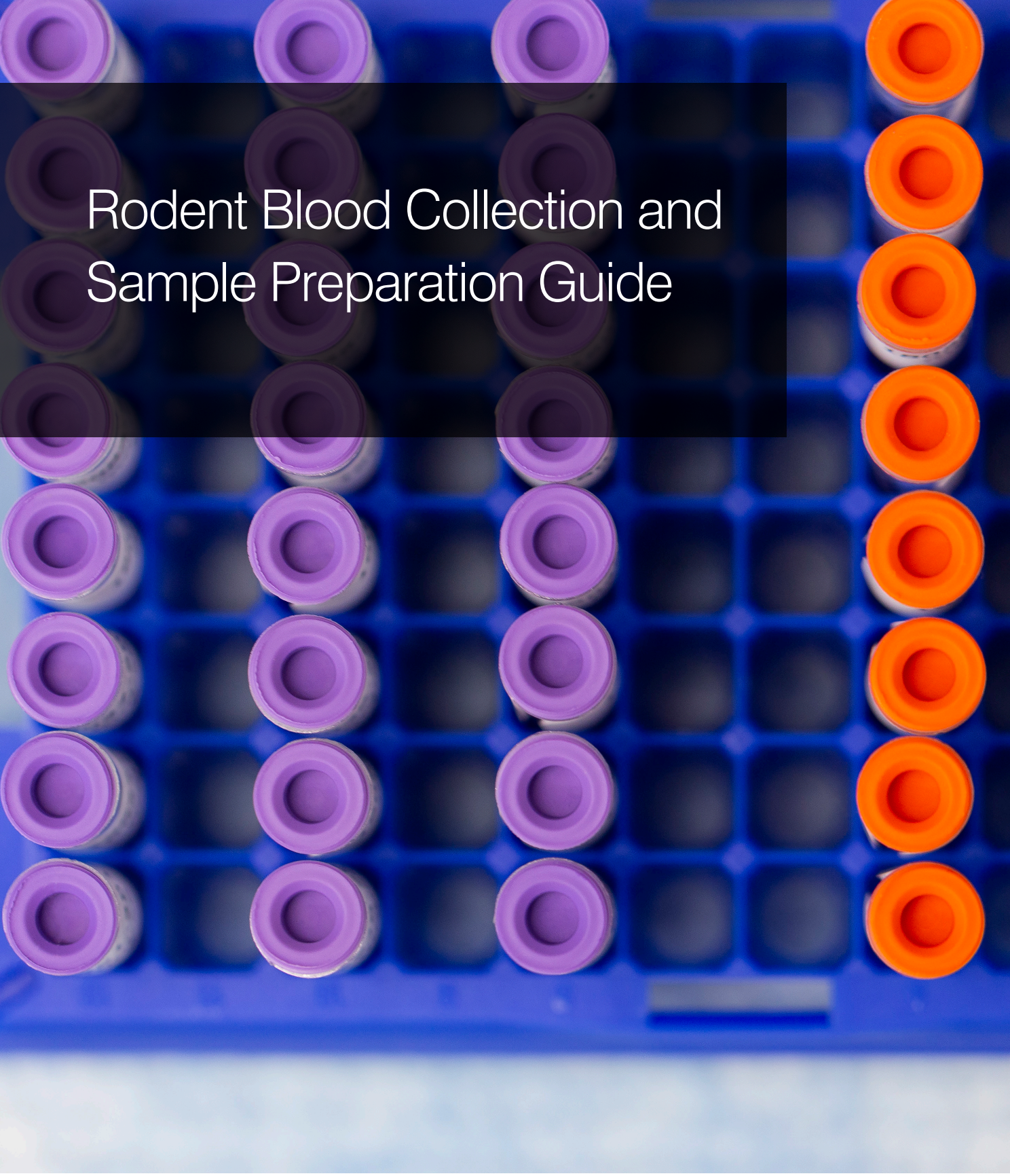


# Rodent Blood Collection and Sample Preparation Guide

Preclinical Services

IDEXX BioAnalytics

# Blood Collection And Sample Preparation For Rodents

## Blood Components

### Whole Blood

**Sample type for: Complete blood count (CBC)**

Blood drawn directly from the animal. Made up of plasma, a fluid component that carries dissolved molecules, and the cellular components, such as red and white blood cells and platelets. Whole blood is drawn into a tube containing anti-coagulant so that cells do not clump together.



