can be collected by fine needle aspiration, non-aspiration techniques, impression smears or scrapings.
- See the following video for aspiration techniques and smear making: [youtube.com/watch?v=DkXDBom_3JI](https://www.youtube.com/watch?v=DkXDBom_3JI)
- See the following video for non-aspiration or needle technique, best used for **lymph node** aspirates: [youtube.com/watch?v=jgTngCWewl4](https://www.youtube.com/watch?v=jgTngCWewl4)
- See the following video for making smears by tissue impression or touch techniques: [youtube.com/watch?v=prggfKrNbl](https://www.youtube.com/watch?v=prggfKrNbl)

- Prepare fluid cytology slides using the wedge or blood smear technique. Mass and lymph node aspirates should be prepared using the squash preparation technique (see below). **Do not** smear imprints.
- Avoid blood dilution, which can be attributed to prolonged aspiration or using too large of a needle.
- Avoid spraying out the aspirated material onto a slide without spreading it.
 - The small drops of cells sprayed onto the smear yield small clusters of poorly spread-out cells. It is usually impossible to adequately visualize the morphology of these cells.
- Avoid making excessively thick smears.
 - Smears that are too thick will not have cells adequately spread out, making the cells virtually impossible to evaluate.

Wedge preparation for fluid aspirates



Squash preparations for mass aspirates



Cerebral Spinal Fluid (CSF) Analysis

- Please contact the clinical pathologist for information regarding method of sample collection, sample preparation and preservation **prior** to performing the CSF tap. Samples can only be accepted from the Winnipeg area on the same day the sample is acquired. Time from acquisition to delivery to the lab is critical as cells start to dissolve within an hour without the addition of a protein source.
- Fluid must be collected in EDTA and red top tubes tubes for cell preservation. A small amount of fluid should be placed in plain sterile red-top serum tubes (do not use tubes with clot activators) for bacterial culture. Do not submit swabs for culture as they are inferior to the direct fluid. Do not refrigerate the sample. **Two direct smears should be made immediately.**
- CSF analysis includes total nucleated cell count (TNCC), RBC count, total protein and microscopic description of findings.
- The samples must be clearly labeled with the animal and owner name.

Bone Marrow Cytology

- Please contact the clinical pathologist for information regarding method of sample collection and smear preparation before acquisition of your sample.
- Bone marrow must be collected in EDTA tubes for cell preservation and to prevent clotting. Three to four direct smears should be made immediately at the time of sample acquisition. Place a thick drop of bone marrow at frosted end of glass slides, tilt the slide lengthwise to let blood run off, and then spread the remaining adherent particles by gentle squash preparation technique.
- Alternatively, the bone marrow sample may be placed into a weigh boat or Petri dish containing a few drops of EDTA. Fine forceps can be used to pick up the fine bone marrow particles and place then on a glass slide. The particle can then be spread using a gentle squash technique.
- Air dry, **do not** stain the smears. The samples must be clearly labelled with the animal and owner name.
- The remaining bone marrow can be placed in a small EDTA tube for automated hematology analysis, flow cytometry, immunochemistry, PCR or other investigations
- Concurrent **CBC** submission, EDTA sample (included with the price) is necessary for accurate cytological interpretation.
- A concurrent bone marrow core biopsy is also advised to gain the most information about the bone marrow sample, ie: fibrosis, fat, bone density and cell arrangement and architecture which are not evident on cytology smears. A core biopsy should be performed **before** the cytology to avoid the hemorrhage caused by aspiration of the bone marrow.
- Further information can be found at:
 - Comparison of sternal, iliac, and humeral bone marrow aspiration in Beagle dogs. Veterinary Clinical Pathology. Open access: doi.org/10.1111/vcp.12036 Bone Marrow Cytologic and Histologic Biopsies: Indications, Technique, and Evaluation.
 - Veterinary Clinics of North America: Small Animal Practice. doi.org/10.1016/j.cvsm.2011.10.001

