Association of Primate Veterinarians
Blood Sampling Guidelines for Nonhuman Primates in Biomedical Research

PURPOSE: Although many institutions have adopted their own set of guidelines for blood collection amounts, this document will provide information for those researchers, institutional animal care and use committees (IACUCs), and veterinary staff without a set blood sampling guidelines for nonhuman primates in biomedical research. It is important to remember these are guidelines and it is the laboratory animal veterinarian in consultation with the PI who must balance the well-being of the animal with the need for the blood collection. The principal investigators (PI) and the professional laboratory animal veterinary staff have the responsibility to weigh the scientific impact of the animal’s physiological response to blood collection and how this collection may affect data interpretation and scientific validity. Researchers and veterinarians should also be diligent to familiarize themselves with the specifics of the NHP species proposed for the research planned.

BACKGROUND: These guiding principles are intended for use with normal healthy nonhuman primates (NHPs). The guidelines may need to be modified by the PI and/or the laboratory animal veterinarian for NHPs which are very young, aged, obese, stressed, undergoing heavy menstruating periods, immunosuppressed or immunocompromised, suffering from cardiac and/or respiratory disease which may not tolerate the loss of blood volumes indicated for healthy animals. Prior to any blood collection, a physical health assessment of the animal(s) by the PI and/or veterinary staff should be conducted prior to the collection. This assessment may include hematological (HCT, HGB, TP, RBC, red blood cell indices, etc.) value(s) which, based on the results, could affect the timing of the procedure or amounts of blood which can be collected during the procedure. For animals undergoing multiple collection procedures, the PI and the veterinary staff should be closely monitoring the wellbeing of the animal. When reviewing NHP protocols with blood collection(s) procedures, the IACUC and veterinary staff should thoroughly review the sample volume(s), collection procedure(s), restraint procedure(s), training and experience of the phlebotomist, and the frequency of collection(s) to address any animal welfare concerns with the procedure(s).

GUIDELINES: Most research protocols and general animal health assessments involving NHPs call for blood collection. These blood collections range from a single blood draw, a single terminal blood draw, or serial (repetitive) blood samplings. Each bleeding schedule may require a calculation of the animal’s circulating blood volume. The average circulating blood volume (CBV) for New World Species is approximately 7-8% of lean body weight, whereas rhesus macaques have approximately 55ml/kg and cynomologus macaques have approximately 65 ml/kg CBV. A recent study done in healthy uncompromised macaques showed a weekly 15% circulating blood volume withdrawal for 4 weeks was well tolerated by a small group of animals.
The authors of the study recommended close monitoring of hematological parameters if this amount is needed beyond the four weeks. Any amounts over 15% CBV should be scientifically justified in an approved animal use protocol. For repeated bleeds at shorter intervals (for example in toxico-kinetic or pharmaco-kinetic studies), some institutions have adopted a policy of a maximum of 7.5% of an animal’s CBV can be removed weekly. Due to the need for hematological assessment of the animal while on study, additional recovery time may be warranted in animal use protocols with repeated or serial blood collection procedures. Those studies which may exceed the blood sampling maximum guidelines noted above may be considered if approved by the IACUC and the veterinary staff. Such approval may depend on increased monitoring or supportive treatment such as fluid or iron replacement. When all blood components are not required, blood samples may exceed the calculated limits if the plasma or red cells collected are returned to the animal with the appropriate fluid replacement(s). Also, it is important animals fasted prior to blood draw have continuous access to water to remain hydrated and water is available ad libitum after blood collection.

**VEINIPUNCTURE TECHNIQUE:** When performing a venipuncture of any vessel, proper aseptic technique and sterile equipment should be used. The most common site for venipuncture in the NHP is the femoral vein. Other venipuncture sites include the saphenous, cephalic veins, lateral tail, and jugular veins. The jugular vein also is a common site for a long term catheter or vascular access ports.

If approved by the IACUC, blood samples may be taken by well experienced phlebotomists from alert New World Species from the femoral or lateral tail veins with a 1-2 ml syringe and a 25-26 gauge needle. Old World Species can also be trained to present a limb for blood sampling, this is much less stressful for the animal than being repeatedly manually restrained and/or sedated. The use of topical skin anaesthetics (EMLA®, Maxilene®, etc.) may also be helpful with un-anesthetized blood collection(s). When numerous blood samples are required, placement of an indwelling catheter is preferable to repeated venipunctures. Catheters increase the efficiency and ease of blood sample collection and may reduce the pain and distress to the animal(s). Surgical implantation of a catheter or vascular access port may also allow for long-term blood collection(s) with very minimal distress to the animal.

**Potential Side Effects of Repetitive Blood Collections:**

Hematoma formation and or hemorrhage from the venipuncture site: Pressure should be placed for an adequate amount of time (e.g. 2-4 minutes) to prevent bleeding and hematoma formation at the site. Hematoma formation is more likely if the femoral artery (which is located lateral to the femoral vein) is accessed. Arteriovenous or Arterial-Venous (AV) fistulas: AV fistulas are an abnormal communication between an artery and a vein. Fistulous connection of the venous and arterial system 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\textsuperscript{8}. Contributing factors in causing this condition are multiple needle sticks at the same site and excess negative pressure for blood collection and not disconnecting tubes from vacutainers prior to removing the vacutainer needle.

**REFERENCES:**

1) Laboratory Animal Medicine, 2\textsuperscript{nd} Edition, ACLAM Animal Medicine Series, 2002


4) The Laboratory Primate, 1\textsuperscript{st} edition, Wolfe-Coote, Elsevier Academic Press, 2005


6) Schlam’s Veterinary Hematology (2010), John Wiley and Sons


