

Methods of Blood Collection in the Mouse

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The author outlines various methods of blood collection from mice.

Collecting blood from mice is necessary for a wide variety of scientific studies, and there are a number of efficient methods available. It is important to remember that blood collection, because it can stress the animals, may have an impact on the outcome of research data. In addition, it is extremely important that those who collect blood become skilled in the techniques they employ, and seek to stress the mice as little as possible. Drawing from experience, personal communication, and published resources¹⁻⁴, the following techniques are described:

- Blood collection not requiring anesthesia:
 - Saphenous vein
 - Dorsal pedal vein
- Blood collection requiring anesthesia:
 - Tail vein
 - Orbital sinus
 - Jugular vein
- Terminal procedures:
 - Cardiac puncture
 - Posterior vena cava
 - Axillary vessels
 - Orbital sinus

The *Guide for the Care and Use of Laboratory Animals* mandates that animal care personnel be appropriately trained⁵. Training is available through accredited programs in veterinary technology; certification programs through organizations such as the American Association for Laboratory Animal Science; or commercially available training materials appropriate for self-study. To minimize pain and stress in the animal, it is recommended that all blood collection techniques be practiced on anesthetized or freshly euthanized mice

until the handler feels comfortable and is able to perform the procedure without causing harm to the animal.

Protocol Approval

The method of blood collection you choose must be described in the approved protocol in which the animals are used. Some IACUCs require justification for collecting blood from the orbital sinus in mice that are intended to recover, since there are other more humane methods available. When not performed correctly, blood collection from the orbital sinus can result in irreversible problems such as blindness or ocular ulcerations. One study showed that the competence of the person performing retro-orbital blood collection in rats had an appreciable impact on the incidence of induced abnormalities⁶.

In addition, the Public Health Service *Policy* requires that, in order to obtain approval to use animals (including mice) in research, personnel must be qualified to perform the techniques described in the protocol.

When deciding which technique is best suited for your study, you will need to consider a number of parameters.

Frequency of Collection

Blood should not be collected from the orbital sinus more frequently than once every two weeks. The tail, dorsal pedal, saphenous, and jugular veins can be used for serial blood collection as often as needed, according to the approved protocol.

Use of Anesthetics

To minimize discomfort to the animal, anesthesia is recommended when

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FIGURE 1. Blood collection from the saphenous vein.

collecting blood from the tail in mice over 28 days old and from the orbital sinus in mice of all ages. Anesthesia is also recommended when collecting blood from the jugular vein to ensure correct positioning of the mouse. When collecting blood from the saphenous or dorsal pedal veins, anesthesia is not necessary, since the procedure causes minimal pain and employs a restraining device that holds the animal in place.

Effect on Blood Parameters

Both the source and method of blood collection can affect blood parameters. Increases in blood hormone and glucose levels are directly related to stressful methods of blood collection. Mice may be stressed by restraining procedures or by sensing impending danger. To decrease stress, acclimate the mice to the restraining device and prevent them from seeing procedures being conducted (move them to the other side of the room or cover cages until they are needed). Packed cell volume (PCV) and hemoglobin measurements have been reported to be higher in blood collected from the tail

vein compared to blood obtained from other sites⁷.

Venous vs. Arterial Blood

Certain methods of blood collection yield arterial blood, others yield venous

blood, while still others yield a mixture of both. The tail yields a mixture of venous and arterial blood. Terminal blood collection from the axillary vessels results in a mixture of venous and arterial blood and cardiac puncture can yield venous or arterial blood, or a mixture of both. The posterior vena cava and the orbital sinus yield venous blood.

Aseptic Blood Collection

The optimal blood collection method will also depend on whether the sample must be taken aseptically. Collection from the orbital sinus and jugular vein are good nonterminal choices when an aseptic sample is needed. Care must be taken to make sure that the blood does not come in contact with the mouse's skin or fur. Cardiac puncture or blood collection from the posterior vena cava are good methods of terminal blood collection when an aseptic sample is needed.

