Guidelines for Handling, Restraint, Injection, and Blood Collection from Small Laboratory Animals

Weill Cornell Medicine

HOSPITAL FOR SPECIAL SURGERY

Memorial Sloan Kettering Cancer Center

RESEARCH ANIMAL RESOURCE CENTER
MEMORIAL SLOAN KETTERING CANCER CENTER
WEILL CORNELL MEDICINE
HOSPITAL FOR SPECIAL SURGERY

CURRENT REVISION DATE: 8.18.2023

Table of Contents

General Considerations……………………………………………………………...………....3

Handling and Restraint……………………………………………………………………3

Mice………………………………………………………………………………….3

Rats…………………………………………………………………………………..5

Hamsters……………………………………………………………………………..6

Guinea Pigs…………………………………………………………………………..7

Rabbits……………………………………………………………………………….7

Ferrets………………………………………………………………………………..9

Parenteral Administration……………………………………………………………..11

Mice………………………………………………………………………………...13

Rats…………………………………………………………………………………20

Hamsters……………………………………………………………………………22

Guinea Pigs…………………………………………………………………………22

Rabbits……………………………………………………………………………...23

Enteral (Oral) Administration

Mice………………………………………………………………………………...25

Rats…………………………………………………………………………………27

Hamsters……………………………………………………………………………28

Guinea Pigs…………………………………………………………………………28

Rabbits……………………………………………………………………………...28

Blood Sampling

Mice………………………………………………………………………………...29

Rats…………………………………………………………………………………34

Hamsters……………………………………………………………………………39

Guinea Pigs…………………………………………………………………………39

Rabbits……………………………………………………………………………...39

Ferrets………………………………………………………………………………40

Chronic Administration and Sampling…………………………………………………41

Table I. Recommended needle sizes and injection volumes for various parenteral techniques...45

Table II. Maximum blood collection volumes for survival and terminal bleeds…………….....45

Guidelines for Handling, Restraint, Injection, and Blood Collection from Small Laboratory Animals

These guidelines have been developed to introduce investigative staff to procedures recommended for handling and restraint of small laboratory animals. In addition, techniques for performing parenteral injections and blood collection are also reviewed. This document is intended to supplement hands-on instruction by an experienced member of your laboratory or a member of the Research Animal Resource Center's (RARC) staff. RARC's Education and Quality Assurance (EQA) staff are available to provide hands-on instruction. You can contact them at RARC_EQA@mskcc.org or RARCEQA@med.cornell.edu to schedule a training session.

There are a variety of other techniques, in addition to those described in this document, which are suitable alternatives. You can contact RARC to discuss their suitability and/or describe them in your animal care and use protocol.

Handling and Restraint

Although there are significant species differences when handling and restraining an animal, there are several important concepts that apply to all species. These include:

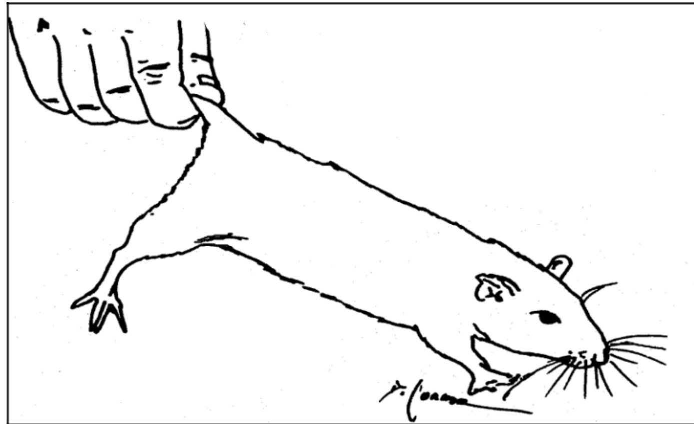
1) Handle animals gently but firmly;
2) Approach an animal with slow but purposeful movements;
3) Wear proper PPE as per facility requirements;
4) Always change your gloves and wash your hands prior to and after handling as odors of other species or blood can be distressing, and your hands can act as a means of spreading infectious agents from one group of animals to another; and,
5) Use a handling method appropriate for the species.

Animals to be used in experimental protocols that involve extensive manipulation should be handled frequently before the onset of the study to allow the animal to acclimate to handling and your scent reducing stress when restrained. Within species, particular stocks or strains may have distinctive behavioral responses.

MICE

Adult mice should be picked up by the tail base by compressing the base of the tail between your thumb and forefinger and gently placing the animal onto a solid surface (Figure 1). You can rest the animal on the cranial aspect of your forearm and transport it short distances. Alternatively, the animal can be placed in a small container with a cover containing holes to allow airflow. Adult mice can also be picked up by grasping the loose skin over the shoulders and gently lifting the animal from its cage or, alternatively, a pair of forceps (toothless) can be used to grasp the mouse by either the tail base or the skin over the shoulders (Figure 2).

Figure 1



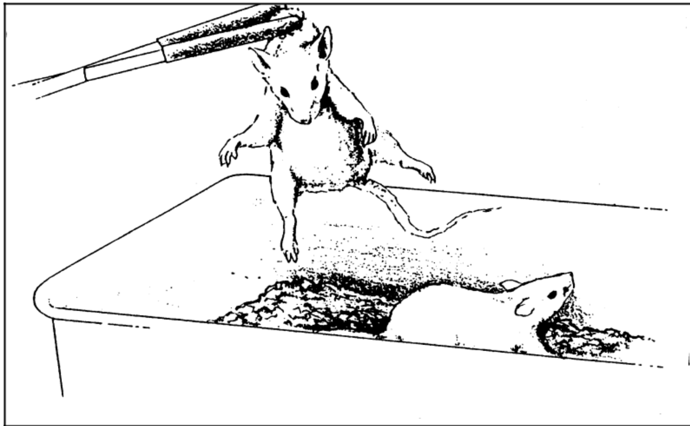


Figure 2

For restraint, the mouse is picked up by the tail as described above and placed over the wire bar lid of the cage and lowered until the mouse grasps the wire with its forefeet (Figure 3a). The excess skin over the animal's back is then grasped between your thumb and forefinger, the hand is rotated so that the mouse is lying on its back within the palm of the hand. The animal's head is closest to your thumb while the tail is grasped with your pinkie (Figure 3a and 3b). The result is a mouse that is immobilized for examination or manipulation.

Devices are available to restrain mice for a variety of procedures. Commercially available plexiglass restraining cylinders provide access to the animal's tail for intravenous injection or blood collection. Homemade devices can be constructed out of plastic syringe casings.

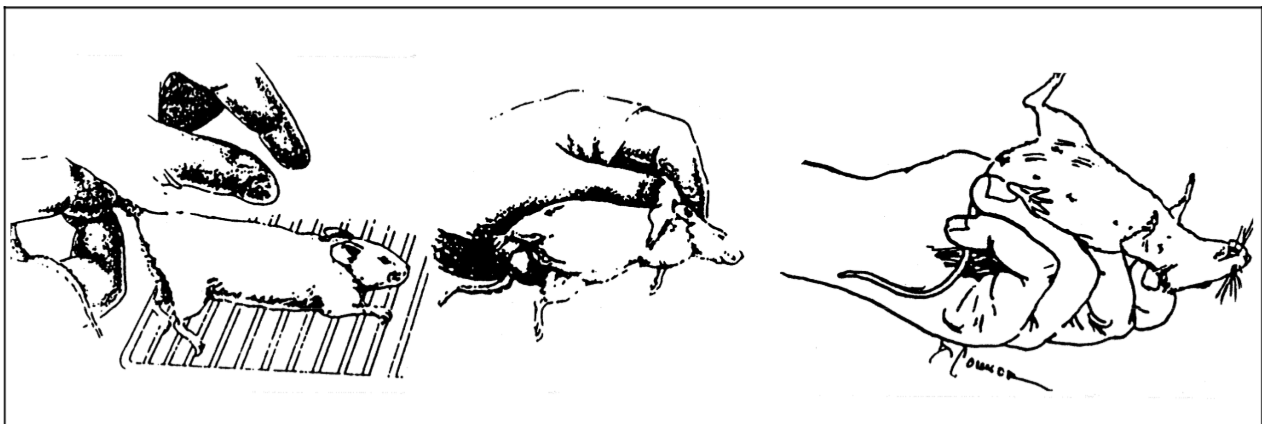


Figure 3a

Figure 3b

Alternatively, the wire bar lid from a shoebox cage which contains a food trough can be used to restrain a mouse to provide access to its tail. The wire bar lid is set on a solid surface so that it rests on the angular food trough. The mouse is directed between the food trough and the end of the wire bar lid. The mouse's tail is directed between the wire bars and gently pulled so that the animal's rear end is held firmly against the lid. This method provides access to the tail, while limiting the mouse's ability to turn around and bite.

RATS

Rats can be picked up by the base of the tail as has been described for mice; however, extreme care must be exercised as an adult rat's body weight is approximately 20-fold greater than an adult mouse, whereas the tail is not 20 times greater in diameter. Therefore, it is easy to injure the rat's tail. Common injuries include fracturing coccygeal vertebrae (the small vertebrae within the tail) or causing the skin to slip off the tail exposing underlying tissue. It is essential that the rat be picked up by the base of the tail as close to the body as possible (Figure 4a). The rat should then be placed on your forearm or a solid surface. A rat should not be carried by their tail for more than a few seconds! Alternatively, rats ($\leq$ 300 grams) may be picked up by grasping the animal's body from above so that the rat's back is held firmly around the thorax with your thumb and forefinger placed on either side of the animal's head at the level of the mandible (Figure 4b). When held firmly, the rat is restrained and is unable to move its head to bite. Large rats may be picked up similarly; however, the hindquarters must be supported with the other hand.

Figure 4a

Figure 4b

As with mice, there are commercial plexiglass rat restrainers that provide access to the rat's tail while protecting the handler from the animal's head. Conical plastic disposable sleeves, referred to as DecapiCones, can also be used (Figure 5). The flexible transparent clear plastic sleeve is conical, open at its base, and has a small breathing hole at the apex. The rat is slid into the cone through the base with its nose resting adjacent to the breathing hole. The cone permits access to the tail while its head and torso are restrained in the cone.

Figure 5

HAMSTERS

Males are generally more docile than females. If precautions are taken, hamsters can be routinely handled with minimal stress to the animal and handler. Awakening the hamster from sleep will frequently be met with an aggressive response. Hamsters can be removed from their cage with the use of a small can or cup, which they will usually enter; they can be scooped out with cupped hands (Figure 6); or, they can be grasped by the abundant loose skin over the dorsal cervical region (Figure 7).