Body Fluid Analysis

- Pericardial, thoracic, peritoneal, trans-tracheal wash (TTW), bronchoalveolar lavage (BAL) and joint fluid must be **collected in EDTA tubes** for cell preservation and to prevent clotting. A small amount of fluid should be placed in a plain sterile red-top serum tube for culture should it be needed. EDTA is bactericidal and not acceptable for culture. Do not use tubes with clot activators.
- The samples must be clearly labelled with the animal, owner name and aspiration site.
- Clearly labeled smears (direct, sediment or both) must be concurrently submitted, especially if there will be a delay in sample submission. Make smears with the blood/wedge smear technique. If the sample is very thick use the squash preparation technique.
- Pericardial, thoracic, peritoneal and joint fluid analysis includes total nucleated cell count (TNCC), RBC count, and total solids if there is sufficient sample. Microscopic description of findings accompanies all submissions.
- For tips on joint taps see: Preanalytical Considerations for Joint Fluid Evaluation, Veterinary Clinics of North America: Small Animal Practice 2017. doi.org/10.1016/j.cvs.2016.07.007

Reference intervals for cavity fluids*

Site	Cell count (x10 ⁹ /L)	Protein concentration (g/L)	Differential cell count
Peritoneal	Dogs and cats 0 - 3	10 - 25	Mononuclear cells and neutrophils
	Horses 0 - 10	10 - 25	
	Cows 0 - 20	10 - 25	
Pleural	0 - 3	10 - 25	Mononuclear cells and neutrophils
Synovial	Large joint 0 - 3	10 - 25	Mononuclear cells neutrophils < 10%
	Small joints 0 - 2		
CSF	Dogs and cats < 0.003	Cisternal < 0.30 g/L	Mononuclear cells
		Lumbar < 0.45 g/L	
	Horses and cows < 0.008	Horses 0.29 - 0.79 g/L	
		Cows 0.11 - 0.33 g/L	

*Cowell and Tyler Diagnostic Cytology and Hematology of the Horse 2nd ed., Cowell and Tyler Diagnostic Cytology and Hematology of the Dog and Cat 4th ed, and various peer reviewed journal articles.

Equine Uterine Cytology

- Uterine fluid for equine uterine cytology should be submitted the same day the sample is acquired for the best cell preservation. If this is not possible, centrifuge a small portion of the uterine fluid at low speed (1600 rpm) for 5 minutes, remove the supernatant and make 2-3 smears from the sediment using the squash technique if the sample is flocculent or cloudy, if the sample is clear use the blood smear technique.

Chemistry

- **Serum** is the best sample for chemistry tests, reference intervals are based on serum samples. Plasma from lithium heparin tubes should only be used for **avian or reptile** submissions. Plasma from EDTA tubes (purple top) is **not** suitable for chemistry tests.
- A **minimum of 0.5 ml of serum** is required for a full biochemistry profile, a fasted sample is preferred. For small volumes use pediatric or microtainer tubes.
- The sample must be clearly labelled with the animal and owner name.
- A red-top tube should be used with or without clot activator. Allow the sample to clot for 20 minutes. The sample should be centrifuged within 1 hour of sample collection to avoid hemolysis and alterations in glucose, and the serum separated into a clean, properly labeled tube for shipping.
- Serum separator tubes (SST) have a gel that facilitates separation of serum from cells and reduces hemolysis, eliminating the need to transfer the sample to another tube.
 - Allow sample to clot for 20 minutes, the SST tubes should then be centrifuged for 10 to 15 minutes for proper separation.
 - **SST tubes must not be used for endocrinology or phenobarbital testing.** Please see [endocrine section](#) for more information.
- Proper sampling and handling are very important to avoid hemolysis of serum or plasma.
 - Excessive hemolysis or lipemia can dramatically alter select test results.
- Refrigerate samples or freeze serum if storage is longer than three days.
 - Repeated freeze and thaw cycles are not ideal for serum samples.

A full biochemistry profile includes:

- **Feline and Canine:** Na, Cl, K, Ca, P, Mg, ALKP, ALT, GGT, CK, HCO₃, bilirubin, urea, creatinine, cholesterol, total protein, albumin, globulin and glucose, amylase and lipase
 - Steroid alkaline phosphatase (SALP) is included on canine submissions.
- **Domestic animal**
 - **Dairy:** includes Na, Cl, K, Ca, P, Mg, ALKP, GGT, GLDH, AST, CK, HCO₃, bilirubin, urea, creatinine, cholesterol, total protein, albumin, globulin, glucose BHBA and NEFA.
 - **Beef:** does not include BHBA, NEFA or cholesterol.
- **Equine:** includes Na, Cl, K, Ca, P, Mg, ALKP, GGT, GLDH, AST, CK, HCO₃, bilirubin, urea, creatinine, cholesterol, total protein, albumin, globulin and glucose.
- **Kidney panel:** includes Na, K, Cl, Ca, P, Mg, urea, creatinine, HCO₃, glucose, TP, albumin, globulin, A:G ratio.
 - T4 and urinalysis can be added if requested.
- **Hepatic panel includes:** TP, albumin, globulins, A:G ratio, urea, glucose, cholesterol, total bilirubin, ALT, GGT and ALP.
 - Canine hepatic panel also includes SALP.
 - Equine panel includes GLDH.
 - Bile Acids and urinalysis can be added if requested.