Volume Required

On average, the total blood volume in the mouse is 6-8% of its body weight, or 6-8 ml of blood per 100 g of body

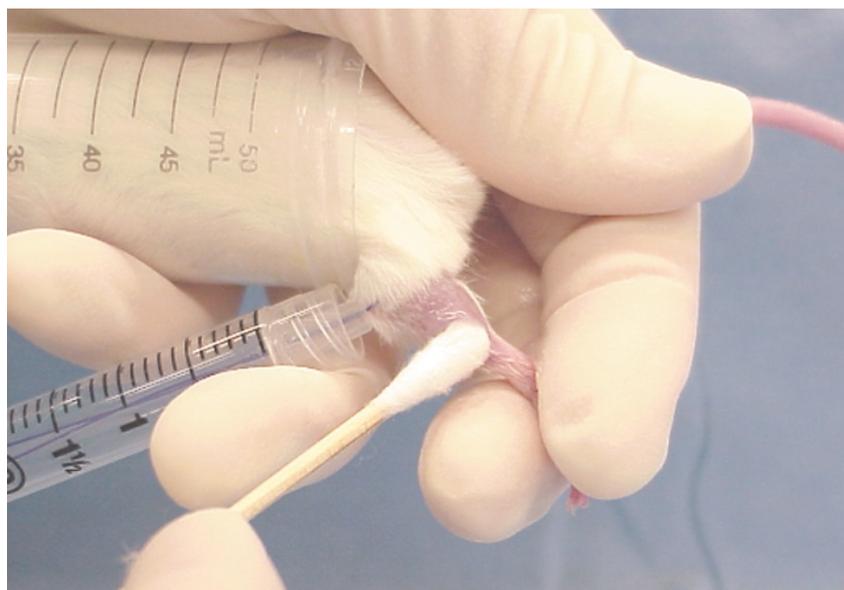


FIGURE 2. Application of petroleum jelly to the blood source.

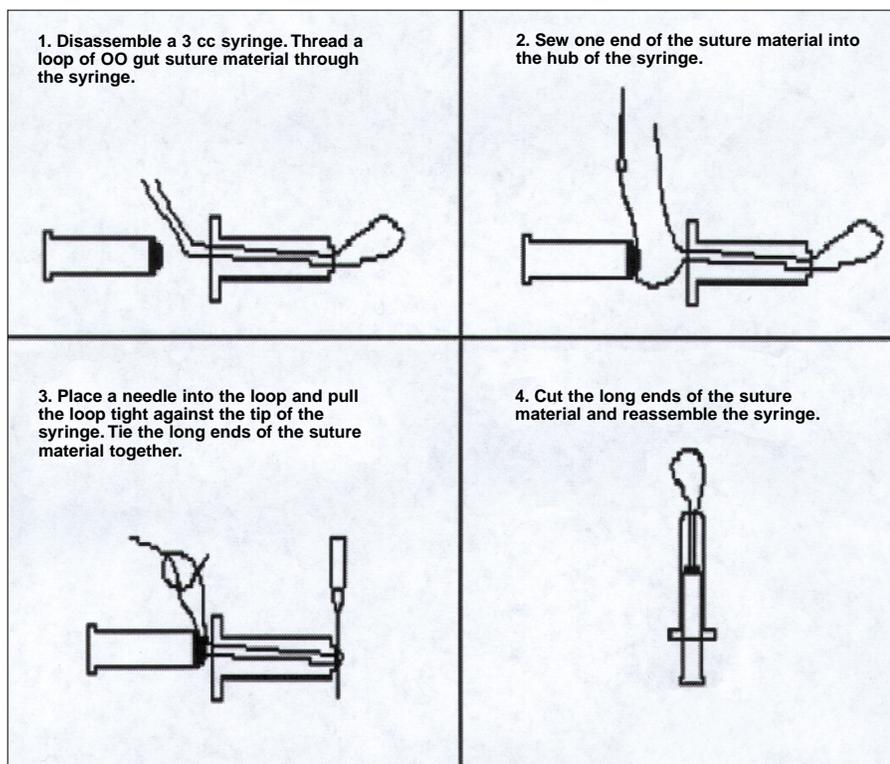


FIGURE 3. Instructions for making a mouse-sized tourniquet.

weight. Thinner animals will have relatively larger blood volumes per body mass, due to greater surface area. Younger animals, especially newborns, have proportionately larger blood volumes than older animals⁸.