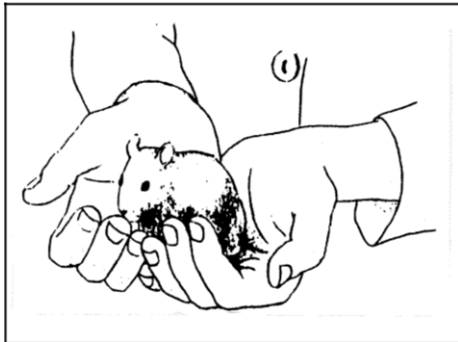


Figure 6

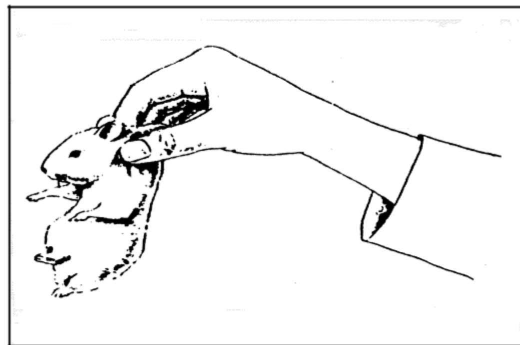


Figure 7

To manually restrain a hamster begin by placing the animal down on a solid surface. The palm of the hand is placed down over the hamster with the thumb near the head. The excess skin is grasped and gathered into your hand until the body wall is snug against your fingers (Figure 8). The animal will be immobile and will not be able to turn its head and bite.

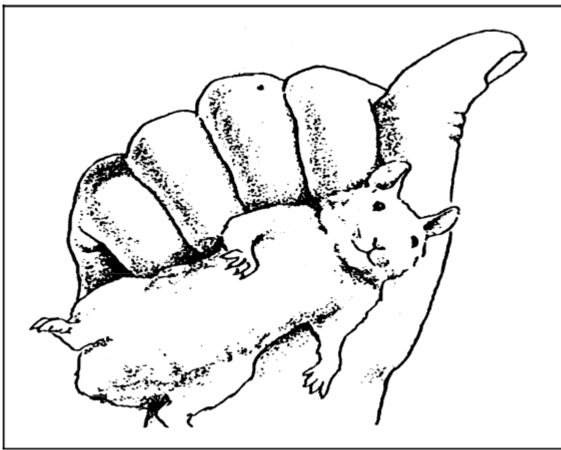


Figure 8

GUINEA PIGS

Guinea pigs should be restrained using two hands. Your dominant hand should be used to grasp the animal's thorax from below, opposing your thumb and fingers on either side of the animal's chest (Figure 9). The second hand is used to support the hindquarters. For greater control, the animal can be held using the same grip, however the animal should be grasped from above the thorax instead of below, and the pelvic limbs should be grasped and extended while the animal is placed in dorsal recumbency on a flat surface.

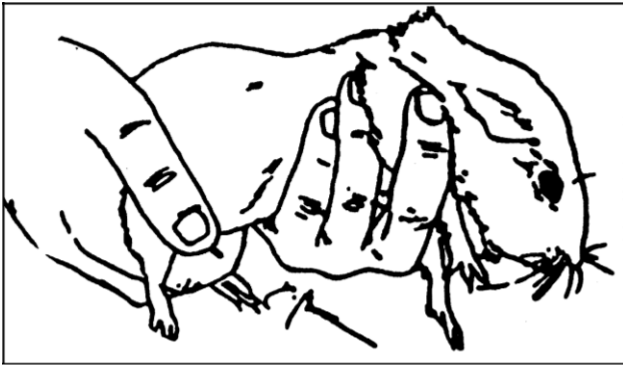


Figure 9

RABBITS

Appropriate technique is essential to prevent the rabbit from being injured. Rabbits should be removed from their cage by grasping the excess skin over the dorsal cervical region (Figure 10). Rabbits **should never** be picked up by their ears. The rabbit's hind end should be supported immediately after removal from its cage. If not supported properly, the rabbit may kick with its powerful hindlimbs, which may induce lumbar vertebral dislocation or fracture (broken back). Rabbits can be moved for short distances by permitting the animal to bury its head at the junction of your body and your bent elbow, supporting the animal's body with your forearm while putting gentle downward pressure over the animals back with your other hand (Figure 11a).

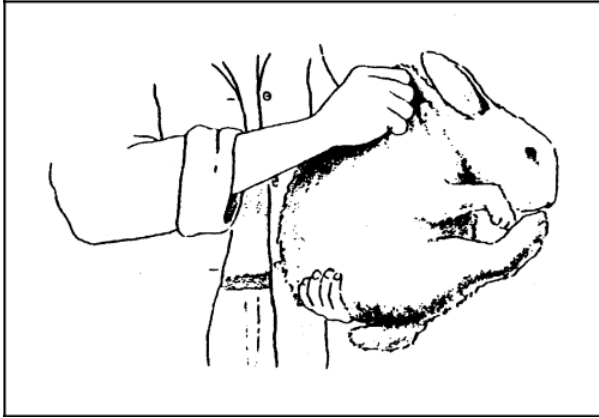


Figure 10

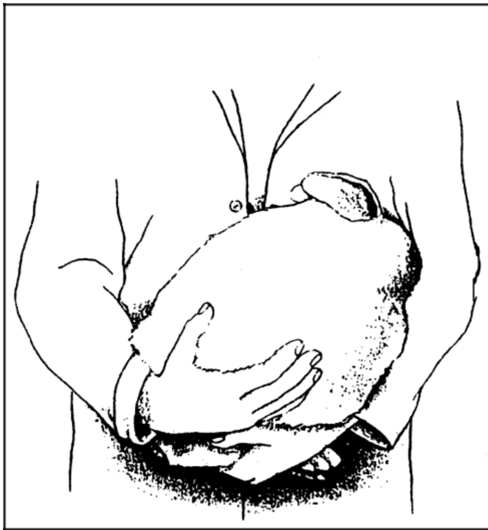


Figure 11a

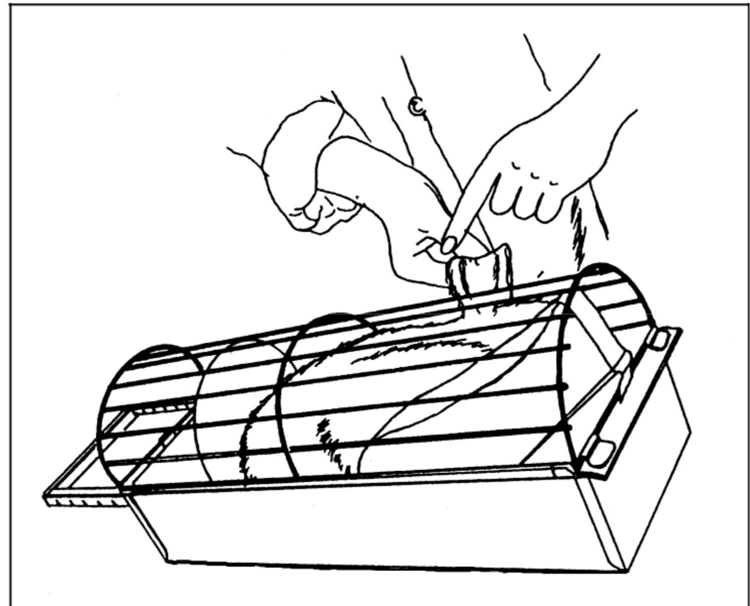


Figure 11b

RARC has stainless steel rabbit restrainers that are extremely useful for transporting or restraining rabbits (Figure 11b). Rabbits are relatively easy to restrain when using an appropriate restraining device. The stainless-steel rabbit restrainers have a solid bottom, a ramp at its far end, a top made from wire bars, and contains a metal sliding plunger. Rabbits are placed into the device by raising the wire bar top and pulling the plunger away from the end with the ramp creating ample space into which the rabbit is placed. The wire bar top is then closed and locked into place, and the plunger slowly pushed in the direction of the rabbit until the rabbit is gently but firmly displaced up the ramp contained within the restrainer such that its nose is at the breathing end of the ramp and the rabbit cannot move. This device provides excellent access to the rabbit's ears, making it very useful for blood collection or IV injections. The restrainer also provides access to the animal's back for subcutaneous or intradermal injections.

Rabbits can also be restrained using a towel (Figure 12). The towel is either utilized to cover the animal's eyes or wrapped around the animal's body to securely enclose the animal's limbs. Either the ears or the rabbit's dorsum is left exposed, dependent on the site that requires access. Clean towels should always be used between different groups of rabbits.

Figure 12

FERRETS

With appropriate handling and socialization, ferrets can be easily managed. Regular handling is necessary to maintain their well-being. Caution should be exercised when handling ferrets from a new shipment or when working with jills (female ferrets) with kits. Jills with litters are protective and may bite if they feel threatened. After adjusting to their new environment, ferrets can usually be handled without difficulty, and without restraint gloves.

The adult ferret is best held by grasping behind the forelimbs with one hand and supporting the hindquarters with the other hand. Restraining its the hind legs will cause it to struggle (Figure 13).

Figure 13

Ferrets can also be restrained using a towel (Figure 14a). The towel is wrapped around the animal's body to securely encase the animal's limbs. An alternative method of restraint is to place one hand across the animal's shoulders, with the thumb and forefinger around the neck and the other fingers around the chest behind the forelimb (Figure 14b).

Figure 14a

Figure 14b

Ferrets can also be scruffed. When the ferret is held by the scruff of the neck, it often yawns and relaxes its body, allowing simple procedures such as nail trimming and intramuscular injections to be performed (Figure 15).

Figure 15

Ferret nails should be trimmed approximately every 4 weeks. This may be easily accomplished by one handler scruffing the ample skin at the ferret's neck and supporting the hind end with the other hand while the other handler trims the toenails.

Parenteral Administration

The ability to administer compounds by injection is essential for many experimental studies employing laboratory animals. Anesthetics and test compounds must frequently be administered to animal subjects by injection. There are five commonly used routes of parenteral administration: subcutaneous (SC), intraperitoneal (IP), intravenous (IV), intradermal (ID), and intramuscular (IM). Not all techniques are routinely conducted for each species. For example, IM injections should be minimized in the mouse because the amount of compound that can be safely injected into the mouse's limited muscle mass is often too small that the technique is not practical. IP injections are almost never administered to rabbits, as other techniques are more suitable. When a study requires repeated parenteral administration over an extended period of time, a more permanent access system is recommended to decrease the risk of pain from multiple injections and minimize animal handling. Please see the "Chronic Administration and Sampling" section below for more details.