- Other add-on analytes at an additional cost include triglycerides and fructosamine.
- Any analyte can be ordered individually.
 - Electrolytes (Na, K & Cl), bicarbonate (HCO_3), urea, creatinine, calcium (Ca), phosphorus (P), magnesium (Mg), ALKP, Steroid AKLP (canines), GGT, total bilirubin, GLDH, AST, ALT, LDH, CK, amylase, lipase, glucose, cholesterol, triglyceride, total protein, albumin, uric acid.

Serum Bile Acids

- Serum bile acids (SBA) assess hepatobiliary function. Bile acids are synthesized by hepatocytes and released into the small intestine (to aid in the absorption and digestion of fats) and are reabsorbed into the portal circulation. Paired fasting and postprandial samples are typically evaluated, post prandial concentrations are generally higher than fasting SBA samples in animals with a gall bladder. In species without a gall bladder, such as horse, a random sample is sufficient.
- If the animal has increased serum bilirubin or clinical icterus, SBA will be concurrently increased and **the test will not provide additional information** on hepatic function. Lipemia, hemolysis and Ursodiol administration may interfere with the test results. Diseases which affect hepatic function secondarily such as hyperadrenocorticism or small intestinal bacterial overgrowth may increase SBA concentrations.
- A number of variables may decrease SBA concentrations including: prolonged fasting, rapid gastrointestinal transit time, concurrent intestinal malabsorption and lack of hepatic parenchyma sufficient to produce bile acids.
- A normal SBA does not rule out hepatic disease.
- Occasionally fasting samples may have a higher SBA than the post-prandial sample, this is due to gall bladder contraction during fasting, an inadvertent recent meal or delayed gastric emptying. According to canine studies by Dr. Center (University of Cornell), SBA concentrations between 15-25 $\mu\text{mol/L}$ are equivocal, while those with serum bile acids $> 25 \mu\text{mol/L}$ usually have hepatic pathology on biopsy. For felines, post bile acids $> 20 \mu\text{mol/L}$ tend to have histopathological changes on hepatic biopsy.
- Protocol for feline and canine:
 - Fast patient for 12 hours
 - Collect 1 ml blood (red top tube), avoid hemolysis in collecting the sample. Separate serum and label appropriately (pre).
 - Feed 2 teaspoons of canned food for small dogs and cats and two tablespoons of food for larger dogs.
 - 2 hours post feeding draw 1 ml of blood (red top tube), separate serum and label appropriately (post).

Fructosamine

- Serum fructosamine measures the amount of glucose bound to blood proteins (mainly albumin). It is an indicator of blood glucose concentration over the prior 2-3 weeks.
- Fructosamine concentrations above the reference interval are typically seen with diabetes mellitus but not with short term stress hyperglycemia enabling differentiation of the two.

- Fructosamine can also be used to monitor response to insulin therapy. Well controlled animals typically have fructosamine either in the upper reference interval or slightly above.

β-Hydroxybutyrate (BHBA) and Non-esterified fatty acids (NEFA)

Dairy

- Non-esterified fatty acids (NEFA) and β-Hydroxybutyrate (BHBA) can be used at both the individual and herd level in the transition period from early postpartum to early lactation (3-14 days of lactation). Samples should be drawn from the jugular or tail vein as the mammary vein values are significantly lower.
- BHBA is a marker of subclinical ketosis.
 - At an individual animal level, values > 1.2 - 1.4 mmol/L in the first two weeks post calving are associated with increased risk of clinical ketosis, displaced abomasum and metritis as well as decreased milk yield.
- NEFAs are a marker of negative energy balance.
 - Prepartum values of > 0.3 - 0.5 mmol/L and post partum values > 0.7 - 1 mmol/L are associated with negative energy balance and increased incidence of displaced abomasum and decreased milk production. For further information see the following references:
 - *Duffield et al J.Dairy Sci. 2009, 92:571-580*
 - *Mahry, et al. J dairy Sci. 2014, 97: 291-298*
 - **Review on herd testing:** *Ospina et al, Vet Clin North America food animal 2013, 29,2, 387-412*

Canine and Feline

- If ketoacidosis due to diabetes is strongly suspected, but the urinalysis dipstick for ketones is negative a BHBA serum test can be run to detect BHBA associated ketones.