If nonterminal blood collection is planned, the orbital sinus, jugular vein, or saphenous vein will typically yield more blood (0.2-0.3 ml in the average size adult mouse) than the tail or dorsal pedal vein. Terminal blood collection from the orbital sinus or by cardiac puncture will yield 0.8-1.0 ml. The posterior vena cava and axillary vessels typically provide a smaller blood volume.

The following guidelines⁹ should be considered when determining a safe volume of blood to withdraw:

- 10-15% of total blood volume or 1% of body weight is the maximum amount of blood that should be collected at one time;

- Blood volume is restored in 24 hours, but erythrocytes and reticulocytes may not return to normal levels for up to two weeks. Therefore, it is recommended that the maximum amount of blood be withdrawn only once every two weeks. Monitoring the PCV (normal range for mice is 39-49%) or hemoglobin can help evaluate whether the mouse has recovered from blood withdrawal;
- Removal of up to 1% of total blood volume daily over time is permissible, however, the effects of stress, site chosen, and anesthetic used must be carefully considered;
- Removal of 2% of the total blood volume is permissible if replacement fluids are given at the time blood is collected. The blood volume removed should be replaced intravenously with twice the volume of warm sterile fluids at a slow, steady rate. If fluids cannot be administered intravenously,

intraperitoneal or subcutaneous routes are other alternatives;

- 15-25% blood loss results in elevated plasma epinephrine, norepinephrine, and corticosterone concentrations to compensate for a decreased level of plasma glucose concentration;
- 20-25% blood loss decreases the arterial blood pressure, cardiac output and oxygen delivery to vital organs leading to hypovolemia and cardiac failure (shock). Muscular weakness, depressed mentation, and cold extremities may also be observed.

Immediately following blood collection, always observe the mouse for signs of distress or anemia (*e.g.*, rapid breathing, pale color of mucous membranes, depressed mentation, or muscle weakness). Observe mice daily for other problems, such as local trauma, infection, or irritation at the blood collection site.

Methods of Blood Collection Not Requiring Anesthesia

Blood Collection from the Saphenous Vein

Warming the mouse immediately prior to blood collection will increase blood flow considerably. Place a lamp over the cage for five minutes or place the cage on a heating pad, on the lowest setting. Place the mouse in a restraining tube so its head is covered and its hind legs are free. Grasp the fold of skin between the tail and thigh (Fig. 1). The saphenous vein is found on the caudal surface of the thigh. Remove hair from the area with clippers. Apply petroleum jelly (Fig. 2) or eye lubricant to prevent migration of blood into the surrounding hair and place a tourniquet around the leg, above the knee (Fig. 3). Puncture the vein with a 25 gauge needle. Collect drops of blood as they appear. The use of collection tubes with capillary action will facilitate blood collection. Apply pressure or use a cauterizing agent such as a styptic pencil (silver nitrate) to stop the bleeding.

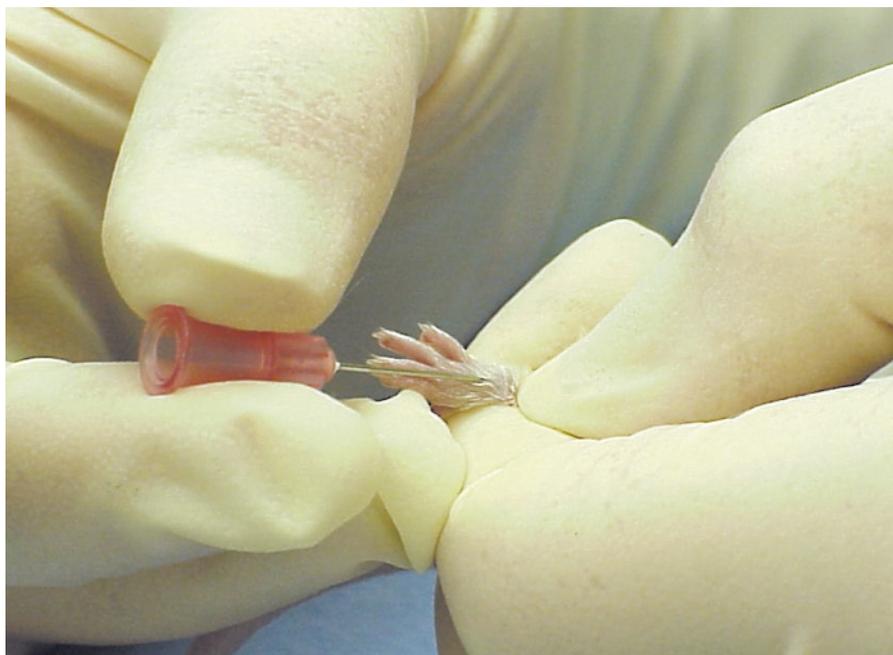


FIGURE 4. Blood collection from the dorsal pedal vein.