It is essential that the appropriate parenteral site be selected. Systemic absorption and distribution differ considerably between sites. Dosage and volume of compound administered must be carefully considered relative to the type of agent, site of injection and species used. The size of syringe and needle must also be considered. In order to assure the delivery of an accurate volume of injected compound, the volume of the syringe should, in general, not exceed the volume of compound to be administered by 10-fold. The length of the selected needle should be long enough that sufficient tissue penetration is achieved but not be so long that it becomes unmanageable or is likely to be inserted too far. The needle's size should be as small (highest gauge) as possible to limit tissue trauma but be large enough so that the injection can be made relatively rapidly and without applying excessive pressure to the syringe plunger. Table I (page 45) contains recommended needle sizes by route of injection for each species. Syringe and needles should be of the locking type in order to prevent accidental dislodgement, which may result in autoinoculation or back spray. To minimize the potential for disease transmission, contamination of injectate, and dulling of needle tips, a new sterile needle should be used for each animal. Proper disposal of used needles and syringes is essential. Needles should **never** be recapped manually, as the risk of accidental injection is highest during recapping, and they should always be disposed of into a designated sharps container. If recapping of the needle is necessary, NeedleSafe® recappers are available in animal procedure rooms (Figure 16). To safely

recap a needle attached to a syringe insert the needle cap into the NeedleSafe® device then insert the needle firmly and fully and pull out the recapped needle with a slight twisting motion.

Figure 16

Most injections should be preceded with local skin/fur antisepsis. A cotton swab containing 70% isopropyl alcohol or an equivalent antiseptic should be applied to the injection site prior to injection. Certain injection methods/location may also require fur removal.

Injection volumes provided in this document are **general recommendations**; however, significant deviations must be scientifically justified in the associated IACUC-approved protocol. Suggested volumes by species and route of administration are provided in Table I. **Under some circumstances it may be inappropriate to inject the recommended volume**. For example, volumes should be reduced when the agent is irritating or hypertonic. Volumes may be increased when giving isotonic fluids for rehydration and fluid maintenance. When performing injections for the purposes of polyclonal or monoclonal antibody production, please refer to the *Guidelines for the Production of Polyclonal and Monoclonal Antibodies in Rodent and Rabbits.*

MICE

Subcutaneous injection

SC injections can be administered easily to mice. The mouse can be restrained by one individual with the injection administered by a second person, or the restraint and injection can be done by one individual. In most cases, anesthesia is not required. The preferred injection site is illustrated in Figure 19. The needle is inserted between the folds of skin into the base of the triangle that is formed when traction is applied to the skin overlying the animal's scruff (between the animal's shoulders or the skin of the animal's flank). The syringe's plunger should be retracted to verify that a vacuum is created and no blood or tissue fluid can be aspirated. Subsequently, the plunger is depressed releasing the compound. In general, no greater than 1 ml should be injected per SC injection site in adult mice ($\geq$ 25 grams) and no more than 3 SC injections should be given to the same animal at one time. Several sites over the animal's back should be used if larger volumes must be administered. In general, needles should be 0.5 – 1 inch long and 23 gauge (G) or larger gauge (smaller needle size).

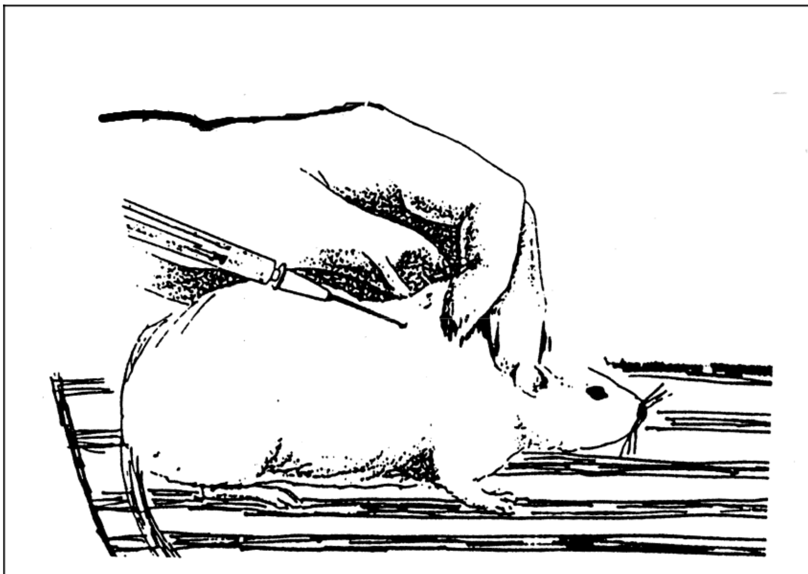


Figure 19

Intradermal injection

Intradermal (ID) injections may be used to immunize animals. In contrast to SC injections where compound is deposited into the space between the skin and body wall, ID injections deposit compound within the layers of the skin (Figure 20). Therefore, the volume of compound which can be administered is very small ($\leq$ 100 µl site; 50 µl recommended). Immediate dissolution of the bleb indicates that the compound has been injected subcutaneously rather than intradermally.

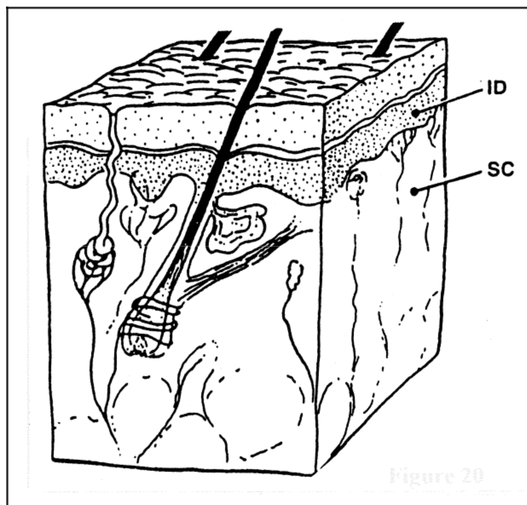


Figure 20

The mouse is anesthetized (e.g., isoflurane inhalation, 2 - 4% in oxygen, to effect). Once the animal is sufficiently immobilized, it is placed on a circulating water heating pad, an infrared or microwave warming pad, or other warming device that offers evenly distributed heat at a finite temperature. If the animal is haired, electric clippers or a depilatory cream is used to expose an area of skin on the dorsum, flank, or abdomen approximately 150% larger than the expected area of the bleb resulting from the injection(s). Loose fur (and cream if used) is removed with a moist gauze pad. If using a depilatory agent, the cream should be removed after no more than 20-30 seconds.

The injection site(s) is wiped with 70% isopropyl alcohol. The skin at the injection site(s) is stretched taut between the index finger and thumb, and a 26 G or smaller needle is inserted bevel up at a shallow angle between the epidermis and dermis ~ 2mm, or the length of the bevel. No more than 100 µL (<50 µL recommended) is injected per site. The site(s) of injection should avoid areas that may compromise the normal movement or handling of the animal (e.g., neck scruff). The injection should create a bleb in the skin, and if multiple injections are performed, they should be placed so that the blebs do not coalesce. The site is assessed for bleeding after injection. If bleeding occurs, pressure should be applied to the site until bleeding stops.

Intramuscular injection

The mouse should be restrained using an IACUC approved restraint technique. The scruff method may be used to secure the foot with the pinky and lower thumb to isolate the quadriceps muscle for injection. Alternatively, a cylindrical mouse restrainer (Figure 21) can be used with the foot gently pulled out of the restrainer. The injection site is wiped with 70% isopropyl alcohol and a 25G or smaller needle is inserted into the cranial aspect of the quadriceps muscle (Figure 22). The syringe's plunger is aspirated prior to injection to assure proper needle placement. If there is no blood, the contents of the syringe can be injected (maximum of 0.05 ml). After withdrawal of the needle, the site is assessed for bleeding. Pressure is applied if bleeding occurs.

Figure 21. Mouse in cylindrical restrainer receiving IM injection.

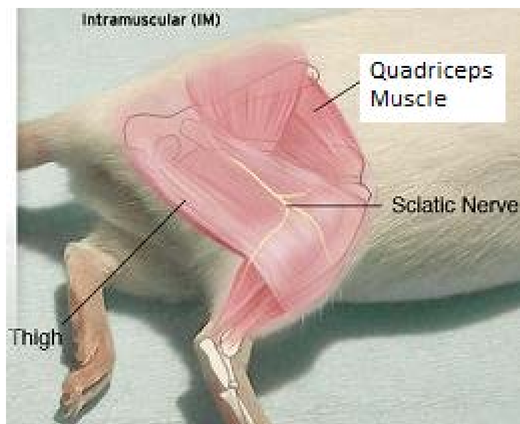


Figure 22. Diagram depicting location of quadriceps muscle where the IM injection should be made

Intraperitoneal injection

The administration of a compound into the peritoneal cavity is frequently performed in mice. The aim of this technique is to administer the compound into the space surrounding the abdominal organs, avoiding injecting an organ. Mice should be restrained and held with their ventrum exposed and head pointed downward. This allows the freely moveable abdominal organs to move towards the animal's diaphragm making accidental puncture of organs less likely (Figure 23). The injection site (lower right abdominal quadrant) should be wiped with 70% isopropyl alcohol. A 1 inch 23 G or higher gauge needle is inserted into the abdominal cavity in the lower right quadrant to avoid the cecum and urinary bladder.

The needle should be directed towards the animal's head at an angle of 40° and inserted approximately 5 mm (Figure 24). Aspiration should be attempted to ensure that an abdominal organ such as the bladder or colon has not been penetrated. If material (e.g., intestinal contents, urine, blood) is aspirated, the syringe should be removed and disposed. Never inject GI tract contents or urine into the peritoneal cavity, as a bacterial or chemical peritonitis will likely result. If no material is aspirated, the syringe contents can be injected. However, it should be noted that the inability to withdraw intestinal contents does not definitively rule out the potential of injecting into the intestinal lumen. After withdrawal of the needle, the site should be assessed for bleeding. Pressure is applied if bleeding occurs. In general, the volume of compound administered IP into an adult mouse should not exceed 2.0 ml.

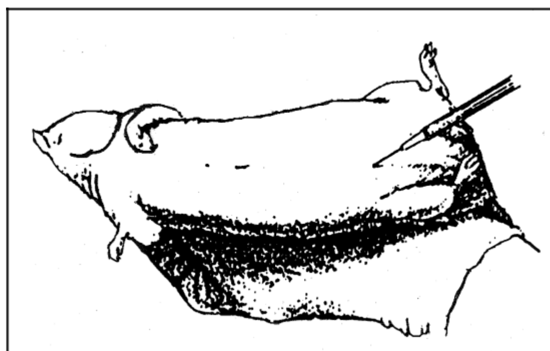


Figure 23

Figure 24

Intravenous injection

The veins on the lateral aspect of the mouse's tail are an excellent site for IV administration. The principal function of these veins is thermoregulation. They will dilate when the mouse's body

temperature rises in order to disseminate heat. Application of heat to the whole animal or locally to the tail can be used to cause venodilation making vascular access easier. The mouse can be warmed prior to cannulation by either placing the animal in a warming incubator set no higher than 37°C, for 15 minutes, or by exposing the animal to a heat lamp (while under constant supervision) for no more than 5 minutes.

