Science Education Collection

Blood Withdrawal II

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Overview

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The collection of blood from mice and rats for analysis can be done through a variety of methods. Each method of collection has variations in the type of restraint required, the invasiveness of the procedure, and the necessity of a general anesthetic.[1]Historically, the use of the retro-orbital sinus cavity has been used, but not without debate. The controversy related to the potential tissue damage,or even blindness,caused by retro-orbital bleeds has led to the development of facial and submandibular vein bleeding methods in mice.Blood collection from the saphenous vein in both mice and rats is another technique that has been developed. These procedures do not require anesthesia and therefore are suitable when the use of anesthetics may confound blood results or other data.

Principles

There is a facial vein in the mouse that runs from the ocular plexus across the cheek, and a submandibular vein that runs along the lower jaw. Both vessels converge into the jugular vein just below the jaw line, making them easily accessible. Serial samples can be taken from either vessel by alternating the side used. However, neither side should be used more often than every 5-7 days.[2]

Precautions need to be taken while performing a facial vein bleed. As the ear canal is located near the facial vein, if the lancet tip is directed caudally, the canal will be pierced. This will result in a siphon effect causing blood to come from the ear. Despite this, blood can still be collected, and the mouse will not sustain any permanent harm. However, this may cause the animal to its shake the head, splattering blood in the cage.

When bleeding from the submandibular vein, the insertion depth of the needle is critical. An insertion depth exceeding 4-5.5 mm may result in trauma to the muscles, nerves, and other vessels that are in the head, neck, and oral cavity. Subsequent complications include excessive hemorrhage resulting in hypovolemia, drowning caused by the fluid in the mouth, and damage to oral structures that interfere with eating and drinking.

Restraint of the animal is crucial for a successful bleed for both facial vein and submandibular vein procedures. If the grip on the scruff is too tight, the blood flow to the facial vein can be restricted. This will result in a reduction in the volume collected. Collection volumes will vary on both the facial vein and submandibular vein bleed. It is imperative to limit collection volumes so that they do not exceed the maximum volume for survival blood collection in accordance with institutional policies and an approved animal protocol. Ensuring hemostasis once the desired amount is collected will prevent additional or excessive blood loss.[2]

Blood collection from the saphenous vein is another viable alternative for serial bleeds. The lateral saphenous vein is a superficial vessel that runs dorsally, and then laterally, across the tarsal joint.[3] Although this procedure may be more aesthetically acceptable than the retro-orbital bleed, because of the preparation required-and no use of anesthesia for this method-it may actually be more stressful to the animal. Complications that can arise from a saphenous bleed are related to the puncture site. If the needle puncture is not directly on the vessel, blood may pool subcutaneously, resulting in a hematoma. Bruising, possible infection, and favoring of the limb are other possible problems. This method requires training, but it is easily grasped. Volumes collected with this method are between 10-150 µL, depending upon the frequency of sampling.[4] The samples are variable in quality as they may contain tissue products. No more than four blood samples should be taken within a 24 hour period from the same leg.

The femoral vein is another option for blood collection on a rat. The femoral vein runs on the medial aspect of the hind leg from the groin to the knee joint before crossing the knee and becoming the lateral saphenous, making it easily accessible. Although this procedure can be done without the use of an anesthetic, it does require two people one person will hold the leg at the point where the leg and the abdomen connect to occlude the vein, while the other performs the venipuncture and collects the blood.

The advantage to using the femoral vein is that a larger volume is more easily collected from it than from the saphenous vein. However, because the femoral vein is large, it is prone to hematoma formation. This can be exacerbated by too firm a grip on the leg, which can cause additional bruising. With this blood collection method, there is variation in the amount of blood collected due to bleeding after the removal of the needle. It is imperative to ensure hemostasis to prevent this excessive blood loss.