### Plasma

**Sample type for: Coagulation testing (e.g., PT, PTT)**

Fluid component of blood. To harvest plasma, blood must be mixed with an anti-coagulant or clotting will occur rapidly, consuming molecules in the plasma that are required for testing. Blood is then centrifuged to separate plasma fluid from the cell components.

### Serum

**Sample type for: Chemistry, immunology, and endocrinology**

Fluid component of clotted blood. During clotting, coagulation factors and some other molecules from plasma are consumed, forming a new fluid called serum. Serum can only form if blood is allowed to clot and cannot be collected using an anti-coagulant. Once a blood clot has formed, the sample is then centrifuged to separate serum fluid from the cell components.

# Sample Submission Tips & Tricks

## Anti-coagulant tubes

Always use anti-coagulant when submitting whole blood or plasma samples. Tests often require a specific type of anti-coagulant for valid results, so always pair the correct tube with the testing desired using the Sample Submission Quick Reference Guide on page 6.

Never use anti-coagulant to collect serum samples. Serum cannot form in these types of tubes. Also, EDTA will interfere with serum biochemistry testing, giving false values for certain tests including potassium and calcium.

**Always follow fill recommendations for anti-coagulant tubes:**

- For EDTA tubes (lavender-top tube/LTT): use minimum and maximum fill lines as guides.
- For citrate tubes (blue-top tube/BTT): fill to the recommended line.
  Note: Underfilling EDTA tubes can cause erroneous results on CBC reports from cell shrinkage. Underfilling citrate tubes can cause erroneous prolonged coagulation testing results. When tubes are overfilled with blood, there is insufficient anti-coagulant to prevent clotting. This can cause platelet clumps and clots to form which prevent accurate measurement of platelet counts on CBC reports, or accurate measurement of clotting times in coagulation testing.

**Mix samples promptly, thoroughly, and gently:**

- Transfer blood to tubes quickly after venipuncture. As soon as tube is filled and capped,
- Invert gently end over end 8-10 times.
  Note: This ensures adequate contact of blood with anti-coagulant and minimizes platelet clumps and clots. Avoid aggressive mixing or shaking which may induce hemolysis.

## Sample processing and storage

### Whole blood

Always submit whole blood CBC samples to the laboratory as soon as possible after collection. These samples degrade rapidly at room temperature and are destroyed with freezing. Refrigerate after collection and in shipment for best preservation. When collecting plasma, blood should be centrifuged promptly after mixing with anti-coagulant. Afterwards, pipette plasma into an empty transfer tube for storage and shipment according to test instructions.

### Serum

When collecting serum, use a sterile non-additive tube or serum separator tube. Allow adequate time for blood to clot (~20 minutes), then centrifuge. Afterwards, pipette serum into an empty transfer tube for storage and shipment according to test instructions.

### Plasma and serum

For plasma and serum samples, it is important to centrifuge and separate fluid from the cell layer within an hour of blood collection. This prevents altered laboratory results that can occur from prolonged contact of the fluid sample with blood cells.

# Sample Submission Tips & Tricks, continued

## Submitting samples

Plasma samples for coagulation testing should be submitted frozen.

Serum samples can be stored and shipped frozen; if storage is short term (within 24 hours of collection), refrigerated samples are also acceptable.

Whole blood samples submitted in a shipping container containing freezer packs should be protected by an inner container to prevent samples from freezing.

## Blood sampling tips to improve sample quality

- To avoid hemolysis, blood samples should be taken immediately after the vein has been raised.
- Avoid "pumping or milking" the blood from the vein as this can induce coagulation and erythrocyte lysis.
- Avoid high negative pressure in the syringe, as this can cause erythrocytes to rupture.
- Do not squirt blood forcefully through the syringe into the tube. Instead apply gentle pressure and allow the blood to run down the side of the tube wall.