The mouse should be restrained so that its tail is accessible (e.g., tail vein injection restrainer). The tail is cleansed with a sterile alcohol wipe prior to injection. A 0.5 inch 25 G or larger gauge needle is used. The vein is located, the needle inserted by directing the needle into the vein with its bevel pointing upward at an angle of approximately 20° (Figure 25). The needle is inserted slowly, visualizing the needle as it enters the vein. Once the vein's wall has been penetrated the needle should be directed cranially approximately 2 mm. Blood should be aspirated into the needle's hub before making an injection. During compound administration the vein should blanch and no compound or swelling should be detectable at the injection site. Compound should be administered slowly to avoid vascular overload or rupture of the vein from excess pressure. No greater than 0.5 ml should be administered IV to an adult mouse. Pressure should be applied over the injection site by gently holding a piece of gauze over the injection site for approximately 30 seconds to prevent hematoma formation. Preferably the needle should be inserted into the vein midway down the tail, permitting additional attempts for venipuncture proximally if the initial attempt is unsuccessful.

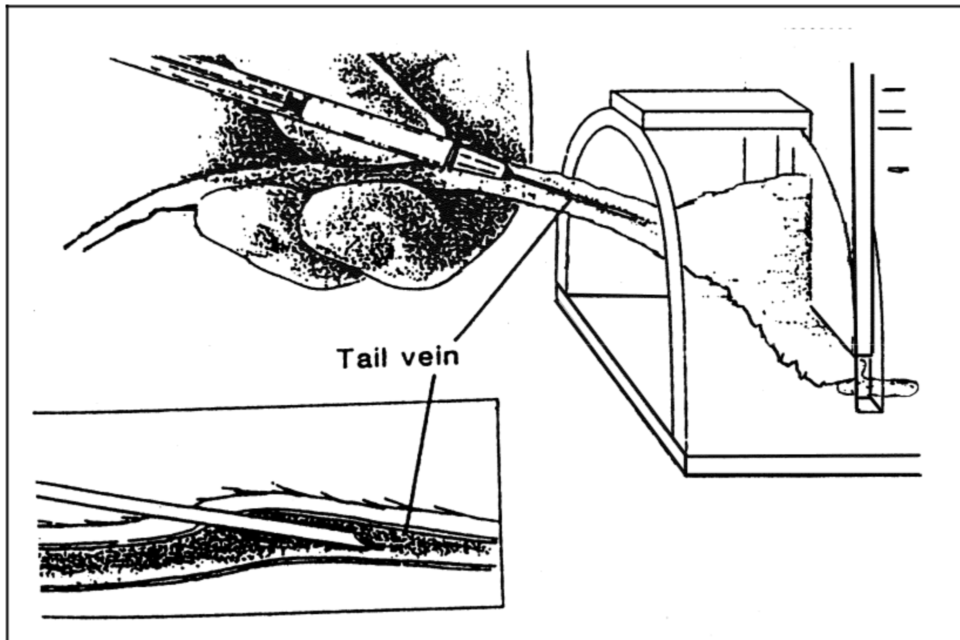


Figure 25

Retro-Orbital injection

The mouse's retro-orbital sinus is a pool of vessels (including the supraorbital vein, inferior palpebral vein, dorsal nasal vein, and superficial temporal vein) caudal to the eye. Injections into the retro-orbital space have been shown to be as effective in distributing compounds systemically as tail vein injections and are generally less technically challenging. However, this technique has the potential to result in more severe post-procedural complications than other methods described; therefore, this technique is recommended only when scientifically justified. The position of the retro-orbital sinus is shown below (Figure 26).

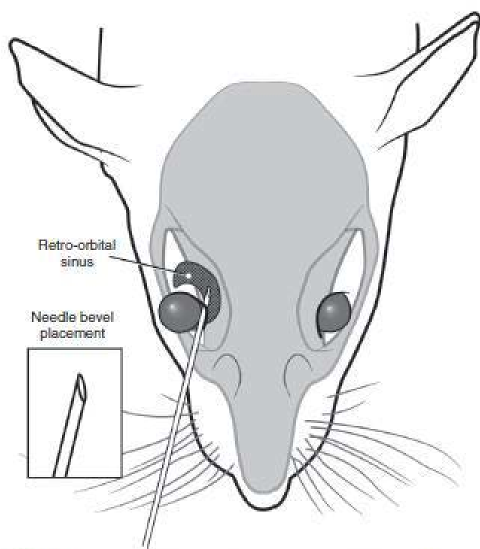


Figure 26

The mouse is anesthetized (e.g., isoflurane inhalation, 2 - 4% in oxygen, to effect). Once adequately anesthetized, the mouse is grasped so that its back rests on the palm of the hand with its head toward the thumb. Alternatively, the mouse is placed in sternal recumbency on a flat work surface. A drop of topical ophthalmic anesthetic (e.g. 0.5% proparacaine hydrochloride ophthalmic solution) may be applied to the eye for additional analgesia. The thumb is placed lateral to the animal's trachea so that the jugular vein on the same side as the retro-orbital space you will be injecting into is partially occluded and the fur on the animal's head is drawn back resulting in the animal's eye to protrude slightly. Care must be taken to ensure that the trachea is not compressed and that the jugular vein is not completely occluded stopping blood flow (Figure 27). If using inhalation anesthesia, you need to work quickly so the mouse does not recover from anesthesia during the injection.

Figure 27

A 27 G or smaller needle (31 G preferred) attached to a 1.0 ml or smaller syringe is inserted into the medial canthus (junction of eyelids closest to the animal's nose) of the eye, ~ 3-5 mm, to a point *behind* the globe. The bevel should be facing down to decrease likelihood of damaging the eye. The plunger should be withdrawn slightly to confirm flashback of blood. If this does not occur the procedure should be repeated. The compound to be administered is injected slowly until completely expelled from the syringe. There should be no resistance during injection. After injection, the needle is slowly removed and the eyelids closed and a dry cotton swab is applied over the eye with gentle pressure to prevent retro-orbital hemorrhage.

The maximum recommended injection volume is 150 µl. If multiple injections are to be given to the same animal, the eye to be injected should be alternated. If the same eye is to receive multiple injections, there should be at least 1 week in between injections. Each eye should receive no more than 3 injections.

Adverse effects of retro-orbital injection can include excessive hemorrhage, corneal lesions, and retro-orbital abscesses. If the retro-orbital space continues to bleed following injection, apply pressure with a clean cotton swab until the bleeding stops. Corneal lesions may occur if the eye becomes scratched during the process. The animal may need to be treated with daily ophthalmic antibiotic ointment, or euthanasia. Retro-orbital abscesses may occur if the injected substance does not disperse from the venous sinus and causes swelling. This will cause the eye to protrude (exophthalmos). If this occurs, consult RARC's Veterinary Service.

RATS

Subcutaneous injection

SC injections are performed in rats using the same technique as was described for mice with the following differences. The volume of compound administered can be increased to approximately 5 ml per site in an adult rat (300 grams). Syringe size should be increased proportionately, and needles should be 22G or larger gauge.

Intradermal injection

ID injections are performed in rats using the same technique as described for mice.

Intramuscular injection

IM injections may be performed in the rat. Injection volumes are limited to 0.2 ml per site because of limited muscle mass. Either the quadriceps muscles located on the cranial aspect of the femur or the hamstrings on the caudal aspect of the femur can be used (Figure 28). The rat can be restrained with a two-person technique. With this technique, one person gently grasps the rat around the shoulders with one hand and then holds the base of the tail with the other hand. The person administering the injection restrains the leg to be injected by gently grabbing the paw and applies 70% isopropyl alcohol over the injection site. Care must be taken to avoid depositing compound on or near the sciatic nerve which runs along the caudal aspect of the femur in the thigh. Therefore, the needle should be directed cranially if injecting the quadriceps or caudally when injecting into the hamstrings. A 23 G 0.5-inch needle or larger gauge should be used. The needle is directed through the skin into the muscle approximately 3-4 mm. Aspiration should be attempted before injecting to determine that accidental penetration of a blood vessel has not occurred. The site is assessed for bleeding after injection. If bleeding occurs, apply pressure to the site until bleeding stops.

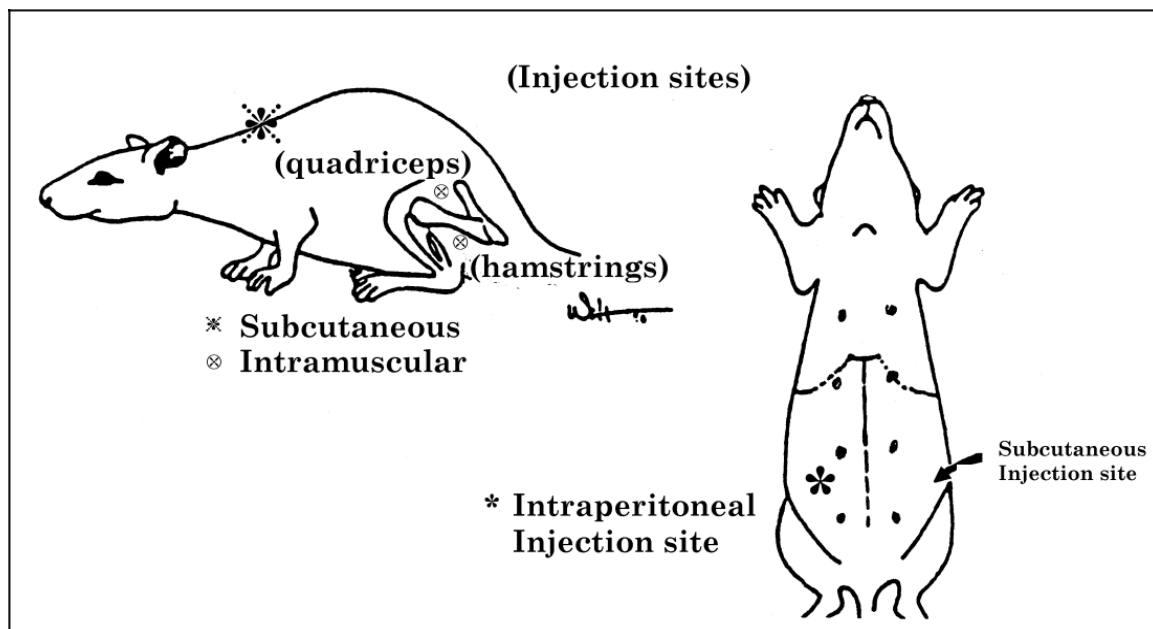


Figure 28

<u>Intravenous injection</u>

IV injection technique for the rat is similar to the mouse. However, the vessels may be more difficult to visualize, especially in adult rats. The skin overlying the vessels in adults becomes quite thick, making vascular access more difficult. The rat should be carefully warmed (e.g. with a heat lamp) to cause venodilation, increasing ease of vascular access. The rat is placed in a restraining device such that the lateral tail veins are accessible. The tail should be cleansed with a 70% isopropyl alcohol wipe prior to injection. A 0.5 inch 25 G or larger gauge needle is directed into a lateral tail vein, bevel up, at an angle of approximately 20°, preferably near the tail base. Once the vein has been penetrated, the needle should be directed cranially a distance of approximately 2 mm. The injectate (no more than 2.0 ml) should be slowly administered, making sure that no swelling is detected cranial to the injection site. Pressure is applied over the injection site after the needle is withdrawn from the vein for approximately 30 seconds with gauze (or similar material) to prevent hematoma formation and ensure that hemostasis is achieved.