Blood Collection from the Top of the Foot

Warm the mouse and place it in a restraining tube as described above. With your thumb and first finger, hold a hind foot around the ankle (Fig. 4). Your thumb should be on top of the foot. The medial dorsal pedal vessel is found on the top of the foot. Apply petroleum jelly or eye lubricant to the foot. Puncture the vein with a 23–27 gauge needle. As drops of blood appear collect them in a capillary tube. Apply pressure or use a cauterizing agent such as a styptic pencil (silver nitrate) to stop the bleeding.

TABLE 1. Supplies and equipment needed to anesthetize mice using a drop jar or Ziploc™ bag.

- 500 ml jar with tightly fitting lid or a Ziploc™ bag
- Cotton or gauze
- Tool to separate cotton or gauze from the animal (e.g., plastic or wire mesh or tissue cassettes)
- Adequate local exhaust ventilation to prevent overexposure of lab personnel (e.g., scavenging devices, fume or snorkel hoods).

Methods of Blood Collection Requiring Anesthesia

Mice can be anesthetized for a short period of time using a drop jar with a tight-fitting lid or a Ziploc™ bag containing an appropriate amount of anesthetic¹⁰. When placed in the container, the mouse should become sufficiently anesthetized to perform procedures, without overdosing from the anesthetic. This method of anesthetizing mice is used when the procedure may cause momentary pain or is difficult to perform when the mouse is able to move about freely, and has been used for many years with anesthetics such as methoxy-fluorane (Metofane®) and ether. Since Metofane® is no longer available and ether presents problems with handling and storage, isoflurane is the current anesthetic of choice. Table 1 lists the supplies needed; Table 2 and Table 3 outline two possible procedures for anesthetizing mice using isoflurane.

Blood Collection from the Tail

Warm the mouse and place it in a restraining tube as described above. Do

not attempt to increase blood flow by rubbing the tail from the base to the tip, as this will result in leukocytosis (increased white blood cell count). Using a scalpel, straight edge razor, or sharp scissors, quickly remove up to 1 cm of the tail. Collect blood in a capillary tube as drops appear. Apply pressure or use a cauterizing agent such as a styptic pencil (silver nitrate) to stop the bleeding. When several samples are needed within a short time period, the original wound can be reopened by removing the clot. When additional samples are needed at a later date, blood samples can be obtained by removing just 2-3 mm of additional tail. Cutting the tail too short may result in trauma to the cartilage and ultimately to the coccygeal vertebrae.

Blood Collection from the Orbital Sinus

Lay the anesthetized mouse on its side on a table or hold it in your hand with its head pointing down (Fig. 5). With your first finger and thumb (finger above and thumb below the eye) pull the skin away from the eyeball, above and below the eye, so that the eyeball is protruding out of the socket as much as possible. Take care not to occlude the trachea with your thumb. Insert the tip of a fine-walled Pasteur pipette (o.d. of

TABLE 2. Procedure for anesthetizing mice using isoflurane in a drop jar.

- Fill tissue cassettes with cotton or gauze and place in the jar or place cotton or gauze in the bottom of the jar and cover with wire or plastic mesh;
- Add 300 μ l isoflurane to the cotton or gauze;
- Quickly close the lid;
- Open the lid, place one mouse in the jar, and quickly close the lid;
- WATCH CLOSELY;
- When the mouse is lying on its side and its breathing has slowed:
 - Open the lid;
 - Remove the mouse from the jar;
 - Quickly close the lid;
- The mouse will be at a surgical plane of anesthesia for 30 seconds to one minute;
- Add another 300 μ l of isoflurane to the jar after every third or fourth mouse.



FIGURE 5. Retro-orbital blood collection.