## Considerations for Optimal Blood Collection

*We recommend working closely with your institutional veterinarian to ensure that all project team members are adequately trained in sample collection and all blood collection protocols are in compliance with institutional and IACUC guidelines.*

When designing an experiment that will require blood collection from rodents, several basic parameters such as the following, should be carefully considered.

- **Survival blood collection versus terminal blood collection**
- **Species of animal**
- **Size of the animal and estimated total blood volume**
- **Amount of blood sample required and frequency of sampling**
- **Type of blood sample**
- **Experience of technical staff**
- **Effect of sampling site and restraint on blood parameter measured**

When using survival blood collection techniques, the frequency and quantity of sample collection must be carefully considered. These factors are dependent on the total circulating blood volume of the animal and the rate of red blood cell turnover which can vary by species.

For mice and rats, the circulating blood volume is approximately 55-70 ml/kg of body weight (1,2). Percentage of the blood volume and frequency of sampling that can be safely performed in mice and rats is listed in the Blood Sample Volumes for a Range of Body Weights table on page 7. More frequent collection or sample volumes greater than those listed in the table can be performed but require fluid or cellular replacement and additional supportive care of the animal.

# Sample Submission • Quick Reference Guide

| Sample Type and Testing | **Whole Blood** For Hematology | **Serum** For Chemistry, Immunology, Endocrinology | **Citrated Plasma** For Coagulation | **Serum or Plasma** For Storage and Shipment |
|---|---|---|---|---|
| **Tube Types** |  <br>**LTT/MINI**<br>  <br>**EDTA anti-coagulant** |  <br>**Microcentrifuge Tube**<br>Non-additive plastic tube<br>  <br>**SST/MINI***<br>Contains clot activator and gel to separate clot from serum (during centrifugation). |  <br>**BTT/MINI**<br>  <br>**Citrate anti-coagulant**<br>Use BTT to collect then transfer to non-additive tube. |  <br>**Microcentrifuge Tube**<br>Sample transfer tube non-additive plastic tube for transport. |
| **Sample Handling** | Promptly fill and thoroughly mix. Observe fill lines to achieve proper blood to anticoagulant ratio. Mix by gently inverting tube 8-10x to prevent clotting and hemolysis. Label the tube with sample ID and collection date. If collecting blood from one animal into multiple different tubes fill LTT last to avoid contaminating other sample tubes with EDTA. | Let specimen clot ~20 minutes, then centrifuge at 1,000 - 2,000g for 10 minutes.<br>Afterwards, promptly pipette serum off of cells or gel layer and into an empty sample transfer tube. Label the tube with sample ID, collection date, and sample type (i.e. serum). | Promptly fill and thoroughly mix. Fill tube to appropriate fill line to achieve proper blood to anticoagulant ratio. Mix by gently inverting tube 10x to prevent clotting and hemolysis. Centrifuge as soon as possible at 1,000-2,000g for 10 minutes. Pipette plasma off of cells and into a sample transfer tube. Label the tube with sample ID, collection date, and "citrated plasma." | Refer to the sample handling directions for the appropriate sample type. |
| **Sample Storage** | Refrigerate at 2-8°C. Do not freeze. | Store samples refrigerated at 2-8°C if shipping within 24 hours of collection. Otherwise, freeze samples in transport tubes at -20°C. | Freeze at -20°C. | Refer to the sample storage directions for the appropriate sample type. |
| **Sample Shipping** | Ship refrigerated on cold packs, ideally same day or day following collection. Samples received 72 hours or more post collection may show siginficant degradation that can affect results. Ensure samples do not come into direct contact with cold packs during shipment. Samples can be placed within an internal container to prevent freezing. | If shipping within 24 hours of collection, samples can be shipped refrigerated on cold packs. If frozen in storage, ship samples frozen on cold packs or dry ice.<br>* Serum separator tubes (SST) CANNOT be used for certain endocrine and specialized testing including: progesterone, and therapeutic drug monitoring (e.g., phenobarbitol). SST tubes CANNOT be frozen- for frozen storage use non-additive tube. | Ship samples frozen on cold packs or dry ice. | Refer to the sample shipment directions for the appropriate sample type. |