<u>Intraperitoneal injection</u>

The technique for IP injections in rats is virtually identical to mice. The rat should be restrained using an IACUC approved restraint technique in a position that exposes the abdomen. The rat is then positioned so that its head is held tilted downward allowing the moveable abdominal organs to shift towards the animal's head, away from the needle, reducing the likelihood of penetrating the gastrointestinal tract. The injection site is located in the lower right quadrant of the animal’s abdomen (from the animal’s perspective). The injection should be performed by inserting a 23-25 G 5/8-inch-long needle approximately 5 mm into the lower right quadrant directing it towards the animal’s head at an angle of ~40°. The plunger is gently aspirated (pulled backwards) prior to injection to assure proper needle placement. If there is no blood, intestinal contents, or urine drawn into the syringe, the contents of the syringe can be injected. However, it should be noted that the inability to withdraw intestinal contents does not definitively rule out the potential of injecting into the intestinal lumen. After withdrawal of the needle the site is assessed for bleeding. Pressure should be applied if bleeding occurs. No more than 5.0 ml is injected IP.

HAMSTERS

Subcutaneous injections

SC injections are performed in hamsters using the same technique as was described for mice with the following differences. The hamster has considerably greater loose skin overlying the injecting site permitting a proportionately larger volume of compound to be administered ($\leq$ 3 ml). Syringe size should be increased proportionately, and needles should be 22 G or larger.

Intradermal injection

ID injections are performed in hamsters using the same technique as described for mice with the following differences. The skin at the injection site(s) is stretched taut between the index finger and thumb, and a 25 G or smaller needle is inserted at a shallow angle between the epidermis and dermis ~ 2mm, or the length of the bevel. No more than 50 µL of physiologically buffered solution is injected per site.

Intramuscular injections

IM injections may be administered to the hamster. The technique is as described for the rat except the injected volume is limited to 0.15 ml site because of the hamster's limited muscle mass. Either the quadriceps muscles located on the cranial aspect of the femur or the hamstrings on the caudal aspect of the femur can be used.

Intravenous injections

IV injections are difficult to perform in the hamster because of the lack of easily accessible veins. RARC staff should be contacted for additional information.

Intraperitoneal injections

The technique for IP injections in hamsters is virtually identical as described for mice and rats. Hamsters should be restrained with their abdomen exposed and their head held downward. The injection site, method and needle size is as described for mice. Because of their larger size $\leq$ 3.0 mls of compound can be administered to an adult hamster.

GUINEA PIGS

Subcutaneous injection

SC injections are performed in guinea pigs using the same technique as was described for mice with the following differences. The preferred injection site is illustrated in (Figure 29). The volume of compound administered can be increased to approximately 5 ml per site in an adult guinea pig. Syringe size should be increased proportionately, and needles should be 22 G or larger.

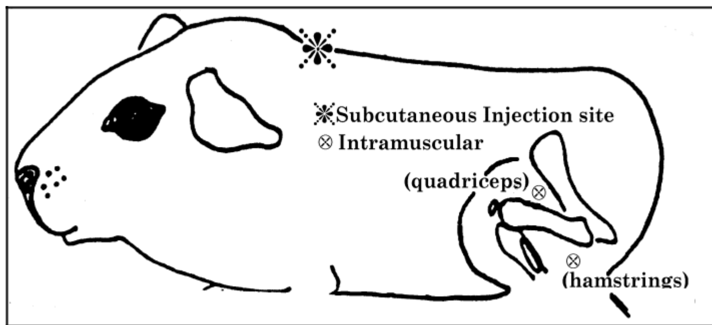


Figure 29

<u>Intramuscular injection</u>

IM injections may be administered to the guinea pig. The sites and methods are similar to those described for the rat (Figure 29). Injection volumes can be increased to $\leq$ 0.3 ml site because guinea pigs have slightly larger muscles.

<u>Intravenous injection</u>

IV injections are very difficult to perform in guinea pigs because of the lack of easily accessible veins. The veins at the base of the tongue, saphenous, or penile vessels (males) may be used. Consult RARC staff for additional information.

<u>Intraperitoneal injection</u>

The technique for IP injections in guinea pigs is virtually identical to mice. Guinea pigs should be restrained with their abdomen exposed and their head held downward. The injection site, method and needle size is as described for mice. Because of their larger size ≤5.0 mls of compound can be administered to an adult guinea pig.

<u>Intradermal injection</u>

ID injections are performed in rats using the same technique as described for mice with the following differences. A 0.5 inch 25 G or larger gauge needle and a 1 ml syringe are recommended. ID injections should be made over the dorsal thoracic and lumbar region. Multiple sites (up to 10) can be used.

RABBITS

<u>Subcutaneous injection</u>

SC injections are easily performed in rabbits because of the laxity of their skin and the large area into which compound can be administered (Figure 30). The technique is the same as described for mice; however, injections should not be administered over the neck as this is the site from which the animal is picked up. A 1 inch 22 G or larger gauge needle is recommended. Volumes should not exceed 5 ml per site unless isotonic fluids are administered. Approximately 20-40 ml of isotonic fluids can be administered per site and multiple sites may be used. Always aspirate before injection.

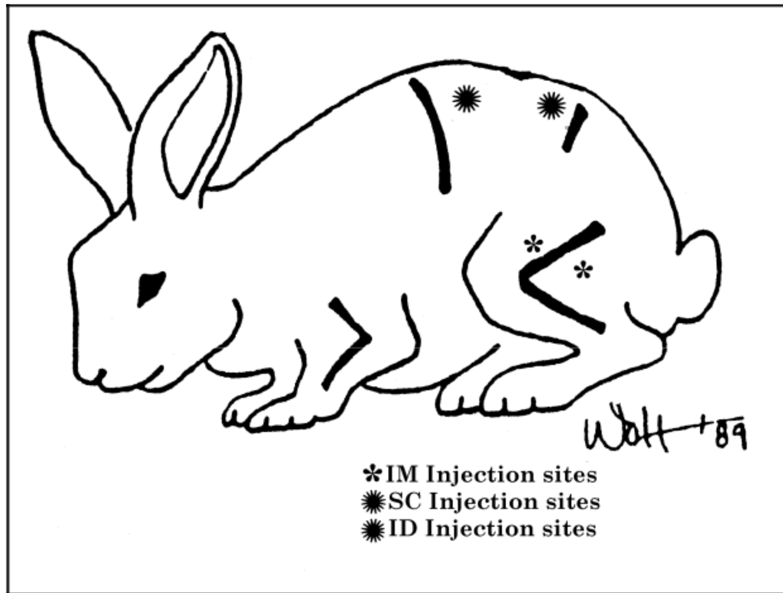


Figure 30

Intramuscular injections

The recommended sites and technique for IM injection in the rabbit are as described for rats. IM injection sites are illustrated in (Figure 30). In addition to the dorsal muscles of the anterior thigh (quadriceps), the dorsal lumbar muscles can be used for IM injections. However, the rabbits larger muscle mass requires a longer needle (1 inch) to adequately introduce the compound deep within the muscle belly, a distance of approximately 7 - 10 mm in an adult rabbit and acceptable volumes to be injected are larger ($\leq$ 1.5 ml).

Intravenous injections

IV injections are straightforward in rabbits because of the ease of vascular access. The marginal ear veins located on the lateral aspect of the rabbit's ears are readily visible (Figure 31). The veins can be made more prominent by occluding the vessel at the base of the ear by gently holding off with your fingers. The vein is swabbed with a 70% isopropyl alcohol-soaked cotton swab. The needle is inserted as described for injecting rodent tail veins. Remember to aspirate to verify placement of the needle within the vein. Remove your occluding fingers prior to injection. Volumes of approximately 5 to 10 mls can be administered if given slowly, however routine volumes are frequently $\leq$ 1 ml. A butterfly needle (Figure 32) or an "over the needle" catheter may be inserted if larger volumes are to be administered or repetitive injections will be given. A 0.5 inch 24 G or larger gauge needle is recommended. Pressure should be applied over the injection site by gently holding a cotton swab or piece of gauze over the injection site for approximately a minute to prevent hematoma formation.

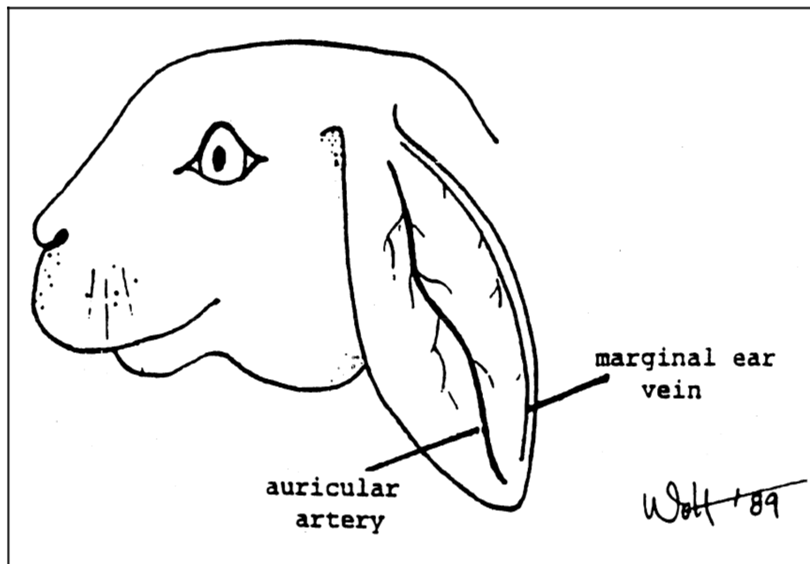


Figure 31

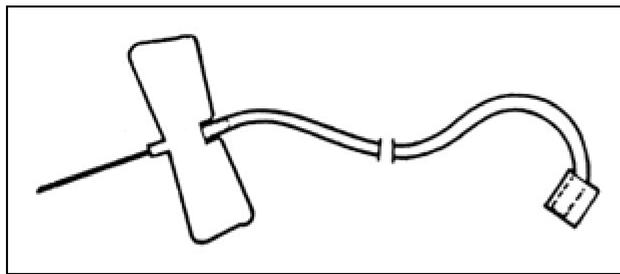


Figure 32

Intradermal injection

ID injections may be used to immunize rabbits. The technique is as described for mice with the following changes. The neck and anterior thoracic region should be avoided for injection as rabbits are handled by grasping this region. Because of their larger size up to 12 sites can be used ($\leq$ 0.1 ml site; 0.05 ml recommended).

