IN-HOUSE DIAGNOSTICS: WHAT THE EMERGENCY CLINICIAN NEEDS TO KNOW

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Collection of blood for diagnostic testing can be challenging in exotic pet mammals, especially in smaller species. Modern technologies allow acquisition of useful information from very small samples, but samples still must be of high quality and meet minimum volume requirements. The challenge of blood collection is compounded in critically ill, hypovolemic, and/or hypothermic patients. In many cases, initial therapy must precede sample collection.

Volume of sample is limited by patient size. A common guideline is to collect no more than 10% of blood volume. Total blood volumes vary from species to species but can generally be assumed to be 6% to 8% of body weight. Clinical judgment may necessitate adjustments to recommended volume limits.

Samples can be run with in-house equipment or sent to reference laboratories familiar with diagnostic testing in these species. The most compelling advantage of in-house diagnostic testing is rapid results. Abaxis (Abaxis, Union City, CA) in-house blood chemistry analyzer can run a chemistry panel in 13 minutes on whole blood samples, and practical minimum volume is 0.15 cc of high quality whole blood. Reference laboratories will often analyze smaller samples, but may use dilution techniques. Individual laboratories should be contacted for instructions on submission of low-volume samples for analysis.

When sample volume does not allow clinical chemistry or complete blood count, a manual complete blood count (CBC) can be obtained on a single drop of blood collected into a heparinized hematocrit tube. Information that can be obtained from a manual in-house CBC includes hematocrit, differential white blood count, and analysis of both red and white blood cell morphology and characteristics, complete blood count utilizing a hemocytometer, and measurement of total serum solids.

Sample collection in small exotic mammals requires practice. Collection site depends on species, patient condition and practitioner preference. The following guidelines are offered based on the author's personal preference and experience, and others may advocate other sites or techniques. The "correct" technique is that which results in consistently good results with optimal patient safety.

RISKS OF BLOOD SAMPLE COLLECTION

Venipuncture inherently involves some degree of risk, including risk of injury or death caused by restraint and handling. Risks from blood collection itself include vessel laceration and hemorrhage, infection, inadvertent damage to adjacent soft tissue structures, and sudden hypotension due to decreased blood volume. Careful patient evaluation, restraint and technique help minimize risk. In the author's opinion, it is best to delay blood sample collection in patients that are hypothermic, hypovolemic, and/or severely depressed. Many patients benefit from gentle warmth and fluid administration for 4 to 6 hours, with frequent re-evaluation.

RESTRAINT

While restraint is relatively easy in larger species such as ferrets and rabbits, it is increasingly challenging in smaller species. The method often depends upon the preferred collection site. In some cases, sedation or anesthesia is appropriate and necessary, especially when considering collection from the vena cava in species other than the ferret. Guidelines for safe sedation and anesthesia are discussed below.

In the author's experience, most ferrets can be safely restrained manually for blood collection by scruffing and stretching, or by rolling the patient in a towel with the head, neck, and forelimbs exposed. It should be noted some ferrets will not tolerate this form of restraint and may require sedation or anesthesia as discussed below.
Rabbits are prone to self-injury during restraint and handling, especially luxation and subluxation of the vertebrae caused by kicking with powerful rear legs. Restraint, therefore, must minimize this risk. The author prefers using a burrito-style towel wrap with either the head or caudal portion of the rabbit exposed, depending on desired collection site. Again, sedation can greatly reduce stress of handling.

Guinea pigs and larger rodents can often be restrained by simply grasping them securely around the shoulders and thorax or with a towel wrap. It becomes exceedingly difficult to safely manually restrain species smaller than the guinea pig for blood collection, and safe sedation and/or anesthesia become even more important.

SEDATION AND ANESTHESIA FOR VENIPUNCTURE

For individuals where manual restraint for sample collection is difficult and risky, or patient struggling is excessive, sedation and/or anesthesia should be considered. The risk of sedation and anesthesia must be balanced against the risk of foregoing diagnostic blood testing. A number of sedation and protocols can be considered, and method should be chosen depending on a number of factors, including overall patient condition. Methods include simple sedation and anesthesia with injectable and/or inhalant agents. The author has found administration of intravenous (IV) or intramuscular (IM) midazolam at 0.25 mg/kg combined with butorphanol at 0.4 mg/kg IV or IM extremely useful to reduce stress for simple procedures such as sample collection and catheterization in exotic companion mammals. It should be noted that collection from the vena cava in smaller patients requires anesthesia to prevent patient injury.