Procedure

1. Facial vein bleed in mice

1. Equipment
    1. Collection of blood is from a free catch into a blood tube or an Eppendorf tube. In some cases, it is desirable to collect blood directly into hematocrit tubes.
    2. Goldenrod lancets will be selected based on the appropriate size for the animal according to age and sex.
        1. Lancets are selected according to the age/size of mice as follows:

4mm lancet: 3-4-week-old mice (under 15 grams body weight)
5mm lancet: female mice under 10 weeks (under ~20 grams body weight)
5mm lancet: male mice under 6 weeks (under ~20 grams body weight)
5mm lancet: for single-drop samples (for blood smears)
5.5mm lancet: female mice over 10 weeks (over ~20 grams body weight)
5.5mm lancet: male mice over 6 weeks (over ~20 grams body weight)
5.5mm lancet: large samples

2. Restraint
    1. Mice are restrained using the scruffing technique.
    2. It is important that side-to-side movement of the head be minimized. This ensures accurate and safe venipuncture with the lancet.

3. Blood withdrawal
    1. The proper-size lancet is held perpendicular to the surface of the skin.
    2. The lancet point is slightly angled, with the tip facing towards the nose.
    3. The lancet blade is best used in a vertical position.
    4. While restraining the mouse, locate the approximate area of the facial vein by measuring the length of the eye below the lateral canthus and the width of the eye caudally.
    5. With the point of the lancet, gently feel for the point at which the jawbone ends.
    6. For better accuracy in puncturing the vessel, position the mouse in lateral recumbency.
    7. Pierce the skin to the shoulder of the lancet at that point. This is done with a firm push and not as throwing a dart.
    8. Upon removal of the lancet, blood will begin to flow.
    9. To assist blood flow, position the mouse with the head lower than the heart.
    10. Collect the blood in the desired collection vessel.
    11. Blot the puncture site and release pressure on the scruff for hemostasis.

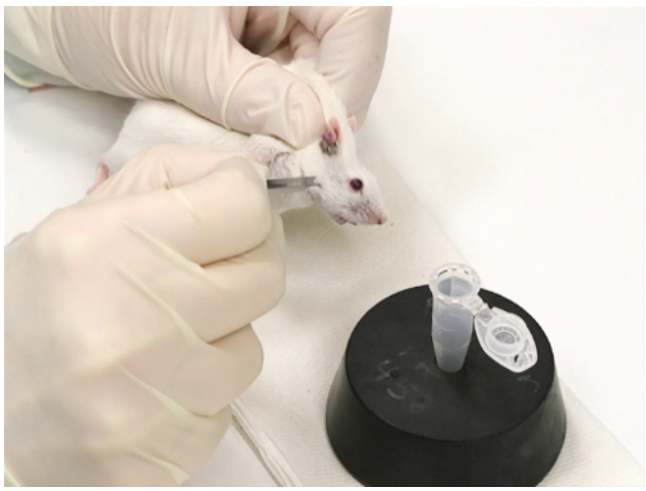

Figure 1. Facial vein bleed in mice.

2. Submandibular bleed in mice: Although very similar to the technique for the facial vein bleed,there are variations in equipment and subtle differences in this bleeding procedure.

1. Equipment
    1. Collection of the blood is from a free catch into a blood tube or an Eppendorf tube. In some cases, it is desirable to collect directly into hematocrit tubes.
    2. 18-22 gauge needles are selected based on the appropriate size for the animal according to age and sex.
        1. Needles are selected according to the age/size of mice as follows:
        22 gauge: 3-4 week old mice (under 15 grams body weight)
        20 gauge: female mice under 10 weeks (under ~20 grams body weight)
        20 gauge: male mice under 6 weeks (under ~20 grams body weight)
        20 gauge: for single-drop samples (for blood smears)
        18 gauge: female mice over 10 weeks (over ~20 grams body weight)
        18 gauge: male mice over 6 weeks (over ~20 grams body weight)
        18 gauge: large samples

2. Restraint
    1. Mice are restrained using the scruffing technique.
    2. It is important that side-to-side movement of the head be minimized. This ensures accurate and safe venipuncture with the needle.

3. Blood withdrawal
    1. The needle is held perpendicular to the surface of the skin.
    2. While restraining the mouse, locate the approximate area of the submandibular vein by the point where a line from the corner of the mouth intersects a line from the lateral canthus of the eye. This coincides with a small, hairless dimple found caudal to the corner of the mouth and slightly below the jaw line.
    3. For better accuracy in puncturing the vessel, position the mouse in lateral recumbency.
    4. Pierce the skin with the tip of the needle at that point. This is done with a firm push and not as throwing a dart.
    5. The needle is not inserted beyond the tip of the bevel.
    6. Upon removal of the needle, blood will begin to flow.
    7. To assist blood flow, position the mouse with the head lower than the heart.
    8. Collect the blood in the desired collection vessel.
    9. Blot the puncture site, and release pressure on the scruff for hemostasis.