Endocrinology

- Testing includes progesterone, cortisol, total T4, TSH and free T4.
 - TSH & Free T4 are canine specific.
- **Serum** is the required sample for endocrinology tests. The sample must be clearly labelled with the animal and owner name.
 - minimum of 0.5 ml of serum is required
- A **plain** red-top tube should be used. The sample should be centrifuged within 30 minutes of sample collection and the serum separated into a clean, properly labeled tube for shipping.

- SST tubes cannot be used for phenobarbital, progesterone or endocrine analysis.
- Refrigerate samples or freeze them if storage is longer than two days.
 - Repeated freeze and thaw cycles are not ideal for serum samples.

Lab Analysis Schedule

- All samples MUST be received before 3 p.m. to be analyzed on that day.

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

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

Endocrine Testing

Baseline Cortisol

- Baseline serum cortisol is a screening test for adrenal insufficiency which should always be interpreted in light of clinical signs, history and pertinent blood work.
- A baseline cortisol of > 55 nmol/L rules out hypoadrenocorticism.
- A value between 28 and 55 nmol/L is equivocal, hypoadrenocorticism should be considered if the clinical signs are consistent, but normal dogs can have a cortisol below 55 nmol/L. An ACTH stimulation test is recommended for diagnosis.
- If the cortisol is < 28 nmol/L hypoadrenocorticism is likely, but an ACTH stimulation test is recommended for confirmation of diagnosis.
- Factors potentially affecting cortisol concentration or its measurement include: stress, mitotane, ketoconazole and topical or systemic glucocorticoids which suppress cortisol concentration. **Glucocorticoids** (prednisolone, prednisone, methylprednisolone, fludrocortisone, cortisone, hydrocortisone) may also **cross-react** with the cortisol assay spuriously increasing cortisol concentration. Dexamethasone does not cross react but can affect cortisol secretion.

ACTH Stimulation Test

1. Differentiates between normal adrenal function and hypoadrenocorticism (Addison's disease) in dogs and cats. The stimulation test is the gold standard test for this disease. In naturally-occurring canine hypoadrenocorticism, baseline and ACTH stimulated cortisol concentrations are most commonly < 28 nmol/L and < 50 nmol/L respectively. Naturally-occurring hypoadrenocorticism although rare in cats does occur, and is generally due to primary adrenocortical disease resulting in an inadequate response with the ACTH stimulation test.
2. May differentiate between normal adrenal function and possible hyperadrenocorticism (Cushing's) in dogs. Normal dogs may have a post ACTH stimulation cortisol value of > 250 but less than 600 nmol/L. Dogs with hyperadrenocorticism of pituitary or hypothalamic origin typically have post ACTH cortisol values > 600 nmol/L, however a value between 250 and 600 nmol/L does not rule out hyperadrenocorticism.
3. Can detect iatrogenic hypoadrenocorticism due to treatment in dogs and cats.
4. Can help to monitor response to therapy (e.g. trilostane or mitotane) in patients receiving treatment for hyperadrenocorticism. While ACTH stimulated cortisol concentrations vary from below detection to low normal, there is typically little change from baseline concentrations.

Canine ACTH Stimulation Protocol

Synthetic aqueous ACTH cosyntropin (Cortrosyn®, generic cosyntropin; 0.25 mg/ml)**

1. Obtain a baseline (0 hr) blood sample, spin, separate and label appropriately (pre).
2. Inject ACTH 5 µg/kg IV Cortrosyn® to maximum 250 ug per dog.
3. Collect a blood sample 1 hour after injection, spin, separate and label appropriately (post).

**NOTE: 0.25 mg vials of these two synthetic ACTH can be divided into four aliquots (62.5 micrograms) and frozen at -20°C in plastic syringes for up to six months. Keep syringes frozen until prior to administration

Canine ACTH for monitoring response to trilostane or mitotane therapy

Synthetic aqueous ACTH cosyntropin (Cortrosyn®, generic cosyntropin; 0.25 mg/ml)**

1. Obtain a baseline (0 hr) blood sample **4-6 hours post pill**, spin, separate and label appropriately (pre).
2. Inject ACTH 1 or 5 µg/kg IV Cortrosyn® to maximum 250 ug per dog.
3. Collect a blood sample 1 hour after injection, spin, separate and label appropriately (post).