1-2 mm) or a microhematocrit blood tube into the corner of the eye socket underneath the eyeball, directing the tip at a 45-degree angle toward the middle of the eye socket. Rotate the pipette between your fingers during forward passage; do not move it from side to side or front to back. Apply gentle downward pressure and then release until the vein is broken and blood is visualized entering the pipette. When a small amount of

blood begins filling the pipette, withdraw slightly and allow the pipette to fill. Do not let the pipette come out of the eye socket. If the pipette is not withdrawn slightly, it may occlude the vein and blood will not flow freely.

Cover the open end of the pipette with the tip of your finger before removing it

from the orbital sinus to prevent blood from spilling out of the tube. Bleeding usually stops immediately and completely when the pipette is removed. It may be necessary to apply gentle pressure on the eyeball for a brief moment by closing the skin above and below the eye using your first finger and thumb. It is recommended that sample collection not be repeated on the same eye for at least two weeks.

Caution: Blindness can occur if the optic nerve is damaged as a result of the blood collection tube coming into contact with the nerve, which attaches to the middle of the ventral surface of the eye. Ocular ulcerations, puncture wounds, loss of vitreous humor, infection, or keratitis may occur as a result of poor technique or uncontrolled movement of the animal.

Blood Collection from the Jugular Vein

Restrain the anesthetized mouse by attaching a loop of string to a gauze square and looping it around the upper incisors. Pull the head up and back while pulling the gauze across the back of the hand and lock the gauze between two fingers (Fig. 6). It may be helpful to place the mouse in your lap. Wet the fur with alcohol or shave the neck area. In this



FIGURE 6. Proper positioning of mouse for blood collection from the jugular vein.

TABLE 3. Procedure for anesthetizing mice using isoflurane in a Ziploc™ bag⁹.

- Fill tissue cassette with cotton or gauze and place in the Ziploc™ bag;
- Add 100-200 ml isoflurane to the cotton or gauze;
- Quickly close the bag;
- Open the bag, place one mouse in the bag, and quickly close the bag;
- WATCH CLOSELY;
- When the mouse is lying on its side and its breathing has slowed:
 - Open the bag;
 - Remove the mouse from the bag;
 - Quickly close the bag.

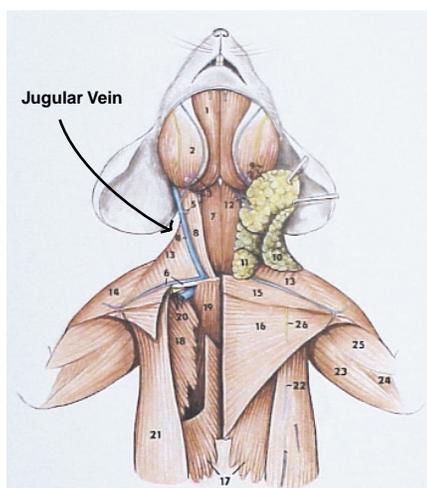


FIGURE 7. Location of the jugular vein in the mouse.

hyperextended position, the jugular veins appear blue and are found 2-4 mm lateral to the sternoclavicular junction (Fig. 7). Using a 1 ml syringe and 25 gauge needle, approach the vessel in a caudo-cephalic direction (from back to front). Insert the needle 1-3 mm deep, 2-4 mm lateral of the sternoclavicular junction, over the sternum; include a small amount of muscle from the sternum to stabilize the needle. Hold the needle very still when blood enters the syringe (Fig. 8). Withdraw blood slowly to avoid collapse of these small vessels.

If the first attempt to draw blood is unsuccessful, withdraw the needle slightly; it may have been placed too deeply. If blood stops flowing, do not continue to draw back on the syringe. The vein may have collapsed or the needle may have attached to the vessel wall. Rotate the needle slightly or apply slight pressure on the needle (either above, below, or to the side of puncture site).

Terminal Methods of Blood Collection Requiring Deep Anesthesia

After performing a terminal blood collection, *always* be certain that the animal is dead before placing the carcass in the freezer. Remember that the animal

will stop breathing before the heart stops. Either perform a bilateral pneumothorax (puncturing both sides of the thorax) or wait for the animal to become rigid.

