Clinical Technique: Amphibian Hematology: A Practitioner's Guide

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Abstract

Amphibian hematology is challenging because of a combination of several factors including small patient size, few venipuncture sites, lack of normative data, and basic variability of the amphibian leukocyte and erythrocyte counts. This variability in amphibian red blood cell and white blood cell counts is based on a number of extrinsic (e.g., temperature, diet, season, light cycle) and intrinsic (e.g., species, gender, life style) factors. If possible, to best guide amphibian hematological interpretation, a conspecific, same gender animal can be sampled for comparison to dispel extrinsic and intrinsic variability. However, the collection of hematological measurements in the single amphibian patient can still provide useful clinical information to guide therapy of even the most diminutive amphibian patient. Therefore, the following brief guidelines are presented in an attempt to guide the clinical practitioner as to collection and interpretive techniques, which can easily be adapted to clinical practice for these fragile jewels of nature. Equipment necessary for venipuncture, venipuncture sites, a venipuncture technique, a technique for determination of an estimated white blood cell count and differential, and a guide for differentiation of leukocytes of the amphibian are given. This guide should by no means supplant a thorough review of the literature or consultation with a clinical pathologist, but will provide general rules of thumb for quick interpretation. Excellent reviews of sampling and complete blood count interpretation are listed in the references.

Key words: **amphibian; hematology; blood collection; venipuncture; clinical pathology**

When collecting blood from amphibian species, one should use appropriate materials for venipuncture, proper handling of the animal, and more commonly, sedation or anesthesia. Use suitable restraint including talc-free, distilled water-washed examination gloves and a deionized water-washed surface; give due consideration to sedation or light anesthesia to give the best chance of successful sample collection and the least chance of harming the amphibian. At the minimum, be sure to record the following information with any amphibian hematology sample: time, date and site of collection; patient species, gender, age, and life stage; and season, diet, and light cycle.

Recommended materials for amphibian blood sampling and blood smear preparation are given in Table 1 and Figure 1. Syringes may be precoated by drawing a small amount of heparin into the syringe and then expelling any excess if a difficult or slow blood collec-

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Table 1. Equipment necessary for amphibian hematological sampling

Equipment	Manufacturer	Comments
Accusure Insulin Syringe 0.3-0.5 mL	Qualitest Pharmaceuticals, Inc., Huntsville, AL	Zero volume hub, attached needle 28 gauge, 1/2''
Selected micro cover glasses	VWR Scientific Inc., Westchester, PA	"Cover slips" for blood smear prep
Gold Seal Rite-On Microscope Slides	Erie Scientific, Portsmouth, NH	1.0-mm thickness
Dog nail trimmers, guillotine style	Resco, Walled Lake, MI	Removal of hub/needle of zero volume attached needle syringes; conserves sample volume
Heparinized hematocrit tubes	Drummond Scientific Company, Broomall, PA	PCV and total solids determination; may be used to create small drop size to facilitate amphibian blood smears
Lithium heparin microtainers	Becton-Dickinson, Franklin Lakes, NJ	May be used to store hematologic samples

tion procedure is expected. Additionally, if sepsis is a concern, consider reserving one drop of blood without anticoagulant for placement onto a mini-tip culturette or directly into an enrichment broth, which then should be incubated at enclosure temperature. When collecting blood for culture, aseptic technique, which includes adequate patient preparation and strict surgical preparation of the skin to avoid culture contamination, is necessary. Contacting the bacteriological laboratory for appropriate materials, such as pediatric blood culture vials, and instructions for sample collection and storage should occur before the procedure for best diagnostic results. Although the collection of samples before antibiotic administration is preferred in a patient where sepsis is a concern, the collection of samples after antibiotic administration is not contraindicated.

Methods of blood collection vary based on the species. Most commonly used venipuncture sites include the ventral caudal tail vein, the ventral abdominal vein, and the heart. Peripheral venipuncture may not require chemical restraint and may be performed in a manner similar to that used on the tail vein of bovids and as in reptilian species for the ventral abdominal vein. For the abdominal vein or cardiopuncture, once the animal is positioned appropriately, it is best to visualize the venipuncture site. For the abdominal vein, visualization may be aided by a bright, cool fiber-optic light source to transilluminate the vein from the side or behind or by ultrasound guidance. The ventral abdominal vein may be visualized without additional aids as it courses down the ventral midline in amphibians with minimally pigmented, translucent skin. The cardiac impulse can be visualized in some species without additional aids. However, the dense skin pigmentation of many species necessitates the use of a Doppler flow probe (Fig 2), ultrasound, or cool light transillumination to confirm the blood flow in the heart or in other vessels before venipuncture. As in other species, cardiac puncture is not without risk and should be performed only in an anesthetized animal with blood aspiration from the ventricle with a small-gauge needle (Fig 3).

A small-gauge needle limits trauma to the amphibian and will not damage blood cells as long as minimal aspiration pressure is applied. Use of an insulin syringe with a zero volume hub also maximizes recovery of the sample; however, the needle must then be removed for

Figure 1. Basic equipment for amphibian hematological sampling. Pictured: Guillotine canine nail trimmers, 0.3-mL insulin syringe with zero hub needle attached, slides, cover slips, heparinized PCV tubes, and lithium heparin microtainers. Additional materials for restraint of the amphibian are necessary (talc-free examination gloves washed with distilled or deionized water, large dog mask for use as induction chamber for isoflurane anesthesia, nonbleached paper toweling or surface which has been moistened with distilled or deionized water).