Feline ACTH Stimulation Protocol

Synthetic aqueous ACTH (Cortrosyn®, generic cosyntropin; 0.25 mg/ml)

1. Obtain a baseline (0 hr) blood sample, spin, separate and label appropriately (pre).
2. Inject 125 ug of synthetic ACTH (Cortrosyn™) IM.
3. Obtain blood sample 30 minutes and 60 minutes after injection, spin, separate and label appropriately (post).

Dexamethasone Suppression Tests Canine and Feline

- The possibility that a patient has hyperadrenocorticism (HAC) is based on history and physical examination. Endocrine tests should be performed only when clinical signs are consistent with HAC and there is a high index of suspicion based on the results of routine blood and urine tests.
- Naturally-occurring HAC may be due to pituitary-dependent hyperadrenocorticism (PDH) or a functional adrenal tumor (FAT) in dogs. The majority of cases are due to PDH (~80-85%).
- The low-dose dexamethasone suppression test (LDDST) is the preferred screening test for diagnosing naturally-occurring HAC in dogs due to greater sensitivity and specificity than the ACTH stimulation test.
- In normal dogs, dexamethasone administration causes rapid and prolonged suppression (>8 hours) of cortisol secretion. The LDDST test allows distinction between pituitary and adrenal origin in ~60% of cases. In dogs with PDH not appropriately suppressed by administration of a low dose dexamethasone 75% of dogs with PDH will suppress after using in the high-dose dexamethasone suppression test HDDST. In patients with an FAT, dexamethasone at any dosage does not suppress cortisol secretion.
- For more information, see Diagnosis of Spontaneous Canine Hyperadrenocorticism: 2012 ACVIM Consensus Statement (Small Animal): doi.org/10.1111/jvim.12192

Low dose dexamethasone suppression test interpretation (canine and feline)

Cortisol concentration		Interpretation
4 hour post	8 hour post	
< 40 nmol/L	< 40 nmol/L	Expected, not hyperadrenocorticism
< 40 nmol/L	> 40 nmol/L	Hyperadrenocorticism
< 50% baseline or < 40 nmol/L	< 50% baseline	Pituitary dependent hyperadrenocorticism (60%)
> 50% baseline or > 40 nmol/L	> 50% baseline	No suppression, functional adrenal tumour or PDH

Canine Dexamethasone Suppression Protocols

Low Dose Dexamethasone Suppression Test (LDDST)

1. Obtain a baseline (0 hr) blood sample between 8 and 9 am, spin, separate and label as 0 hour.
2. Inject 0.01 mg/kg dexamethasone IV.
3. Obtain a 3 or 4-hour post and 8-hour post injection blood samples, spin, separate and label appropriately.

High Dose Dexamethasone Suppression Test (HDDST)

1. Obtain a baseline (0 hr) blood sample between 8 and 9 am, spin, separate and label as 0 hour.
2. Inject 0.1 mg/kg dexamethasone IV.
3. Obtain a 3 or 4-hour post and 8-hour post injection blood samples, spin, separate and label appropriately.

Feline Dexamethasone Suppression Protocols

Low Dose Dexamethasone Suppression Test (LDDST)

1. Obtain a baseline (0 hr) blood sample between 8 and 9 am, spin, separate and label 0 hour.
2. Inject 0.1 mg/kg dexamethasone IV.
3. Obtain a 3 or 4-hour and 8-hour post injection blood samples, spin, separate and label appropriately.

High Dose Dexamethasone Suppression Test (HDDST)

1. Obtain a baseline (0 hr) blood sample between 8 and 9 am, spin, separate and label 0 hour.
2. Inject 1.0 mg/kg dexamethasone IV.
3. Obtain a 3 or 4-hour and 8-hour post injection blood sample, spin, separate and label appropriately.

Dexamethasone Suppression Tests Equine

- The overnight (19 hr) low dose dexamethasone suppression test was previously considered the gold standard for the diagnosis of antemortem equine pituitary pars intermedia dysfunction (PPID). However, it is now known the test only has high sensitivity and specificity in end stage PPID when numerous clinical signs are evident. It has shown poor performance in diagnosing PPID in the early stage and has resulted in false negative results. In addition, seasonality affects the test response and seasonal reference intervals have not been established.
- A cortisol value at 19 hours of ≤ 28 nmol/L rules out PPID (indicates suppression of adrenal axis).

