Standard Operating Procedure: Blood Collection in Rodents (Revised 10/26/2017)

These guidelines have been developed to assist investigators and the Howard University Institutional Animal Care and Use Committee (IACUC) in their evaluation of investigator choice and application of survival rodent bleeding techniques. These guidelines are based on peer-reviewed publications as well as on veterinary data and experience. It is the responsibility of the investigator to use techniques and procedures that result in the least procedural pain and distress with minimal postprocedural impact on the animal; while adequately addressing protocol objectives and requirements. Training and experience of the technician in the chosen procedure are also of paramount importance. Training opportunities and resources, including access to experienced personnel, are available to new personnel. Blood collection should be recorded on the cage card. The procedures utilized must be reviewed and approved by the IACUC prior to their implementation.

Factors to consider in choosing the blood withdrawal technique include:

- The species to be bled
- The size of the animal to be bled
- The type of sample required (e.g. serum, whole cells, etc.)
- The quality of the sample required (sterility, tissue fluid contamination, etc.)
- The quantity of blood required
- The frequency of sampling
- The health status of the animal being bled
- The training and experience of the technician

Permissible Collection Volumes: IACUC approval is required in advance of performing these procedures.

Both the quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal. As a general rule the blood volume of any individual animal is 6 to 8% of the total body weight. Up to 10% can be removed from an animal at two week intervals without having to give fluid replacement (warm saline). The approximate blood volume of a mouse is 78 to 80 ml/kg and 50 to 70 ml/kg for the rat. Please see the tables (1 and 2) below (and in the attachment) for permissible blood volume removal amounts.

Maximum Blood Collection Volumes

Blood Collection Interval	Maximum Percentage of Blood Volume to be Removed	Percentage of Body Weight	Fluid Replacement Required (Y/N)	Mouse with body weight 23g – 35 g Blood volume 2 to 2.75 ml	Rat with body weight 250g – 500 g Blood volume 15 to 30 ml
One Time	15%	1.5%	Yes	0.3-0.42 ml	2.25-4.5 ml
Every 2 wks	10%	1.0%	No	0.2-0.28 ml	1.5-3.0 ml

Weekly	7.5%	0.75%	No	0.15-0.21 ml	1.125-2.25 ml
Terminal	30 to 50%	3.0 to 5.0%	Not applicable	1-1.5 ml	o.81-1.08 ml

If **blood** is taken on several occasions over 24 hours and then not for 4 weeks, the maximum amount may be increased to 10% of circulating **blood** volume

For a single survival collection: If you require more than 10 % of an animal's blood volume, scientific
justification and IACUC approval is required in advance of performing the procedure.

For a single collection followed by immediate euthanasia: If you require more than 35% of an animal's blood volume the animal must be anesthetized for the procedure followed by humane euthanasia. If anesthesia cannot be used, a scientific justification is required in advance for IACUC review and approval.

For multiple survival blood collections: If you require more than 15% of an animals' blood volume in any given two-week timeframe (with 7.5% collected once a week and with a 7-day interval in-between), scientific justification is required in advance for IACUC review and approval.

Collection Sites:

The following guidelines refer to the most frequently used survival sampling sites: a) Tail vein; b) Facial or Mandibular artery or vein, Saphenous vein and; d) Retro-orbital sinus (discouraged).

Blood withdrawal by cardiac puncture or axillary cut down are considered terminal procedures and must be performed only after ensuring that the animal is under surgical anesthesia. Issues that should guide the choice of survival blood collection route(s) are listed below. It is important to note that samples collected from different sites may show differences in clinical pathology values. Please refer to the information above regarding permissible collection volumes

A. Mandibular Vein/Artery:

- Can be used in both rats and mice by piercing the mandibular vein or artery with a needle [20G], lancet or stylet.
- Obtainable volume: medium to large [100-200 ul, mouse; 0.4-0.5 ml rat]
- Repeat sampling is possible.
- Sample quality is good.
- The procedure is customarily done on an unanesthetized animal, but effective restraint is required.
- Arterial sampling produces large volumes very rapidly.
- Venous sampling produces medium volumes more slowly.
- Ensure that gentle pressure is applied for approximately 30 seconds post-collection to ensure hemostasis.