Enteral (Oral) Administration

The most common routes of enteral administration are oral delivery and oral gavage. These methods are convenient and do not require the use of chemical restraint. They can be used in rodent and non-rodent species and when performed by skilled personnel, they are well tolerated and minimally stressful to the animals. Habituation to restraint prior to oral gavage can reduce the stress associated with the procedure. The maximum volume administered should not exceed 10 ml/kg. Oral gavage is most commonly performed in mice and rats.

Mouse oral gavage

The mouse is restrained vertically using an IACUC approved restraint technique. A gavage needle is chosen assuring that the length of the needle and the size of the ball at the tip of the needle is appropriate for the size and age of the mouse (generally 22-20 G). The diameter of the ball must be small enough to slide down the esophagus, but large enough so that it does not easily pass into the trachea. Before inserting, the gavage needle is aligned along the mouse's body so the ball sits at the level of the last rib. This allows a determination as to how far the needle should be inserted (Figure 33).

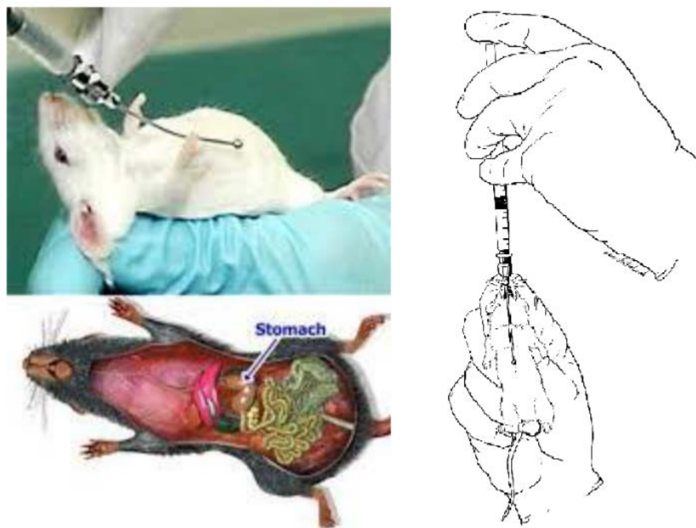


Figure 33

The needle is placed into the animal's mouth at approximately a 45° angle with the horizontal plane. As the needle is advanced, it is raised so that it aligns with the axis of the body. The needle and syringe are gently advanced. Once the ball has reached the level of the stomach, the contents of the syringe, is delivered, which should not exceed 1 ml (0.5 ml or less is preferred). The plunger is then drawn back slightly and the needle removed (Figure 34).

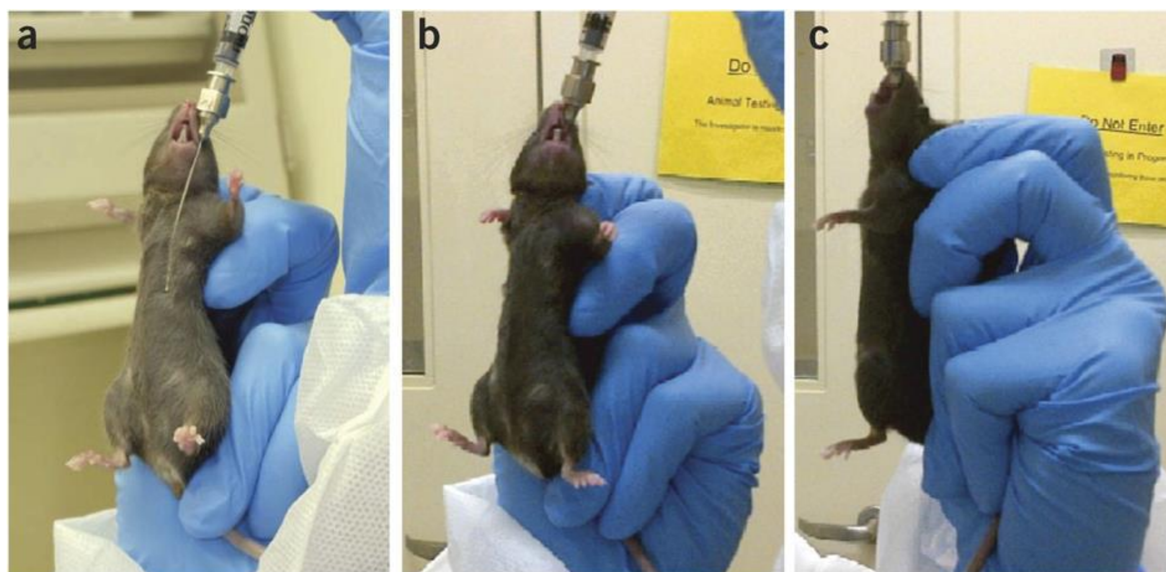


Figure 34. (a) Measurement of the length of the gavage needle against the animal's body; (b, c) Administration of the solution upon verification of proper placement of the gavage needle.

Rat oral gavage

Rat oral gavage is performed in rats using the same technique as described for mice with the following differences. A gavage needle is chosen assuring that the length of the needle and the size of the ball at the tip of the needle are appropriate for the size and age of the rat (generally 20-18 G). Once the ball has reached the level of the stomach, the contents of the syringe, which should not exceed 5 ml in an adult rat, are delivered (Figure 35 and 36).

Figure 35

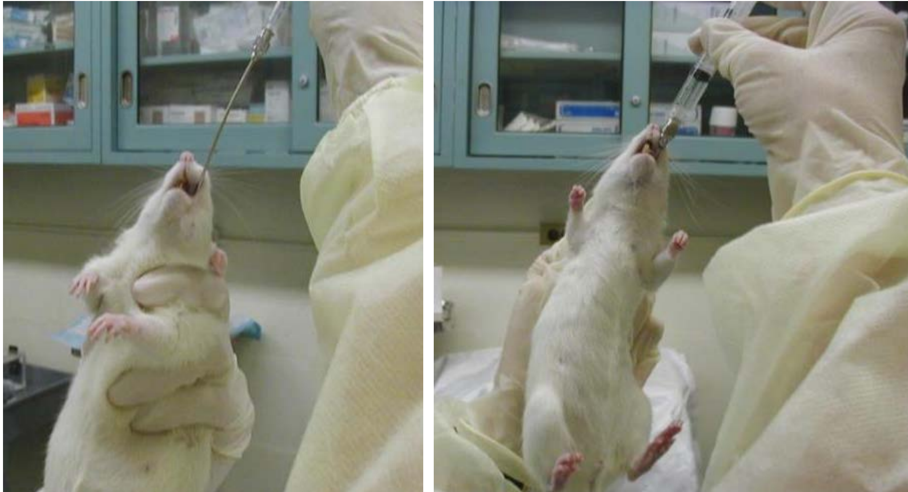


Figure 36

Hamster oral gavage

With the hamster manually restrained, a curved or straight, flexible or rigid, 18–20 G gavage needle (with a ball head) is inserted into the mouth. The length of the gavage needle should be a little shorter than the distance from the tip of the animal's nose to the end of its rib cage (~4 cm). When using a straight needle, the needle is slid caudally along the roof of the animal's mouth while using the needle as a lever to assure the hamster's head is in a straight line with the remainder of its body. At times, slight dorsoflexion of the animal's head, using the needle, makes the intubation easier. With gentle pressure the needle is advanced down the esophagus and into the stomach. The animal is generally not anesthetized for this procedure and if anything more than mild resistance is felt the gavage needle should be removed and redirected. Even with a small ball head on the gavage nee dle the chance of entering the trachea is extremely low and is readily detected via the animal's gag reflex. A maximum volume of 20 ml/kg body weight can be administered (10 ml/kg ideal).

Guinea pig oral gavage

With the guinea pig manually restrained, a curved or straight 18-20 G 1.5 inch stainless steel gavage needle (with a terminal ball) is inserted into the mouth. A simple mouth gag may be used to enable intragastric intubation of the guinea pig by one person without the use of sedation or anesthesia. A maximum volume of 20 ml/kg body weight can be administered (10 ml/kg ideal).

Rabbit oral administration gavage

Rabbits will refuse food or water that has disagreeable odor or flavor. To administer small quantities of liquid, the rabbit is restrained wrapped in a towel, and a syringe is introduced into the corner of the mouth (Figure 37). The liquid is slowly dispensed as the rabbit is observed to ingest it. Mixing a substance with honey or sweetened yogurt may increase its palatability.

Figure 37

Blood Sampling

Collection of blood from laboratory animals is frequently necessary for a variety of experimental uses including determination of pharmacokinetics, antibody production, clinical pathology evaluation, etc. Blood may be collected from animals that are to survive the procedure or at sacrifice as a terminal event. Whereas there is no limitation on the amount of blood that may be collected terminally, the volume collected from animals surviving the collection is limited to

prevent anemia and hypovolemia. As a general guide the 1-3-6 rule should be followed. The rule states that the average blood volume of most laboratory animals is 6% body weight (60 ml/kg); the most blood that can be reasonably expected from a terminal sacrifice is 3% body weight (30 ml/kg); and no more than 1% (10 ml/kg) body weight may be collected during any 2-week period from animals surviving the blood collection. Although venipuncture is generally a satisfactory method for survival blood collection, catheterization of a peripheral vessel may be necessary for animals requiring frequent collection of small quantities of blood. Table II (page 41) contains maximum blood collection volumes by species for survival and terminal bleeds. It is extremely important to apply pressure to the blood collection site, especially when penetrating an artery, for several minutes post blood collection to prevent hematoma formation. Please, refer to the Chronic Administration and Sampling Section for repeated blood collection.

MICE

There are a variety of methods that are utilized to collect blood from mice. The techniques described below are recommended for survival (tail vein, orbital venous sinus, submandibular, saphenous vein, or mental [chin]), or terminal (cardiac) blood collection.