BLOOD SAMPLE COLLECTION SITES

Sample site is based on clinical experience and practitioner preference. Author preferences are listed in Table 1. The greatest limiting factors with regard to site selection are patient size and ability to safely restrain.

Table 1. Suggested Blood Collection Sites for Selected Exotic Mammal Species, in Order of Author Preference

Species	Method	Comments
Ferret	Cranial vena cava	Vena cava approach especially easy and safe due to particular anatomy of the ferret, even with manual restraint
	Jugular	
	Tail vein	
Rabbit	Auricular or Central ear artery	Reported risk of vessel damage with auricular method, rare in author's experience
	Lateral saphenous	Restraint relatively easy
	Medial femoral	Restraint relatively easy
	Cranial vena cava	Manual restraint for vena cava approach extremely stressful and not recommended
	Cephalic	Reduced size makes adequate sample collection difficult
Guinea Pig	Lateral saphenous	Restraint relatively easy, is located more proximally than expected. Shaving enhances visualization.
	Cranial vena cava	Manual restraint for vena cava approach is extremely stressful, and potentially dangerous, and therefore not recommended
	Cephalic	Reduced size makes adequate sample collection difficult
Rodents	Cranial vena cava	Manual restraint for vena cava approach is extremely stressful and potentially dangerous, and therefore not recommended
	Lateral saphenous	
	Tarsal vein	Note: Manual restraint for collection from any site increasingly difficult
	Lateral tail vein	
Hedgehogs, gliders	Cranial vena cava	Manueal restraint for vena cava approach is extremely stressful and potentially dangerous, and therefore not recommended
		Manual restraint impossible in all but extremely debilitated patients

Notes on Sample Collection from the Vena Cava

Exotic practitioners have utilized the vena cava as a safe and effective method of sample collection for many years. Careful understanding of the anatomical relationship of the vessel, the heart, and external landmarks greatly reduces risk. In the ferret, the vena cava is exceptionally long due to relative caudal placement of the heart in the thoracic cavity. The cava is surrounded and protected by fat, and is accessible just below the notch of the sternal manubrium. Correct needle placement is very shallow, and poses no risk to inadvertent penetration of the heart.

The cava is shorter in other species, and the distance between accessible vessel and the heart is progressively smaller as patient size decreases. Use of small, short needles (1/2- and 5/8-inch, 25- to 27-gauge) reduces risk. In all but very small guinea pigs, these needles will prevent inadvertent puncture of the heart. In other smaller species, however, risk of cardiac penetration is greater, and the practitioner must avoid advancing the needle into the thoracic cavity. While not ideal and absolutely to be avoided, it should be noted that in most cases, inadvertent cardiac puncture is not associated with severe complications or death in the still, anesthetized patient. It should be stressed that sedation and often general anesthesia are absolutely required for collection from the vena cava. A possible exception is venipuncture of calm, well-restrained ferrets.

The general procedure for vena cava puncture is as follows:

- Isolate the center of the cranial sternum and manubrium
- Insert a 25- to 27-gauge needle to the right or left of the manubrium at a slight angle aiming for the opposing hip
- Advance the needle while applying negative pressure, redirecting slightly until a flash of blood is detected. The vessel is very close to the surface under the cranial sternum, and it is not necessary to advance the needle very far
- Blood should flow readily after detecting the flash. Failure to flow often means the needle was advanced into and through the vessel, or the bevel is obstructed by the vessel wall. Pull out, rotate and/or redirect slightly until blood flow improves

SAMPLE HANDLING

Analysis of blood samples is markedly impacted by sample quality. Poor sample quality will result in no results (sample failure) or inaccurate results. When working with small exotic companion mammals, the author prefers collection using smaller sized needles and heparinized syringes (1 cc syringe with 27- to 25-gauge needles), or in case of particularly small vessels, venipuncture with a needle only, and collection from the hub into heparinized hematocrit tubes. While the use of heparin may interfere with some analytes, collection without heparin resulted in unacceptably high rate of coagulated samples, and samples containing large fibrin clots. Samples for in-house analysis are processed immediately (preparation of blood film, determination of packed cell volume [PCV] and total serum solids, and in the author's practice chemistry panel run on whole blood using an in-house analyzer [see above]).