- A combined dexamethasone suppression with TRH stimulation test or a plasma endogenous ACTH test before the fall season may be of added value in the diagnosis of early PPID.
- For more information, see the PPID working group (Equine Endocrinology Group) for recommendations sites.tufts.edu/equineendogroup/
- Also see Vet Clin Equine 35 (2019) 327–338 doi.org/10.1016/j.cveq.2019.03.005

Equine Dexamethasone Suppression test -19 hour

1. Obtain a baseline blood sample between 4 – 6 p.m., spin, separate and label 0 hour.
2. Inject 40 µg/kg dexamethasone IM.
3. Obtain blood sample between 11 a.m. – 1 p.m. the following day; spin, separate and label as 19 hours post.

Thyroid Tests

Canine Hypothyroidism

- Hypothyroidism is the most common endocrine disorder in dogs. The clinical signs are variable but can include weight gain, mental dullness and hair or coat changes. Clinicopathological findings often include mild normocytic, normochromic non-regenerative anemia and fasting hypercholesterolemia. Most hypothyroid dogs have primary disease (originating within the thyroid gland) resulting in a low baseline serum total thyroxine (total 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.
- A mid to upper range total T4 rules out hypothyroidism. Diagnosis of hypothyroidism using baseline serum total T4 concentration alone is often not possible due to suppression of total T4 concentration when nonthyroidal illness is present. Glucocorticoids, phenobarbital, and mitotane can also cause a decrease in total T4. In addition, sight hounds have a lower total T4 than other breeds.
- The free T4 is believed to be less affected by non-thyroidal illness and drug administration.
- A concurrent canine specific endogenous TSH (cTSH) is increased in 60-70% of dogs with hypothyroidism.
- Current recommendations for the diagnosis of canine hypothyroidism, particularly in the face of concurrent illness, include serum total T4, free T4 and TSH.
- **Therapeutic monitoring** of treatment (levothyroxine) includes both measurement of total T4 and resolution of clinical signs. Serum total T4 should be measured 4 - 8 weeks after initiation of treatment; if there is no response to treatment; if signs of thyrotoxicosis develop; or if there is a change in dosage.
- Peak total T4 concentration, sample **taken 4 - 6 hrs post-pill**, should be in the upper half of or just above the RI (i.e. 39 - 77 nmol/L). Serum total T4 concentrations should be > 19 nmol/L regardless of time interval between pill administration and sampling
- Measurement of TSH concentration may be useful in treated dogs in which the TSH concentration was above the RI at the time of diagnosis. TSH concentration should be within the RI when measured 4 - 6 hrs after adequate thyroid hormone supplementation.

Feline Hyperthyroidism

- Hyperthyroidism is the most common endocrine disorder in older cats and is usually due to the presence of nodular hyperplasia or adenoma of the thyroid gland. Clinicopathologic findings commonly seen are erythrocytosis, increased ALT and/or ALP.
- The diagnosis is usually easily confirmed based on a high baseline serum total T4 concentration. However, baseline total T4 concentration may be within the RI in cats suspected of having hyperthyroidism if there is concurrent disease or non-thyroidal illness (euthyroid sick). Underlying kidney disease is especially common in cats with hyperthyroidism. Under these circumstances, additional diagnostic options are: measurement of free T4 by equilibrium dialysis (send out test), repeat total T4, or T3 suppression.
- Free T4 by dialysis identifies most cats with mild hyperthyroidism or those with nonthyroidal illness. The false positive rate is 5 - 6 %. The values should be interpreted in the context of the clinical signs and the total T4.
- **Therapeutic monitoring** post initiation of treatment is recommended depending on the therapy type. A total T4 should be repeated at 2 - 4 weeks post oral medication initiation, and 2 weeks after a dose change. By 2 - 4 weeks the peak and trough levels should be about equal. A repeat biochemical panel is also recommended due to possible changes in cardiac output and glomerular filtration that occur with treatment.

Other

Phenobarbital

- **Initiating phenobarbital therapy:** Serial phenobarbital concentrations should be assessed at 2 weeks (the first steady state concentration point) and 6 weeks (steady state clearance point) after the initiation of phenobarbital to assess if enhanced clearance has occurred. Trough levels are advised for these time points. Hepatic enzymes should also be monitored at this time.
- **Maintenance therapy:** In most dogs and cats that have received phenobarbital for > 6 weeks, fasting trough or peak phenobarbital levels do not differ significantly. But, for comparison between samples, fasting and consistency in time of sampling to avoid diurnal variations and food interference is recommended.
- The most efficacious and safe therapeutic range for dogs (according to the ACVIM consensus) is 65-151 $\mu\text{mol/L}$, although efficacy can be seen at lower concentrations. Monitoring at 6 month intervals and evaluation of hepatic enzymes for hepatotoxicity is recommended.
- The administration of phenobarbital does not interfere with the response to exogenous ACTH or the low dose dexamethasone suppression test. However, total and free T4 concentration may be low in dogs treated with phenobarbital, when testing for hypothyroidism care needs to be taken in the interpretation of the results.
- See the 2015 ACVIM Consensus Statement: doi.org/10.1016/jvim.13841