# Rodent Blood Sample Volumes

## for a range of body weights

| Body weight (g) | *CBV (mL) | 1% CBV every 24 hrs ** | 7.5% CBV every 7 days | 10% CBV every 2 – 4 wks ** |
|---|---|---|---|---|
| 20 | 1.10 - 1.40 | 11 - 14 µL | 90 - 105 µL | 110 - 140 µL |
| 25 | 1.37 - 1.75 | 14 - 18 µL | 102 - 131 µL | 140 - 180 µL |
| 30 | 1.65 - 2.10 | 17 - 21 µL | 124 - 158 µL | 170 - 210 µL |
| 35 | 1.93 - 2.45 | 19 - 25 µL | 145 - 184 µL | 190 - 250 µL |
| 40 | 2.20 - 2.80 | 22 - 28 µL | 165 - 210 µL | 220 - 280 µL |
| 125 | 6.88 - 8.75 | 69 - 88 µL | 516 - 656 µL | 690 - 880 µL |
| 150 | 8.25 -10.50 | 82 - 105 µL | 619 - 788 µL | 820 - 1000 µL |
| 200 | 11.00 -14.00 | 110 - 140 µL | 825 - 1050 µL | 1.1 - 1.4 mL |
| 250 | 13.75 -17.50 | 138 - 175 µL | 1.0 - 1.3 mL | 1.4 - 1.8 mL |
| 300 | 16.50 - 21.00 | 165 - 210 µL | 1.2 - 1.6 mL | 1.7 - 2.1 mL |
| 350 | 19.25 - 24.50 | 193 - 245 µL | 1.4 - 1.8 mL | 1.9 - 2.5 mL |

* Circulating blood volume (1mL = 1000 µL)
** Maximum sample volume for that sampling frequency

References:

1. Everds N (2007). Hematology of the laboratory mouse. The Mouse in Biomedical Research, 2nd ed. Vol 3 Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, and Smith AL (Eds.), Burlington, MA: Academic Press, pp. 142-48.

2. Koch M (2006) Experimental Modeling and Research Methodology. The Laboratory Rat, 2nd ed. Suckow MA, Weisbroth SH, and Franklin CL (Eds.), Burlington, MA: Elsevier Academic Press, pp 593-594.

3. National Institutes of Health (NIH) Animal Research Advisory Committee Guidelines (2015). Survival Bleeding of Mice and Rats. https://oacu.oir.nih.gov/animal-research-advisory-committee-guidelines

# Blood Collection Technique Comparison Guide

## Survival Procedures

| Technique | Volume | Advantages | Disadvantages |
|---|---|---|---|
| **Chin Bleed** | <0.2 mL* | • Can be done with or without anesthesia<br>• Ease of locating target vessels for sampling with training<br>• Moderate to large sample volume<br>• Easy to stop post collection bleeding<br>• Can be performed rapidly, decreasing risk of hemolysis and platelet clumping<br>• Alternation between left and right submental veins possible if more frequent collection needed | • Prolonged restraint can cause difficulty in breathing<br>• Can cause bleeding from oral cavity |
| **Lateral Saphenous Vein** | <0.2 mL* | • No anesthesia required<br>• Ease of locating target vessel for sampling with training<br>• Moderate sample volume<br>• Can use the same site multiple times by removing scab at puncture site<br>• Can be performed rapidly, decreasing risk of hemolysis and platelet clumping<br>• Alternation between left and right hind limbs is possible if more frequent collection needed | • May be difficult to stop post collection bleeding<br>• Prolonged procedure technique can induce temporary lameness of the animal |
| **Cheek Bleed** | <0.2 mL* | • Can be done with or without anesthesia<br>• Moderate to large sample volume<br>• Can use same site multiple times by alternating sides of the face<br>• Can be performed rapidly decreasing risk of hemolysis and platelet clumping | • Challenging to visualize target veins for sampling<br>• May be difficult to stop post collection bleeding |
| **Lateral Tail Vein/Ventral Tail Artery** | <50 µL* | • No anesthesia required<br>• Ease of locating target vessels with training<br>• Can use the same site multiple times<br>• Easy to stop post collection bleeding | • Small sample volume<br>• Slower blood flow can increase risk of platelet clumping and hemolysis of sample |