Lateral tail vein venipuncture

The veins located on the lateral aspect of the mouse's tail are useful for collecting small volumes (< 0.3 ml) of blood. The mouse is carefully warmed (e.g. with a heat lamp) to cause vasodilation, increasing ease of vascular access. The mouse is placed in a clean and disinfected restraining device such that the lateral tail veins are accessible. The tail is cleansed with a sterile alcohol wipe prior to blood collection. A small puncture is placed in the vein with a sterile disposable #15 scalpel or a sterile 23-26 G needle with the bevel pointing up. The initial puncture should be performed distally (~2-3 cm from the tip of the tail), to allow repeat collections to be performed above the initial puncture site avoiding the formation of a hematoma. Droplets of blood, which collect at the puncture site, are collected with a capillary tube or microfuge tube held at a 45° angle to the skin. The use of collection tubes with capillary action facilitates blood collection. Hemostasis is achieved by applying gentle pressure with a gauze sponge or cotton swab to the puncture site for approximately 30 seconds. Alternatively, a cauterizing agent, such as a styptic pencil, may be used to achieve hemostasis. The puncture site is inspected visually to ensure adequate hemostasis before returning the mouse to its cage.

No more than 1% (10 ml/kg) of the mouse's body weight should be collected every two weeks. This amount may be collected in up to 5 collections. When multiple collections are performed, blood should not be collected from a single vein more than once a day and the right and left veins should be alternated to allow adequate healing of the vessel.

Submandibular blood collection

Adequate restraint will be provided by grasping the mouse's skin between the shoulder blades using the non-dominant hand. The caudal aspect of the mandible is determined by gentle palpation and the approximate area of the temporomandibular junction (the junction of upper and lower jaw) is identified. The cheek and underlying vascular bundle immediately caudal to this area will be punctured using a commercially available lancet (Goldenrod Lancet, MEDIpoint, Inc.). A thin layer of petroleum jelly or eye lubricant may be applied to the fur overlying the vessel allowing the blood to form clean droplets on the surface of the skin.

The appropriate sized lancet is chosen based on the age of the mouse: 2-8 weeks old 4 mm point, 2-6 months old - 5 mm point, over 6 months of age - 5.5 mm point. The lancet is repositioned if bone is hit. Once blood begins to drip, a blood collection or a microhematocrit tube is used for collection. Blood is collected by holding one of the aforementioned tubes under the site to collect the blood droplets. If blood is observed to flow from the ear, collection may be attempted or the lancet repositioned slightly cranial and ventral from the previous site once blood flow from ear has ceased. Blood loss through the ear must be accounted for in total blood volume collected from mouse. After collecting the desired sample amount, gentle pressure will be applied to the puncture site using a 3x3 inch gauze square until bleeding stops. After cessation of bleeding, the mouse is observed for signs of trauma and if normal, is returned to its cage. No more than 1% (10 ml/kg) of the mouse's body weight should be collected every two weeks. Sampling may be done as frequently as once every 3 days provided alternating submandibular veins are used.

Retro-orbital venous sinus blood collection

The sinus surrounding the globe of the mouse's eye is a useful site for collecting larger volumes of blood from surviving animals. The schematic provided in Figure 38 illustrates the location of the sinus. General anesthesia must be provided when collecting from this site.

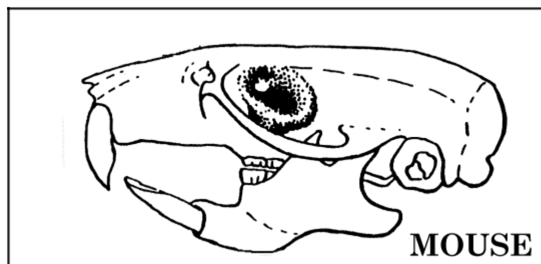


Figure 38

Under general anesthesia, the mouse is grasped so that its back rests on the palm of your left hand (right hand if you are left-handed) with its head toward your thumb. The thumb is placed just lateral to the animal's trachea so that the jugular vein on the same side as the eye from which you are collecting blood is occluded and the fur on the animal's head is drawn into the palm of your hand. This causes the animal's eye to proptose (bulge) slightly. Be careful not to occlude the trachea. A 50 µl microhematocrit tube which has been broken in two is directed into the medial canthus (junction of eyelids closest to the animal's nose) of the eye rotating slightly as the tube is directed to a point directly behind the glove, inserting the non-broken end first (Figure 39a). Sufficient pressure must be applied to cut through the fibrous layer which surrounds the sinus. Blood flows through the tube and occasionally around the tube once the sinus had been penetrated (Figure 39b).

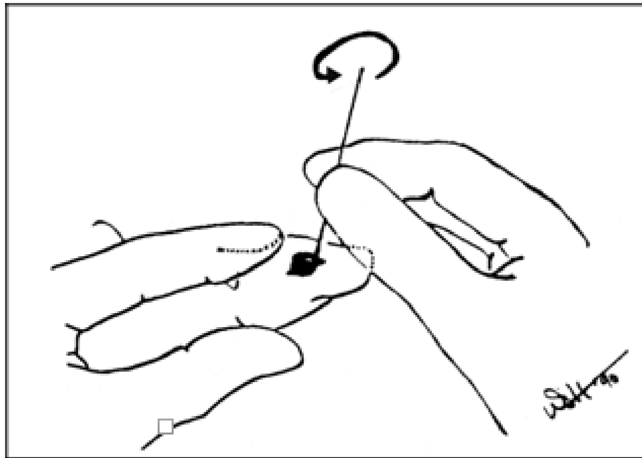


Figure 39a

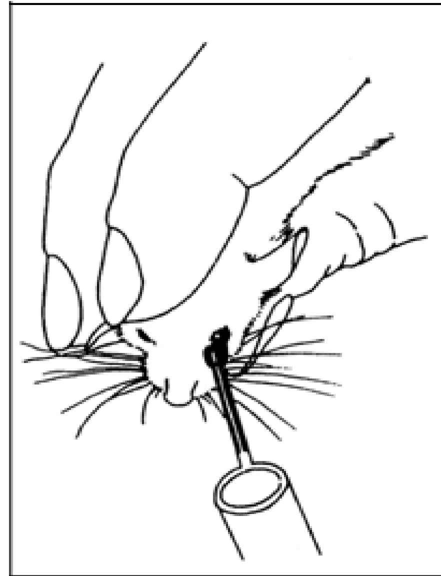


Figure 39b

After blood collection, the tube is removed, the eyelids closed and dry gauze is applied over the eye with gentle pressure to prevent retro orbital hemorrhage. In general blood should not be collected from the same eye more than 3 times, both eyes cannot be used in the same day, allowing at least 1-2 weeks between collections. No more than 1% (10 ml/kg) of the mouse's body weight should be collected every two weeks.

Saphenous vein blood collection

The mouse should be warmed immediately prior to blood collection to increase blood flow by either placing a heat lamp over the cage for three - five minutes (watching carefully for signs of overheating) or placing the cage on a circulating warm water pad. The conscious mouse is then placed in a clear, perforated restraining tube so that its head is furthest inside the restraint devise. The hind leg is extended and immobilized in an extended position by applying gentle downward pressure immediately proximal (superior) to the knee joint. Extension stretches the skin over the leg, making the saphenous vein more accessible. The saphenous vein is found on the caudolateral aspect of the hind leg. The area may be shaved with clippers (#40 blade) to make the vein more visible. The area should be cleaned with 70% isopropyl alcohol on a cotton swab or gauze sponge. A thin layer of petroleum jelly or eye lubricant is applied to the skin overlying the vessel allowing the blood to form clean droplets on the surface of the skin as it leaves the vessel. Application of gentle pressure between the tail and the thigh will reduce venous return making the vessel more visible. A small puncture (do NOT lance) is placed in the vein with a sterile disposable #15 scalpel or a sterile 23-26 G needle with the bevel pointing up. Droplets of blood, which collect at the puncture site, are collected with a capillary tube or Microfuge tube held at a 45° angle to the skin. The use of collection tubes with capillary action facilitates blood collection. Hemostasis is achieved by applying gentle pressure with a gauze sponge or cotton swab over the puncture site. The operator s grip is also released to aid in hemostasis. Alternatively, a cauterizing agent, such as a styptic pencil, may be used to achieve hemostasis. The puncture site is inspected visually to ensure adequate hemostasis before returning the mouse to its cage.

Only small blood volumes (< 80 µL) can be collected by this method. No more than 1% (10 ml/kg) of the mouse’s body weight should be collected every two weeks. When multiple

collections are performed, blood should not be collected from a single vein more than once a day and the right and left veins should be alternated to allow adequate healing of the vessel.

Tail tip (tail clip, tail snip) blood collection

Anesthesia is recommended, but not required. Alternatively, the use of a systemic analgesic or local anesthetic (e.g., bupivacaine) should be considered. The mouse may be carefully warmed (e.g., with a heat lamp) to cause vasodilation, increasing ease of vascular access. The mouse is placed in a clean and disinfected restraining device such that the distal tip of the tail is accessible. The tail is cleansed with a sterile alcohol wipe prior to blood collection. Using clean, sharp scissors or a scalpel, 0.5–1 mm of tissue can be cut from the tail tip distal to the tail vertebrae/bone at an angle perpendicular to the tail. The cutting instrument should be disinfected with 70% isopropyl alcohol between uses on individual mice. Blood can be obtained by direct flow or by gently massaging ('milking') the tail until a droplet of blood forms at the tail tip. Note that rubbing the tail from base to tip may result in leukocytosis in the sample. The cut is made only once. Small amounts of blood can be collected from the tip of the tail multiple times by warming and massaging the tail, or if necessary, removing the scab or blood clot from the tail tip with a gauze pad soaked in saline. Blood can be collected with a capillary or microfuge tube. After collection and before returning the mouse to its cage, the tail tip should be checked for hemostasis. If necessary, gentle pressure can be applied with sterile gauze or a cauterizing agent, such as a silver nitrate, to achieve hemostasis.

The amount of blood collected by this method ranges from 10-100 µL and the cumulative amount of blood collected should not exceed 1% (10 ml/kg) of the mouse's body weight. This technique should only be used once per mouse and may not be used in mice that were previously sampled for genotyping by tail amputation.

The sample will contain both venous and arterial blood and may be contaminated with tissue fluid. Due to the invasiveness of this blood collection technique and the availability of alternatives, less invasive blood collection methods should be considered and a justification for the use of this method should be provided.

Mental region (chin) blood collection

Restraint is provided by grasping the mouse's skin (scruff) between the shoulder blades using the non-dominant hand being careful not to restrict airway and/or blood flow. Sufficient restraint and retraction of the skin and subcutaneous tissue is imperative for adequate visualization of the anatomic landmarks (Figure 40). The ventral neck may be shaved for better visualization, but is not required, and may make visualization of landmarks more difficult. Locate the medial hair whorl under the chin, and subsequently the dark area, cranio-lateral to the hair whorl on either side of midline, where the facial and inferior labial veins converge. Using a 4- or 5-mm lancet, based on the age and size of the mouse, smoothly puncture the skin and underlying vessel with the lancet, which is inserted perpendicular to the area, and then withdraw. Collect blood droplets in a blood collection or microhematocrit tube. The expected volume is between 100-150 µL. After collecting the desired sample amount, the mouse is released, which typically results in cessation of bleeding. If needed, gentle pressure is applied to the puncture site using a gauze or a cotton pledget until bleeding stops. After cessation of bleeding, the mouse is observed until normal behavior returns. No more than 1% (10 ml/kg) of the mouse's body weight should be collected every two weeks. Sampling may be attempted from each site as frequently as once every 3 days provided alternating sides of the submental region are used.