Fecal Occult Blood

- This test is useful:

- for cases of suspected GI ulceration or neoplasia
 - when microcytic hypochromic anemia is detected
 - when there is anemia with low total protein and no obvious signs of blood loss
- A positive fecal occult blood supports blood loss into the GI tract; however, false positives do occur as naturally occurring peroxidases and catalases in red meat, some fruits and vegetables that are frequently found in dry/wet dog food can cause a positive reaction.
 - The animal should be on a strict diet prior to fecal occult blood analysis to minimize both false positives and false negative results. **Do not feed** red meat, raw vegetables or fruits for 3 days prior to testing to prevent false positive tests. Vitamin C supplements or vitamin C enriched foods should also be avoided for 3 days prior to testing as they can cause false negative results. Results should be interpreted in light of sample collection, medications and clinical signs.
 - See the Cornell EClinPath website for more information: eclinpath.com/chemistry/iron-metabolism/fecal-occult-blood/

Ethylene Glycol

- When ethylene glycol (EG) or antifreeze is ingested it is converted by the liver into toxic by-products that can cause acute kidney injury and death.
- This is a **qualitative test** for ethylene glycol and metabolites.
- Serum or plasma can be used for the test. Separate plasma or serum immediately, store at 2-8°C.
- Collect sample before the administration of diazepam, activated charcoal or other gastrointestinal absorbents. These products contain substances similar to ethylene glycol such as propylene glycol, glycerol and sorbitol and may cause **false positives**.
- Ethanol and Fomepizole (4-methylpyrazole) antidotes do not interfere with the test.
- **False negatives** can occur if not enough EG has been ingested (as is often the case with cats) or if the time from ingestion is greater than 24- 48 hours. Peak absorption is typically 1-6 hours.

Appendix 1- Blood Collection Tubes

Appendix 2 - Chemistry Reference Intervals

Test	Units	Canine	Feline	Equine	Bovine (Dairy)	Porcine
Sodium	mmol/L	140 - 154	147 - 157	136 - 144	132 - 145	140 - 150
Potassium	mmol/L	3.8 - 5.4	3.6 - 5.2	3.1 - 4.3	3.9 - 5.6	4.7 - 7.1
Na/K Ratio		29 - 37	30 - 41	33 - 44	28 - 36	21 - 30
Chloride	mmol/L	104 - 119	114 - 123	95 - 104	90 - 113	99 - 105
Bicarbonate	mmol/L	15 - 25	14 - 21	25 - 36	17 - 26	-
Anion gap	mmol/L	13 - 24	15 - 26	6 - 21	13 - 22	-
Urea	mmol/L	3.5 - 9.0	6.0 - 12.0	4.2 - 8.9	3.0 - 8.0	3.0 - 8.5
Creatinine	µmol/L	53 - 124	71 - 186	71 - 133	35 - 80	90 - 240
Calcium	mmol/L	2.50 - 3.00	2.22 - 2.78	2.75 - 3.35	2.10 - 2.80	1.80 - 2.90
Phosphorus	mmol/L	0.90 - 1.85	0.80 - 2.29	0.73 - 1.71	1.47 - 2.63	1.6 - 3.4
Magnesium	mmol/L	0.70 - 1.00	0.80 - 1.10	0.60 - 1.00	0.82 - 1.30	0.8 - 1.6
Amylase	U/L	299 - 947	482 - 1145	-	-	-
Lipase	U/L	25 - 353	12 - 32	-	-	-
ALKP	U/L	22 - 143	12 - 60	119 - 329	25 - 127	0 - 500
Steroid ALKP	U/L	0 - 84	-	-	-	-
GGT	U/L	0 - 7	0 - 6	7 - 54	11 - 51	10 - 400
Total bilirubin	µmol/L	0 - 4	0 - 4	21 - 57	0 - 5	0 - 8
Unconj. bilirubin	µmol/L	0 - 3	0 - 3	18 - 52	0 - 3	0 - 4
Conj. Bilirubin	µmol/L	0 - 1	0 - 1	2 - 10	0 - 2	0 - 4
GLDH	U/L	-	-	1 - 7	3 - 45	-
AST	U/L	-	-	259 - 595	44 - 153	-
ALT	U/L	19 - 107	31 - 105	-	-	-
CK	U/L	40 - 255	94 - 449	108 - 430	44 - 211	0 - 800
Glucose	mmol/L	3.3 - 7.3	4.4 - 7.7	3.7 - 6.7	2.5 - 4.3	3.6 - 5.3
Cholesterol	mmol/L	3.60 - 10.20	2.00 - 12.00	1.70 - 2.70	1.73 - 7.73	2.0 - 5.0
Triglycerides	mmol/L	0.20 - 1.30	-	-	-	-
Total Protein	g/L	55 - 74	60 - 82	58 - 75	66 - 86	61 - 81
Albumin	g/L	29 - 43	30 - 44	30 - 37	30 - 42	27 - 39
Globulin	g/L	21 - 42	23 - 42	26 - 41	30 - 53	34 - 43
A/G Ratio		0.7 - 1.8	0.7 - 1.4	0.8 - 1.3	0.6 - 1.3	0.5 - 1.2
Calculated Osmolality	mmol/L	276 - 306	296 - 316	269 - 287	265 - 295	280 - 320
BHBA	mmol/L	< 0.20	< 0.20	-	0.40 - 1.30	
NEFA	mmol/L	-	-	-	0.10 - 0.37	
Fructosamine	µmol/L	170 - 338	219 - 347	-	-	
Bile acids - fasting	µmol/L	0 - 5	0 - 5	-	-	
Bile acids - 2 h post prandial	µmol/L	0 - 15	0 - 10	0 - 6	11 - 60	