## Terminal Procedures (Volumes refer to Mice)

| | | | |
|---|---|---|---|
| **Intra-cardiac Puncture**<br>This method must be performed under appropriate anesthesia | <1 mL* | • Easy to perform with training<br>• Large sample recovery | • Can cause increased hemolysis if inappropriate size needle and syringe or vacutainer used |

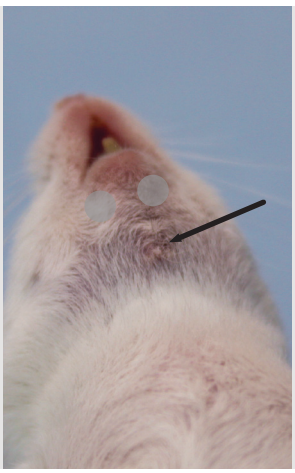
*Estimated total blood volumes per collection based upon size of an adult mouse. Actual collection volumes should be determined by time point, weight of animal, and approved IACUC animal guidelines.

# Survival Blood Collection Procedures

## Chin Bleed

### Materials needed

- 4 mm sterile lancet
- Blood collection tubes
- Sterile gauze squares or cotton balls



Find fur whorl (black arrow). Submental veins are approximately 0.5 cm rostral and lateral of the whorl, toward the lower jaw (yellow circles).

### Collection Steps

- **Label** blood collection tubes and place in a tube rack.
- **Remove the lid** from the first sample tube.
- **Restrain the mouse** with the non-dominant hand by grasping the loose skin over the shoulder and behind the ears. The skin should be taut under the chin but not so tight as to preclude breathing.
- **Locate the fur whorl** landmark.
- **Locate the puncture site** (when the chin is shaved the vessels should be visible approximately 0.5 cm rostral and lateral toward the lower jaw).
- **Prick the blood vessel** firmly using the tip of a sterile blood lancet or sterile needle and blood should flow immediately.
- **Collect the blood sample** with a collection tube until the target volume is reached.
- **Release the mouse** into the home cage.
- **Apply mild pressure** to the puncture site with a sterile gauze sponge if bleeding continues. Bleeding typically will stop following the release of the animal.
- **Ensure** that all animals have stopped active bleeding before returning cage to the rack.

  Note: For more frequent bleeding intervals, blood collection can be performed by alternating sides for each collection. This allows the previous vessel time to heal.

  As the vasculature of the chin can be difficult to assess when initially learning this procedure, it may be beneficial to shave the chin and neck area of the mouse to help enhance visualization of the blood vessels.

References:
1. Constantinescu, G.M and Dufee, N.M. (2017). Letter to the Editor: Comparison of Submental Blood Collection with the Retroorbital and Submandibular Methods in Mice (Mus musculus). JAALAS, Nov; 56(6):711-712. PMC: 5710148

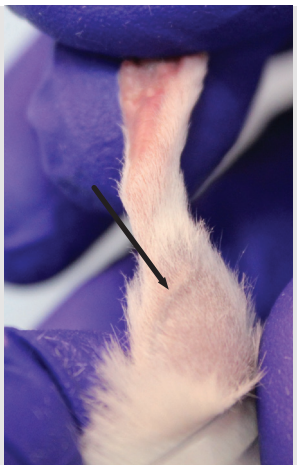
2. Regan, R.D., Fenyk-Melody, J.E., Tran, S.M., Chen, G., Stocking, KL. (2016). Comparison of Submental Blood Collection with the Retroorbital and Submandibular Methods in Mice (Mus musculus). JAALAS, Sept; 55(5):570-576. PMC: C5029828.

# Survival Blood Collection Procedures

## Lateral Saphenous Vein

### Materials needed

- Restraint device (modified 50 ml conical tube with breathing holes or disposable plastic cone-shaped restrainer)
- Small rodent fur clippers
- 25-gauge sterile needle
- Sterile gauze squares or cotton balls
- Blood collection tubes



Apply gental pressure below the knee to hold leg against side of restraint device inducing occlusion of blood flow. Lateral saphenous vein can be easily visualized.

