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Association of Primate Veterinarians Blood Collection Guidelines

Purpose

This document is intended to serve as a reference for the establishment of new policies or the assessment of current policies regarding blood volume collection limits and blood collection techniques in nonhuman primates (NHP).

Background

There is a paucity of literature available on safe blood collection volumes and practices, especially for nonmacaque and New World primate species. Blood collection practices for research or clinical purposes should be designed to prevent both harm to the animals and the introduction of experimental confounds associated with excessive blood collection.

Guidelines

Determining safe collection limits.

Blood collection limits should be expressed as a proportion, or percentage, of an individual animal's circulating blood volume (CBV) that may be collected over a set period.

Estimating CBV. CBV is dependent on lean body mass. Lean body mass may be measured directly using bone densitometry (DXA) scan or estimated using body weight (BW) and body condition scoring (BCS). The positive correlation between BCS and % body fat has been validated in NHP.[16]

Safe collection volumes. The percentage of CBV that may be safely collected typically ranges from 10% to 20%. This percentage should take into consideration the period of collection. The time needed for full recovery from blood collection may vary among individuals depending on immune status, age, sex, and disease state. For healthy animals, a 'rolling sum' is often used wherein the total volume of blood collected must not exceed the policy limit over a given period, for example, 15% of CBV over a 21-d period. This allows 21 d of recovery after a single maximum blood collection and takes into consideration the cumulative effects of multiple submaximum collections. There is little evidence in the NHP literature regarding recovery times needed after large single collections compared to recovery times needed for smaller, more frequent collections. The values reported here represent common industry standards. This is an area in need of further investigation.

Practical application. A 'rule-of-thumb' that is commonly applied at biomedical institutions is the '10%:10% rule,' wherein the maximal sample volume allowed is 10% of the blood volume, and the blood volume is estimated as 10% of the body weight (approximately 100 mL/kg).[9,15,19] The '10%:10% rule' was borne of mathematical convenience rather than scientific evidence and has been shown to overestimate the maximum blood collection limits in overweight/obese animals. In light of this, institutions are encouraged to incorporate algorithms and mathematical formulas for blood collection limits with the assistance of their medical records so that a specific animal's total volume of blood available for withdrawal may be recorded. Institutions with electronic medical record systems may be able to utilize tracking functions to record volumes withdrawn and automatically subtract from the total available during a given period. In the example of a 21-d 'rolling sum,' a large blood withdrawal from 21 d prior falls out of consideration on day 22. Thereby, the amount available increases on day 22, so blood collections may be planned in advance to avoid potential over-draws.

Formulas for estimating CBV for macaques: Body weight and body condition (or percentage of body weight that is composed of fat) are the primary predictors of CBV.[11]

Body condition may be determined objectively using DXA. When an objective value for percentage body fat is available, CBV (in mL/kg) may be calculated using the following formula:

$$CBV = 79.814 - (fat\% \times 0.981)$$

Because DXA may be an impractical method for determining body condition for routine CBV calculation, BCS may be used for predicting CBV (in mL/kg) using the following formula:

$$CBV = 113.753 + (0.752 \times \text{body weight [kg]}) - (18.919 \times BCS)$$

, with BCS graded on a scale of 1 [emaciated] to 5 [obese].[7]

Phlebotomy technique, sites, and considerations for choosing a site.

Technique. Proper technique and sterile equipment should be used. The site is typically shaved and wiped with an alcohol swab prior to venipuncture. Shaving may not be recommended for animals with alopecia, a history of overgrooming, or stereotypies. Depending on the site chosen, the vessel may be either visualized or palpated.

Common venipuncture sites. The femoral vein is the most common collection site for Old World monkeys (OWM) and New World monkeys (NWM) and can be used for large volume collections.[6,14,19] The cephalic, saphenous, and lateral tail veins are often used for smaller volume collections (< 3 mL OWM and < 0.2 mL NWM).[6,10] The jugular vein may be used if a femoral collection is unsuccessful, however for safety reasons the primate should be fully anesthetized.[15] Jugular veins are difficult to access in species with well-developed air sacs, such as pigtail macaques. Because jugular veins are frequently used for indwelling catheters and vascular access ports, caution should be taken to minimize damage from percutanous puncture, depending on study objectives. The ear capillaries may be used for very small samples (~500 µL), especially if repeated collections are needed (for example, blood glucose monitoring).[13]

