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Bird Perfusions with BriteVu®

1. Give 1000 U/Kg animal weight heparin (IV, IP or IM) 30 minutes prior to flushing blood.

2. Anesthetize the animal as per your IACUC, or other regulatory research, protocol.

3. Place an appropriately sized catheter in the basilic (wing), jugular or medial metatarsal vein. Use the largest catheter that will safely fit in the vein.
   Basilic vein: Direct the catheter towards the heart. Once the catheter is in the vein, place a piece of butterflied radiolucent tape around the catheter hub. Suture the tape ends to the skin around the elbow being careful to not lacerate the underlying vessels. This step will help reduce catheter movement.
   Jugular vein: Direct the catheter towards the heart. Once the catheter is in the vein, place 1-2 encircling ligatures around the jugular vein housing the catheter. The ligatures should be tight, however not compressing the catheter. Apply butterflied radiolucent tape around the catheter hub and suture to the surrounding skin. The portion of the jugular vein opposite the catheter may need to be clamped or ligated to prevent leakage.
   Medial metatarsal vein: Direct the catheter towards the heart. Apply butterflied radiolucent tape around the catheter hub and tape to the foot and lower leg.

   Attach a flush filled IV extension line to the catheter. Make sure there are no bubbles/air pockets in the extension line. When flushing, do not put any pressure or apply movement to the catheter end. Use the free end of the extension line to attach syringes for flush and BriteVu® mix perfusion.

4. Use warmed fluids to flush the blood from the vascular system.
   A. Cut a vessel distant from your catheter site. For example, if a basilic (wing) vein catheter is placed, cut one or both medial metatarsal vein(s).
   B. Use warm (38-41°C [101-106°F]) physiologic solution (0.9% NaCl, PBSS, etc) as vascular flush.
   C. Use 30-40% of body weight in flush. Use 300-400 cc (ml) per Kg of bird body weight.
   D. Use a syringe and determine ideal flush pressure using your hand. A syringe pump can be used. However, there is a risk of vascular rupture if a constant pressure pump is used and not adjusted during the perfusion.
   E. Blood and flush solution should be noted exiting the cut vessel. Excessive delivery pressure and/or speed may result in vessel rupture. As the flush progresses, the exiting fluid should turn from (blood) red to light pink.

5. Prepare BriteVu® mix.
   1. Consider plain (distilled water), added 1-2% BriteVu® Enhancer, Phenol or other preparations. See 'Protocols' for more details.
   2. Plan for 30-40% of body weight in BriteVu® mix. Use 300-400 cc (ml) per Kg of bird body weight.

6. Perfuse subject with BriteVu® mix.

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Keep in a cool dry place. Keep out of the reach of children. Avoid contact with eyes and inhalation
In case of accidental exposure call 1-800-535-5053 (North America) or 1-352-323-3500 (International).

A. xAs with the flush solution, deliver the BriteVu® mix via hand perfusion. A syringe pump can be used. However, there is a risk of vascular rupture if a constant pressure pump is used and not adjusted during the perfusion. If delivered too fast or with too much pressure (via hand or syringe) there is a risk of micro or large vascular rupture.
B. BriteVu® mix should be seen exiting the distant cut vessel after 20-30% of the calculated BriteVu® mix is delivered.
C. Monitor the tongue, skin and peripheral vessels for evidence of adequate perfusion.

7. Once the perfusion is complete, cap off catheters, tie off vessels or apply pressure bandages to prevent leakage.
   A. Place the subject in ice water to speed solidification and stop heat related tissue damage.
   B. Clean the skin and feathers of any leaked BriteVu® mix. BriteVu® mix can simply be washed off with warm water, wiped or picked off. Once cooled, excess BriteVu® mix (inside and outside the body) may be removed as solid pieces.

8. Once the BriteVu® mix has solidified, the subject is ready for scanning or storage in fixative.
   A. Harvested tissues, regions or whole body can be stored in formalin indefinitely and scanned later.
   B. Tissues can also be stored in various fixatives for histology, electron microscopy, etc.