### Collection Steps

- **Label** blood collection tubes and place in a tube rack.
- **Remove lid** from the first sample tube.
- **Encourage mouse or rat** to enter the restraint device while maintaining a gentle hold on the tail.

  Note: holding the restraint device at a slight angle in front of the mouse or rat will often induce the animal to enter by free will. However, some animals may require a gentle push from behind to entice them to enter the device. Never dangle an animal over a device and forcefully shove into the restrainer.
- **Gently position** the hind leg and extend outside of the restraint device, while keeping the animal in the restrainer. Note: Hold the tail base with your index finger to help extend the limb.
- **Shave** the hair of the leg with mini fur clippers.
- **Apply gentle pressure** below the knee, using your hand, to hold the leg against the side of the restraint device rim. This will occlude blood flow, allowing blood to pool and the lateral saphenous vein will become prominent. Note: When using a Decapicone, gently apply pressure below the knee with your finger tips to help occlude blood flow.
- **Gently puncture the vein** using a blood lancet or 25-gauge needle, being careful to not push the needle all the way through the vein and into the leg.
- **Quickly remove the needle** and drops of blood will form at the venipuncture site. Keep the leg extended with slight pressure below the knee for the best blood flow.
- **Draw blood into the tube** by touching the rim of the blood collection tube to the venipuncture site.
- **Apply gentle pressure** to the venipuncture site using sterile gauze or cotton ball once blood collection is complete. Allow the leg to bend, releasing any pressure on the back of the knee.
- **Return the mouse** to the cage once bleeding has stopped at the venipuncture site.
- **Ensure** that all animals have stopped active bleeding before returning cage to the rack. Note: For more frequent bleeding intervals, blood collection can be performed by alternating legs for each collection. This allows the previous vessel time to heal.

# Survival Blood Collection Procedures

## Cheek Bleed

### Materials needed

- 4 mm sterile lancet (shorter lancets do not work)
- Sterile gauze squares or cotton balls
- Blood collection tubes

Locate fur whorl/bald spot (black arrow). Puncture site will be slightly above and caudal to the fur whorl at the back of the jawbone where upper and lower jaws meet (yellow area).

### Collection Steps

- **Label** blood collection tubes, and place in tube rack.
- **Remove the lid** from the first sample tube.
- **Restrain the mouse** with the non-dominant hand by grasping the loose skin over the shoulder and behind the ears. The head should be adequately restrained in order to prevent movement.
- **Locate the fur** whorl/bald spot landmark.
- **Puncture** just behind the point at which the upper and lower jawbones meet, avoiding the jawbone. The puncture site will be slightly above and caudal to the fur whorl and will be at the back of the jaw, slightly behind the hinge of the jawbone toward the ear. .
- **Prick the blood vessel** quickly and firmly, using the sterile lancet. Blood should flow immediately.
- **Collect the blood sample** with a collection tube until the target volume is reached.
- **Gently apply pressure** to the puncture site using a sterile gauze or a sterile cotton ball to stop blood flow.
- **Release the mouse** into the home cage.
- **Apply mild pressure** with a sterile gauze sponge if bleeding continues.
- **Ensure** that all animals have stopped active bleeding before returning cage to the rack.

  Note: As the submandibular/facial veins can be difficult to assess when initially learning this procedure, it may be beneficial to gently palpate the jaw line to find where the upper and lower jaws meet before performing venipuncture.

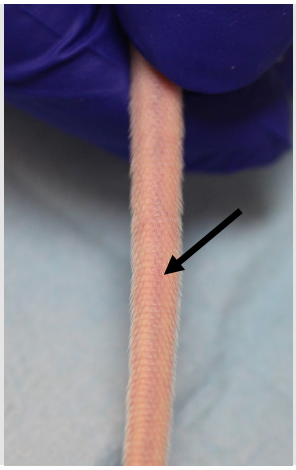
References: Golde, W.T., Gollobin, P., and Rodriguez, L.L. (2005). A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. Lab Animal, 34:39-43. PMID: 16195737, DOI:10.1038/laban1005-39.