Blood Collection by Cardiac Puncture

Three possible approaches:

- Hold the mouse by the scruff of skin above the shoulders so that its head is up and its rear legs are down. Use a 1 ml syringe and a 22 gauge needle. Insert needle 5 mm from the center of the thorax towards the animal's chin, 5-10 mm deep, holding the syringe 25-30 degrees away from the chest (Fig. 9);
- Lay animal on back and push syringe vertically through sternum;
- Lay animal on side and insert the needle perpendicular to chest wall.

If blood doesn't appear immediately, withdraw 0.5 cc of air to create a vacuum in the syringe. Withdraw the needle without removing it from under the skin and try a slightly different angle or direction. When blood appears in the syringe, hold it still and gently pull back on the plunger to obtain the maximum amount of blood available. Pulling back on the

plunger too much will cause the heart to collapse. If blood stops flowing, rotate the needle or pull it out slightly.

Blood Collection from the Posterior Vena Cava

Open the abdominal cavity of anesthetized mouse by making a V-cut through the skin and abdominal wall 1 cm caudal to the rib cage. Shift the intestines over to the left and push the liver forward. Locate the widest part of the posterior vena cava (between the kidneys). Use a 23-25 gauge needle and a 1 ml syringe. Carefully insert the needle into the vein and draw blood slowly until the vessel wall collapses. Pause to allow the vein to refill and then repeat three or four times or until no more blood is available.

Blood Collection from the Axillary Vessels

Lay the anesthetized mouse on its back. Stretch out a forelimb and pin the front foot. Make a deep incision in the axilla (armpit) at the side of the thorax. Hold the skin at the posterior part of the incision using forceps to create a cupped area. Incise the blood vessels in the area



FIGURE 8. Blood collection from the jugular vein.

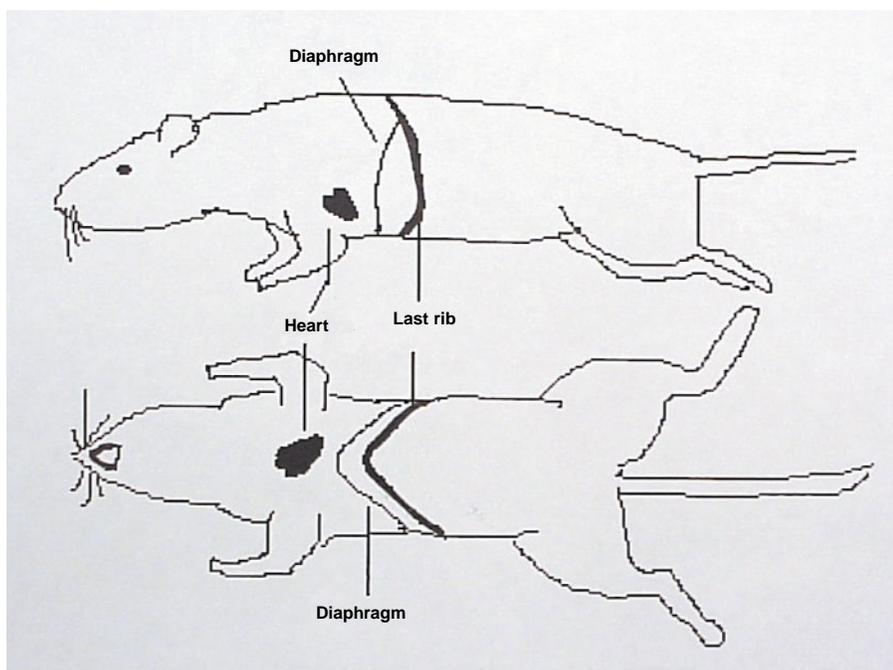


FIGURE 9. Relative locations of the heart and diaphragm in the mouse thorax.

with a scalpel or straight edge razor and collect blood as it pools. It may be important to consider that tissue fluids will contaminate the blood sample.

Blood Collection from the Orbital Sinus

Quickly remove the eyeball from the socket with a pair of tissue forceps. Hold the mouse in the palm of your hand over a collection tube. Massage the body of the mouse with your hand by squeezing the rear half, then the middle, then the head of the mouse so that you are “milk-ing” the blood toward the eye. When done properly, large drops of blood will flow from the orbital sinus.

Received 1/27/99; accepted 9/19/00.

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