Considerations. If repeated blood draws are necessary, it is recommended to rotate vessels to prevent adverse effects. Whenever possible, positive reinforcement training (PRT) techniques for repeated blood collection are encouraged.[8,17] PRT has been shown to desensitize animals to stressful stimuli, which reduces fear and anxiety and associated variability. In addition, the animals gain a sense of control and mental stimulation, which improves their overall well-being. PRT also reduces the need for sedation, which reduces variability caused by pharmacological agents. Various studies have shown the positive effects of training animals to either present an arm using a "blood sleeve" or to present a leg through a feeder slot. The use of ear capillary blood collection devices may be well suited for primates that are chair trained especially if they have a head restraint, and

for primates that are trained to present an ear cage-side. NHP with longer tails (for example, cynomolgus macaques) can also be trained to present their tails for capillary sampling.

Adverse effects from venipuncture.

Hematoma and/or hemorrhage from venipuncture site is the most common complication of phlebotomy. The most common clinical sign of hemorrhage is bruising, or ecchymosis, at the site of collection. If severe, hematomas may cause anemia and cardiovascular shock, especially in small animals. Late-developing hematomas may present as mild to severe lameness during cage-side observation.6 Due to concerns of hematoma formation and bruising, pressure should be placed for an adequate amount of time (for example, 2 to 4 min) after phlebotomy to prevent bleeding and hematoma formation at the site.

Arteriovenous or arterial-venous (AV) fistula. AV fistulas are an abnormal communication between an artery and a vein. Fistulous connection of the venous and arterial systems can be an undesirable side effect of lower extremity venipuncture in monkeys. Initial presentation of an AV fistula is usually a palpable 'thrill' in the femoral region which is audible on auscultation. As the fistula matures, clinical signs such as distal limb swelling, disuse and guarding of the limb, prominent vasculature in the femoral groove, and venous congestion caudal to the fistula site may be seen.[20] Multiple needle sticks at the same site may cause penetrating trauma and injury to the vessel, and therefore may be a contributing factor.[5]

Anemia. A single large volume blood collection or sequential blood collections that occur too frequently may result in anemia. Acute blood loss can cause hypovolemic shock, physiological stress, or even death. Therefore, it is important to monitor the red blood cell status of NHP requiring large volume blood collection or frequent blood collections. Clinical signs of excessive blood collection, hematologic monitoring parameters, and treatment options are listed below.

Monitoring NHP during frequent phlebotomy.

Clinical signs of excessive blood collection.[1] In general, healthy animals are able to compensate for mild anemia without demonstrating clinical signs. However, if the animal is unable to compensate for moderate to severe anemia, clinical signs may become evident, including inappetence, weight loss, weakness, lethargy, social isolation, increased heart rate and respiratory rate, and pallor. If an animal becomes hypotensive, nausea and emesis may present.

Monitoring parameters. Anemia presentation and monitoring in NHP is similar to most domestic species (elevated heart rate, decreased systolic blood pressure, changes in complete blood count (CBC).[1,15] CBC reference ranges below are for rhesus macaques (Macaca mulatta).[3] Ranges for other species and parameters may also be found in the APV formulary or other references.[16]

	Adult Males	Adult Females
Hemaglobin (Hb)	4.95–6.09 106/µL	5.46–6.0 106/µL
Hematocrit (Hct)	35.9–44.3 %	38.3–42.3 %
Mean corpuscular volume (MCV)	70–76 fL	67–73 fL

Recovery time and supplementation/treatment. Recovery time varies depending on the amount of blood collected, the frequency of collection, and the individual animal's health status. Therefore, evaluation of hematologic parameters is important for any animal suspected to be anemic. This includes evaluation of these parameters at baseline to determine an animal's suitability for volume blood removal. There are supplementation and treatment modalities that can aid in a faster recovery and provide relief and support after large and/or frequent blood collections. Supplementation with hematinics, such as iron dextran (10 mg/kg IM, maximum dose 100 mg) and cyanocobalamin (250 to 500 µg SQ every 1 to 2 wk), may be beneficial in animals undergoing repeated blood collections due to the potential to develop iron deficiency over time.3 It is important to note that if long-term iron supplementation is required, oral iron supplementation should be considered, as iron overload can occur with long-term parenteral iron.12 Additionally, it may be indicated to halt or decrease blood collection frequency or volume until monitoring parameters return to baseline. Supplemental intravenous or subcutaneous fluids can minimize the effects of hypotension following a single large volume collection.[15] Antiemetic medications may be considered in animals exhibiting emesis or signs of nausea.

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