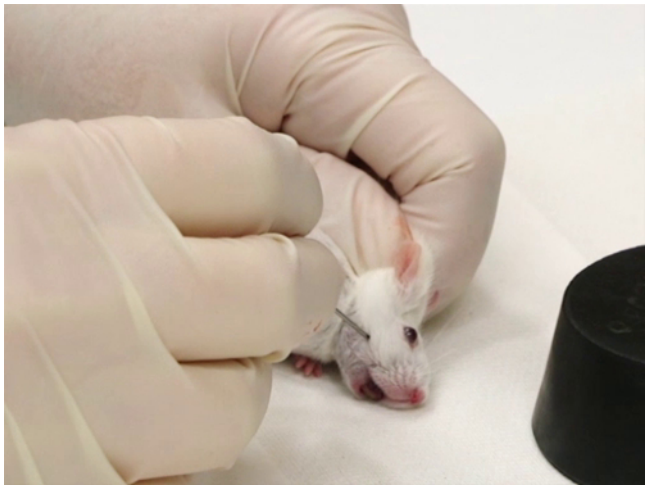

Figure 2. Submandibular vein bleed in mice.

3. Saphenous bleed

1. Equipment
    1. Clear plastic, flexible restraint cones can be used for either the mouse or the rat. For mice, modified 50 milliliter plastic conical tubes can be used for restraint. For rats, modified Plexiglas restraint tubes-with a slot wide enough to extend the hind leg-can be used.
    2. When utilizing the plastic cone, it is measured against the body length of the animal, and an oval hole is cut at the level of the thigh.
    3. A conical tube can be modified for a mouse for this blood collection method by cutting off the end of the tube to allow a breathing hole. A slot is cut from the cap end of the tube about ½ inch wide and 2 inches long. The edges are covered in cloth tape or smoothed for animal safety.
    4. A tourniquet is manufactured using a 3 cc syringe and a length of 2-0 nonabsorbable suture.[3]
    5. Triple antibiotic ointment or white petroleum jelly is used as a moisture-proof barrier on the skin.
    6. A 22 gauge needle is the preferred size for the venipuncture.
    7. Blood is collected directly into hematocrit tubes.

2. Restraint
    1. Flexible plastic cones
        1. The mouse or rat is placed into the cone nose-first.
        2. The end of the cone is folded and closed using a small binder clip to prevent the animal from exiting the restraint cone.
        3. The hind leg is gently pulled through the oval opening to the groin.
    2. Conical tube for mice
        1. The mouse is placed into the tube nose-first.
        2. The hind leg is gently guided into the slot.
        3. The middle finger is placed over the end of the tube to prevent the mouse from exiting the tube.
        4. The index finger and thumb stabilize the mouse's leg.
    3. Plexiglas restraint tube for rats
        1. The rat is placed into the tube nose-first.
        2. The hind leg is gently guided into the slot.
        3. The end of the tube is secured to prevent the rat from backing up and exiting the tube.
        4. The index finger and thumb stabilize the rat's leg.

3. Blood withdrawal

1. The hair is removed from the lateral aspect of the leg from the hock to the stifle. This can be done by plucking, shaving, or using a depilatory cream.
2. Once the hair has been removed, a small amount of ointment is applied and spread in a very thin layer on the hairless area.
3. The tourniquet is applied as far cranially as possible and tightened.
4. The saphenous vessel running across the outer surface of the leg from the knee to the ankle will begin to fill and will become raised and easy to visualize.
5. The needle is held perpendicular to the surface of the skin directly over the blood vessel.Puncture the vessel. Be careful not to insert the needle deeply into the leg, to avoid puncturing the muscle or hitting bone.
6. The blood will bead up on the surface of the leg for collection with the hematocrit tube.
7. Once the blood has been collected, loosen the tourniquet and apply pressure over the puncture for hemostasis.
8. Once bleeding has stopped, remove the animal from the restraint and return it to the home cage.