B. Saphenous/Lateral Tarsal:

- Blood collection from the saphenous or lateral tarsal vein can be used in both rats and mice by piercing the vein with a needle [23-25G: mouse, 21-23G: rat].
- The procedure is customarily done on an unanaesthetized animal, but effective restraint is required.
- Two people can more easily perform the procedure if the animal is not under anesthesia. Light anesthesia can be used to chemically restrain the animal if only one person is performing the procedure.
- The leg of the animal is shaven over the vein if required wiped with alcohol and the area permitted to dry. The bleeder applies gentle pressure to the leg or foot above the site of collection to dilate the vessel. Using a 25g needle, the vein is pierced and blood is collected via capillary tube or allowed to drop into a collection vial. After collection is completed a gauze is used to apply gentle pressure over the collection site for 15 to 30 seconds.
- Obtainable blood volumes: small to medium [mouse: 100 ul; rat: 0.4 ml]
- Repeat sampling is possible.
- Variable sample quality.
- Can be more time-consuming than other methods due to time required for site preparation.
- After training, it requires more practice than tail or retro-orbital sampling to reliably withdraw more than a minimal amount of blood. Prolonged restraint and site preparation time can result in increased animal distress when handling an unanesthetized animal.
- Temporary favoring of the limb may be noted following the procedure.
- Care must be taken to ensure adequate hemostasis following the procedure.

C. Lateral Tail Vein or Ventral/Dorsal Artery:

- For tail vein collection the animal is usually placed in a restraint device that permits the tail to be extended from the device.
- Aseptic technique should be used. It is recommended that a local anesthetic cream (e.g. EMLA cream) be applied to the tail 30 minutes prior to blood sampling.
- Tail vein blood collection by nick or syringe requires the animal's tail to be warmed in order to dilate the blood vessel prior to taking the sample. This may be stressful and can cause dehydration due to salivation, in addition to increasing metabolic rate, which may affect the experimental data. Warming can be accomplished by placing a heat lamp over the tail 2 to 5 minutes before collection; making sure the lamp is not placed too close to the tail. A gauze pad soaked in warm (not hot) water can also be used after heat lamp warming.
- Tail vein collection by needle can be used in both rats and mice by inserting a 23G needle or by nicking it superficially perpendicular to the tail respectively. Tail nick can also be accomplished by using a sterile scalpel blade to make a small nick over the lateral tail vein on either side (with a superficial but deep enough nick blood should well up in the nick). For needle stick, using a 23g needle (a 1" butterfly catheter is recommended) attached to a syringe, insert the needle into the lateral tail (When the blood appears in the hub slowly pull back the plunger to collect the blood).

- After withdrawing the needle apply gentle pressure with gauze over the collection site for 15 to 30 seconds and affirm that bleeding has stopped.
- Obtainable volumes: Mouse - small to medium [50-100 ul] Rat – medium [0.2-0.4 ml]
- Sample collection using a needle minimizes contamination of the sample, but is more difficult to perform in the mouse.
- Sample collection by nicking the vessel is easily performed in both species, but produces a sample of variable quality that may be contaminated with tissue and skin products. It is not recommended rat for repeat samples. In this case, blood is collected directly into a vial or into a capillary tube. Stroking the tail from base to tip after nicking may aid in increasing blood flow.
- Sample quality decreases with prolonged bleeding times and tail stroking.
- Repeated collection is possible.
- After obtaining the blood sample apply direct pressure over the blood collection site for 15 to 30 seconds to effect hemostasis. Alternatively, some apply a small amount of sterile surgical glue over nicks to stop bleeding.
- The procedure is relatively non-traumatic.
- This procedure is routinely performed without anesthesia, although effective restraint is required.
- In the rat arterial collection requires analgesia and anesthesia, however it yields larger volumes and is faster, but special care must be taken to ensure adequate hemostasis. A cannula is recommended for repeat samples.
- If the animal was anesthetized, monitor the animal until it is fully awake and able to walk normally.
- Clean restraint devices between animals.

D. Retro-orbital:

*Note: Due to the increased risk of complications associated with this procedure, the IACUC recommends that other routes of blood collection be considered prior to use of this method. The mandibular technique permits an equivalent volume of blood to be collected in a rapid manner with less risk or complications.

- Retro-orbital sinus blood collection can be used in mice by penetrating the retro-orbital sinus with a glass capillary tube [0.5 mm in diameter] or via the retro-orbital plexus in rats with a capillary tube.
- The animal is anesthetized and a drop of ophthalmic anesthetic is placed in the eye. The skin around the head and neck are gently pulled taut to cause the eyeball to slightly protrude; but being sure not to obstruct breathing. A sterile capillary tubs is inserted into the inner corner (medial canthus) of the eye. The capillary tube or pipette must be directed towards the midline, and towards the back of the head. Caution must be exercised so as not to break the tube or scratch the eye. Gentle pressure is applied in advancing the tube into the retro-orbital sinus or plexus. When blood flow commences, flow is facilitated by directing the tube downward; so flow is assisted by gravity down into the collection tube. After blood collection is completed the collection tube is removed and gentle pressure is applied to the eye using gauze for 15 top 30 seconds. Ophthalmic antibiotic ointment can be applied to the eye after hemostasis is achieved.
- **Must be performed by a skilled operator.**