Samples intended for outside laboratory analysis should be processed and delivered per reference laboratory instructions and recommendations for the handling of smaller samples.

SAMPLE ANALYSIS

While a full complete blood count and chemistry panel are ideal, the minimum database for the critical care patient is PCV/total serum solids (TSS), examination of the blood film (differential), blood glucose, and blood urea nitrogen (BUN).

In the critical care setting, in-house testing represents a significant advantage over submission of samples to reference laboratories in terms of speed of test results. In-house testing allows analysis and decision making prior to initiation of specific therapy. Critical patients can seldom wait hours to days for test results. As mentioned above, several in-house chemistry analyzers can perform speedy analysis of small test samples. The author prefers the Abaxis in-house chemistry analyzer, which can accept samples as small as 0.15 mL high quality whole blood. In-house hematology analyzers have for the most part not been validated in exotic pet species, but can be useful as well.

Comments on specific analytes are listed below:

PCV and Total Serum Solids

Determination of PCV with total serum solids can reveal anemia, or conversely suggest dehydration, and are important when planning treatment. In general a low PCV and TSS suggests anemia due to blood loss whereas a low PCV and elevated TSS suggests anemia due to other causes. Normal PCV with elevated TSS suggests dehydration.

Blood Smear (Differential)

The blood smear is evaluated for rough cell numbers and cell quality. Abnormalities such as left shift, white cell toxicity and absence of polychromasia in the face of anemia are important in the evaluation of the critical patient.

Glucose

Exotic companion mammals are prone to stress hyperglycemia. If the glucose is higher than 300, measurement of blood glucose should be repeated and/or evaluated with urine glucose. As in small mammals, diabetes is documented with repeatable high blood glucose with a glucosuria.

Hypoglycemia is seen occasionally, especially in any sick anorectic animal. Pancreatic islet cell tumors of ferrets (insulinoma) are of special concern, and commonly produce mild to profound hypoglycemia. Diagnosis is based on measurement of hospital-monitored fasting blood glucose of 65 mg/dL or lower. Most experts recommend fasting for 6 hours; however blood glucose should be measured at the first indication of symptoms suggesting hypoglycemia, including weakness, lethargy, nausea, drooling, and hind limb weakness.

Measurement of glucose is straightforward. Practitioners have investigated the use of human hand-held glucometers, especially in patients requiring serial or repeated monitoring. It should be kept in mind that many human units are not calibrated to be accurate at levels typical of hypoglycemia exotic mammals. Any hand-held device should be checked against standard measurement of glucose to determine accuracy at lower glucose levels.

Blood Urea nitrogen (BUN)

BUN is reduced in patients with liver disease and elevated in patients with pre-renal, renal, or post-renal azotemia. A quick estimate of the BUN can be obtained by placing a drop of blood on a commercially available Azostick (Bayer, Pittsburgh, PA) and evaluating the color change. Because this test is subject to operator error and the subjective ability to assess color changes, actual measurement of BUN is preferred. Assessment of BUN can be very helpful in evaluating animals with vomiting and anorexia, as the underlying cause of illness may be renal disease rather than primary gastrointestinal disease. Whenever possible, a urine sample should be obtained prior to administering fluids. Concentrated urine with an elevated BUN is consistent with dehydration, whereas isosthenuria with azotemia generally indicates primary renal disease.

OTHER DIAGNOSTIC TESTS

Additional tests are performed based on history, physical exam, and results of other diagnostic testing. Routine hematology and chemistry analysis includes a CBC and at a minimum liver enzymes, glucose, BUN, creatinine, calcium, phosphorus, albumin, and electrolytes. Hematology and biochemistry tests are important in the diagnosis of infectious, metabolic, inflammatory, and some toxic diseases.