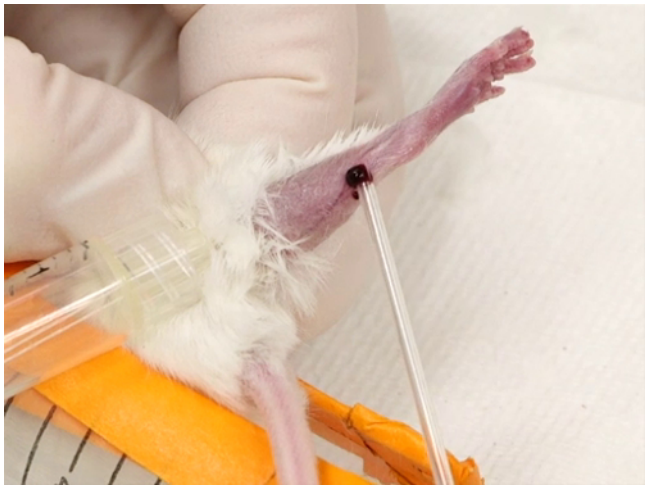

Figure 3. Saphenous vein bleed in mice.

4. Femoral vein for rats

1. Equipment
    1. Blood is collected into a hematocrit tube.
    2. Triple antibiotic ointment is needed to create a barrier between the skin/hair and the blood droplet.
    3. A 22 gauge needle is used to puncture the vein.
    4. A small, portable hair clipper with approximately a 1" wide blade is used to clip the hair from the leg.

2. Restraint
    1. Rats are restrained using a clear, flexible plastic restraint cone.
    2. The plastic cones are measured against the body length and an oval hole cut at the level of the thigh. The cone is cut such that the hind leg can be exteriorized with access to the vein.

3. Blood withdrawal
    1. The hair is shaved from the inner surface of the leg from the groin to the knee.
    2. Triple antibiotic ointment is applied in a thin layer at the puncture site.
    3. The restraint person occludes the vein and holds the rat with the inner surface of the leg facing the phlebotomist.
    4. Use the needle to puncture the vein. The needle is held perpendicular to the blood vessel and the puncture is done directly on the vein.
    5. The puncture is made as close to the knee as possible, allowing for additional sampling anterior to the first blood collection site.
    6. The vessel is superficial. Therefore, the depth of the puncture should not be deeper than the length of the bevel of the needle.
    7. To assist blood flow, position the rat with the leg lower than the heart.
    8. Collect the blood in the hematocrit tubes as it beads on the skin surface.
    9. Release pressure on the leg and apply pressure on the puncture site to achieve hemostasis.

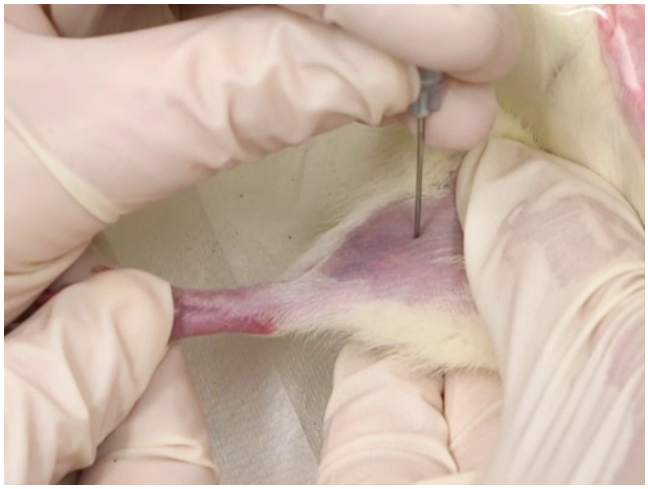

Figure 4. Femoral vein bleed in rats.

Summary

The method of blood collection can cause a variation in the analysis of the sample. The skill level of the technician performing the sample collection has an impact on the quality of the sample and the welfare of the animal. The use of anesthetics can also affect the sample quality. The methods described here are all performed without the use of anesthesia, thus that variable has been eliminated. Also, all of these techniques can be used for serial sampling with minimal discomfort to the animal.

References

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