- **Follow-up required 24-48 hours after blood collection. If complications such as squinting or** bulging of the eye are noted, an animal health report must be completed.
- Obtainable volume: medium to large
- Collection is limited to once per eye.
- In the hands of an unskilled operator, retro-orbital sampling has a greater potential than other blood collection routes to result in the following complications:
    - Hematoma and excessive pressure on the eye resulting from retro-orbital hemorrhage
    - Corneal ulceration, keratitis, rupture of the eyeball or micro-ophthalmia caused by pressing on the eye to stem persistent bleeding or from a hematoma
    - Damage to the optic nerve and other intra-orbital structures leading to vision deficits or blindness
    - Fracture of the bones of the orbit and neural damage by the pipette; loss of vitreous humor due to penetration of the eyeball

Postprocedural Monitoring for Survival Blood Collection Procedures

Anesthetized animals must be monitored until they gain lateral recumbancy. Unanesthetized animals should likewise be monitored for one hour and at 24 hours after blood collection. Observe the recovery or postprocedural anesthetized or unanesthetized animal for bleeding from the collection site as well as other signs of illness including but not limited to labored breathing, discharge from the nose or mouth, ruffled fur, hunched posture, lethargy, immobility, etc. Euthanasia may be warranted based on postprocedural outcomes as stipulated by the Veterinarian.

Terminal and post-mortem blood collection
Blood withdrawal by cardiac puncture or axillary cut down are considered terminal procedures and must be performed only after ensuring that the animal is under surgical anesthesia. The post-mortem collection from the aorta is performed immediately after euthanasia.

A. Cardiac Puncture

- Cardiac puncture can be used in both rats and mice by penetrating the heart with the thoracic cavity open or closed. With a closed chest a needle (25g for mice and 23g for rats) fitted with a syringe is inserted just below the xiphoid cartilage and advanced slightly towards the left shoulder at a 30 degree angle. The syringe plunger is pulled back slightly and advanced until blood appears in the hub of the syringe. At that point, advancement is stopped and the plunger is pulled back until collection is completed.
- Cardiac puncture must be performed by a skilled operator.
- Medium to large volumes of blood are obtainable.
- The animal must be euthanized immediately after blood collection.

B. Axillary cut down

- Can be used in both rats and mice.
- Axillary vessels are cut with a scalpel blade **or scissors** and the pooled blood is collected via capillary tube.

- Obtainable volume: medium to large.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia.

C. Pre-mortem collection from the aorta or vena cava

- Can be used in both rats and mice as a pre-mortem procedure on anesthetized animals.
- Blood is collected using a needle.
- Animal must be euthanized immediately after blood collection prior to recovery from anesthesia
- Obtainable volume: medium to large.

D. Post-mortem collection from the aorta

- Can be used in both rats and mice as a post-mortem
- Aorta is cut and the blood pools in the pleural cavity.
- Blood is collected in a mini capillary tube. The tube must be held continuously in a horizontal position during the blood draw.
- Obtainable volume: medium to large.

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9. Murdoch University Animal Ethics Committee: Blood Collection: Volume and Frequency
10. KMJ/ Drafted March 2010/ Updated September, 2012 KG

Blood Collection: Volume and Frequency

Reference Source: Murdoch University Animal Ethics Committee

Bleeding Interval	Maximum Volume Removed
Greater than 4 weeks	10% of circulating blood volume
Weekly	7.5% of circulating blood volume
Daily	1% of circulating blood volume
Less than 24 hours between bleeds*	Total taken in 24 hours not to exceed 1% of circulating blood volume

* If **blood** is taken on several occasions over 24 hours and then not for 4 weeks, the maximum amount may be increased to 10% of circulating **blood** volume

Circulating Blood Volume by Species

Species	Blood Volume* (ml/kg)	Species	Blood Volume* (ml/kg)
Cattle	60	Chicken	60
Cat	47-66	Dog	79 -90
Ferret	75	Goat	70
Guinea Pig	67-92	Horse	75
Mouse	78-80	Pig	65
Rabbit	44-70	Rat	50-70
Sheep	60	Tammar Wallaby	93.5
Kangaroos	87.5	Common Brushtail Possum	51 - 63.8

For example; A dog weighing 20kg with a circulating blood volume of 80mg/kg has a total circulating blood volume of 20 x 80 = 1600mls. 10% (minimum interval 4 weeks) would be 160mls