# Survival Blood Collection Procedures

## Lateral Tail Vein/Ventral Tail Artery

### Materials needed

- Restraint device (modified 50 ml conical tube with breathing holes or disposable plastic cone-shaped restrainer)
- 4 mm sterile lancet or 25-gauge sterile needle
- Sterile gauze squares or cotton balls
- Blood collection tubes



The dorsal/ventral tail arteries (black arrow) and lateral tail veins (not shown) can be easily visualized.

### Collection Steps

- **Label** blood collection tubes and place in a tube rack.
- **Remove lid** from the first sample tube.
- **Encourage mouse or rat** to enter the restraint device while maintaining a gentle hold on the tail. Note: holding the restraint device at a slight angle in front of the mouse or rat will often induce the animal to enter by free will. However, some animals may require a gentle push from behind to entice them to enter the device. Never dangle an animal over a device and forcefully shove into the restrainer.
- **Gently grasp** the tail and extend outside of the restraint device, while keeping the animal in the restrainer.
- **Visualize** the dorsal and ventral tail arteries along the top and bottom of the tail. The lateral tail veins can be easily visualized along the sides of the tail.
- **Gently puncture** the tail artery or vein using a sterile blood lancet being careful to not push the lancet all the way through the blood vessel.
- **Quickly remove** the needle and drops of blood will form at the venipuncture site.
- **Touch the rim** of the blood collection tube to the venipuncture site to draw blood into the tube, working quickly. Note: Let the blood flow freely. Attempting to “milk” the blood from the venipuncture site can induce blood clotting of the sample.
- **Apply gentle pressure** to the venipuncture site using a sterile gauze or cotton ball once blood collection is complete.
- **Return the mouse** to the cage once bleeding has stopped at the venipuncture site. Note: Before returning cage to the rack, ensure that all animals have stopped active bleeding.

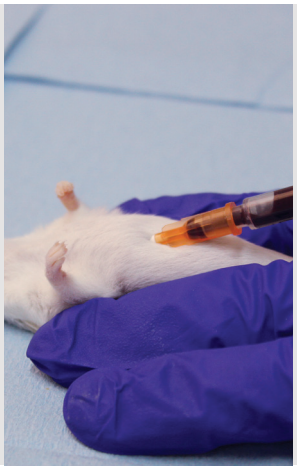
  Note: For more frequent bleeding intervals, blood collection can be performed by alternating sides of the tail or by moving down the tail to a new site. Alternatively, if a scab is present at the venipuncture site, gentle removal will allow additional blood collection.

# Terminal Blood Collection Procedures

## Intra-cardiac Puncture

### Materials needed

- 25-gauge sterile needle
- Sterile gauze squares or cotton balls
- Blood collection tubes



Insert needle at a 30-40° angle under xiphoid process and gently pull plunger back to collect blood sample.

This method of collection must be performed under appropriate anesthesia.

### Collection Steps

- **Label** blood collection tubes and place in a tube rack.
- **Remove the lid** from the first sample tube.
- **Work quickly** if collecting blood at necropsy to prevent blood clotting. If animals are anesthetized be sure that they are under deep anesthesia and not responsive to stimuli.
- **Place animal** on work surface in dorsal recumbency (belly side up).
- **Locate the xiphoid** process along the midline of the animal (boney prominence of the sternum).
- **Insert needle** at a 30-40° angle under the xiphoid process and just slightly to the left of midline (center) with need pointed toward the animal's head.
- **Retract the plunger** slightly to create a vacuum inside the syringe and gently advance the needle until you see blood appear in the needle hub. Note: If you do not see blood in the needle hub gently reposition the needle being careful to not pull out of the animal.
- **Continue to aspirate** until a sufficient amount of blood has been collected.
- **After collecting blood** follow IACUC guidelines for terminal procedures.

**Do you have any questions regarding blood collection or sample preparation?** Contact Client Support Services at 1-800-669-0825 and we will be glad to assist you or email us anytime at idexxbioanalytics@idexx.com.

**idexxbioanalytics.com**