Appendix 3 - Hematology Reference Intervals

Test	Units	Canine	Feline	Equine	Bovine (Dairy)	Porcine
Leukocytes	x10 ⁹ /L	4.9 – 15.4	4.2 – 13.0	5.1 – 11.0	5.1 – 13.3	11.0 – 22.0
Neutrophils segmented	x10 ⁹ /L	2.9 – 10.6	2.1 – 8.3	2.8 – 7.7	1.7 – 6.0	4.0 – 9.5
Neutrophils band	x10 ⁹ /L	0.0 – 0.3	0.0 – 0.3	0.0 – 0.2	0.0 – 0.2	0.0 – 0.6
Eosinophils	x10 ⁹ /L	0.0 – 1.3	0.0 – 1.6	0.0 – 0.7	0.0 – 1.2	0.0 – 2.2
Basophils	x10 ⁹ /L	0.0 – 0.1	0.0 – 0.26	0.0 – 0.1	0.0 – 0.2	0.0 – 0.3
Lymphocytes	x10 ⁹ /L	0.8 – 5.1	1.1 – 8.1	1.3 – 4.7	1.8 – 8.1	6.0 – 12.6
Monocytes	x10 ⁹ /L	0.0 – 1.1	0.0 – 0.5	0.1 – 0.8	0.1 – 0.7	0.3 – 2.2
Platelets	x10 ⁹ /L	117 – 418	93 – 514	83 – 270	160 – 650	325 – 700
MPV	fL	7 – 14	8 – 21	6 – 11	4.6 – 7.4	-
Plateletcrit	%	0.14 – 0.47	-	-	-	-
Erythrocytes	X10 ¹² /L	5.8 – 8.5	6.2 – 10.6	6.9 – 10.7	4.9 – 7.5	5.0 – 8.2
Hemoglobin	g/L	13 – 197	93 – 153	112 – 169	84 – 120	99 – 158
Hematocrit	L/L	0.39 – 0.56	0.28 – 0.49	0.28 – 0.44	0.21 – 0.30	0.32 – 0.50
MCV	fL	66 – 75	39 – 52	42 – 53	36 – 50	51 – 68
MCH	pg	21 – 25	13 – 17	14 – 18	14 – 19	17 – 22
MCHC	g/L	321 – 360	317 – 350	328 – 364	380 – 431	300 – 341
RDW	%	11 – 14	14 – 17	16 – 20	16 – 20	-
Reticulocytes	x10 ⁹ /L	<80	<60	-	-	-
Total solids	g/L	55 - 75	60 – 80	57 – 75	60 – 80	50 – 80
Fibrinogen	g/L	-	-	1 - 4	3 - 7	-