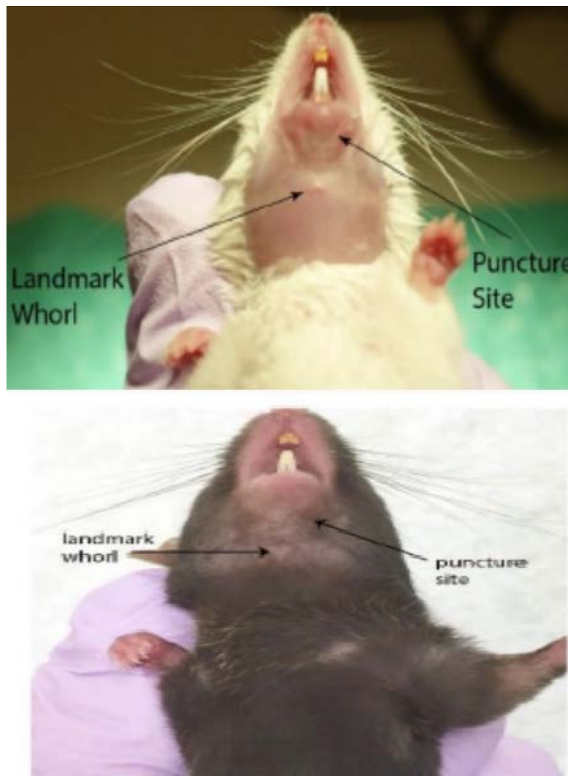
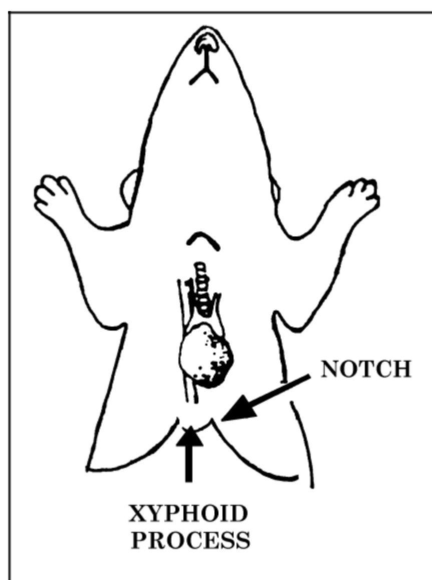


Figure 40. Puncture sites are located on either side of midline cranial to the landmark hair whorl. The puncture site is visible as a darker area on the black mouse.

Cardiac puncture (diaphragmatic approach)

Cardiac puncture is the preferred technique for terminal collection of large blood volumes. Carbon dioxide euthanasia or general anesthesia is administered, and the animal is placed on a solid surface with its ventrum exposed. The xyphoid process is palpated at the caudal aspect of the animal's sternum (Figure 41a). A notch is present on both sides of this process. A 1 inch 22 G or larger gauge needle attached to a 1 - 3 ml syringe is inserted into either notch and directed toward the midline at an ~ 40° angle as determined by palpating for the apex beat (Figure 41b). Negative pressure should be applied, by placing slight backward pull on the plunger, once it has been inserted beneath the skin. Reflux of blood is apparent once the needle has penetrated the heart. The animal must be sacrificed at the completion of the procedure prior to awakening from anesthesia. Death is verified by confirming the absence of a heartbeat by palpation by placing the thumb and index finger on opposing sides of the animal's chest. If a heartbeat is detected euthanasia must be completed by exposure to carbon dioxide for at least 15 minutes.



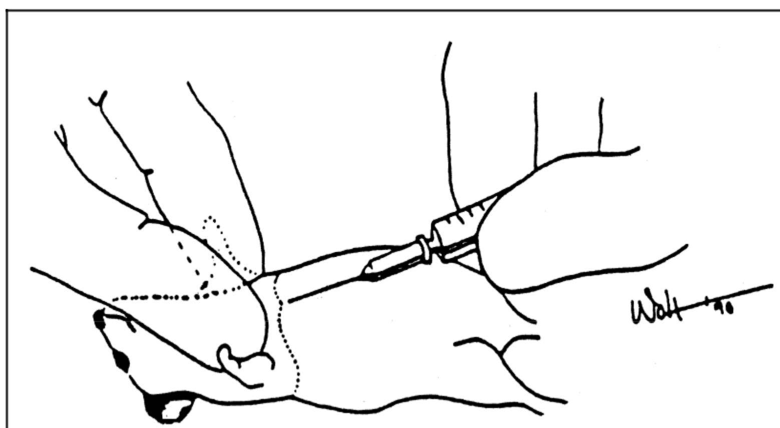


Figure 41a **Figure 41b**

RATS

Lateral tail vein venipuncture

The rat is carefully warmed (e.g. with a heat lamp) to cause vasodilation, increasing ease of vascular access. The rat is placed in a clean and disinfected restraining device such that the lateral tail veins are accessible. The tail is cleansed with a sterile alcohol wipe prior to blood collection. A small puncture is made in the vein with a sterile disposable #15 scalpel blade or a sterile 23-26 G needle with the bevel pointing up. The initial puncture should be performed distally (~2-3 cm from the tip of the tail), to allow for repeat collections to be performed above the initial puncture site avoiding the formation of a hematoma. Droplets of blood, which collect at the puncture site, are collected with a capillary tube or microfuge tube held at a 45° angle to the skin. The use of collection tubes with capillary action facilitates blood collection. For larger volumes (0.5-1.0 ml) of blood, a butterfly winged infusion set (25 G x 0.75, 3/5 inch tube) with a 1 ml syringe attached, is inserted into the lateral tail vein. Once blood enters the tubing, gentle aspiration is applied to the syringe to create blood flow. If blood flow is slow to fill the syringe, the tail can be gently pumped to improve flow but the tail should not be rubbed aggressively or 'milked' as this causes painful inflammation and sloughing of the skin. Hemostasis is achieved by applying gentle pressure with a gauze sponge or cotton swab over the puncture site for approximately 30 seconds. Alternatively, a cauterizing agent, such as a styptic pencil, may be used to achieve hemostasis. The puncture site is inspected visually to ensure adequate hemostasis before returning the rat to its cage.

No more than 1% of the rat's body weight, which equals approximately 10-15% of the rat's total blood volume (60 ml/kg) should be collected every 2 weeks. This amount may be collected in up to 5 collections. When multiple collections are performed a single vein should be accessed no more than once a day and the right and left veins alternated to allow adequate healing of the vessel.

Orbital venous plexus blood collection

The technique describing blood collection from the mouse's orbital venous sinus should be followed for orbital venous plexus collection in the rat. The only difference is that the vessels

surrounding the rat's globe are a network of small veins rather than a blood-filled sinus (Figure 42) and the fibrous connective tissue surrounding the plexus is quite dense. Therefore, the broken end of the hematocrit tube, which provides a cutting edge, should be inserted into the plexus. The broken edge should be examined carefully to ensure that no loose pieces of glass are present. Remember this technique must be performed under general anesthesia and post-bleeding hemostasis is essential to prevent complications.

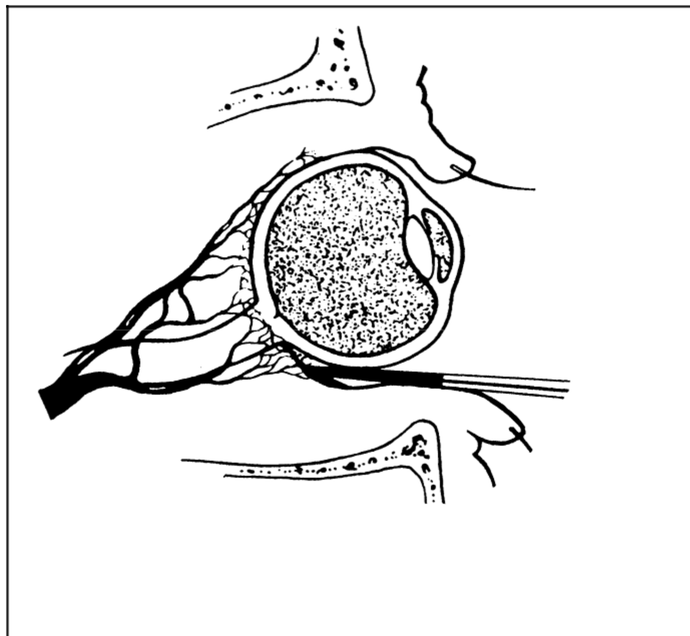


Figure 42

Under general anesthesia, the rat is grasped so that its back rests on the palm of the hand with its head toward the thumb or with larger rats, > 300 g, rest the rat in sternal recumbency on a flat work surface. The thumb is placed lateral to the animal's trachea so that the jugular vein on the same side as the eye from which you are collecting blood is occluded and the fur on the animal's head is drawn back. This causes the animal's eye to proptose (bulge) slightly. A microhematocrit tube, or glass pipette of similar or smaller diameter, is directed into the medial canthus (junction of eyelids closest to the animal's nose) of the eye rotating slightly as the tube is directed to a point directly behind the globe. Sufficient pressure must be applied to penetrate the dense fibrous layer that surrounds the plexus. Blood flows through the tube and occasionally around the tube once the venous plexus has been penetrated. After blood collection, the tube is removed and the eyelids closed and a dry cotton pledget is applied over the eye with gentle pressure to prevent retro-orbital hemorrhage. Blood should not be collected from the same eye more than 3 times, allowing at least 1-2 weeks between collections from the same eye. Both eyes should not be used in the same day. No more than 1% (10 ml/kg) of the rat's body weight should be collected every two weeks.

Saphenous vein blood collection

The rat should be warmed immediately prior to blood collection to increase blood flow by either placing a heat lamp over the cage for 3-5 minutes (watching carefully for signs of overheating) or placing the cage on a circulating warm water pad. The conscious rat is then placed in a clear restraining tube or a soft plastic restraining sleeve so its head is furthest inside the restraint device. The hind leg is extended and immobilized by applying gentle downward pressure immediately proximal (superior) to the knee joint. Extension stretches the skin over the leg, making the saphenous vein more visible on the caudolateral aspect of the hind leg. The area is shaved with clippers (#40 blade) and cleaned with 70% isopropyl alcohol on a cotton swab or gauze sponge. A thin layer of petroleum jelly or eye lubricant is applied to the skin overlying the vessel to aid in the formation of blood droplets on the surface of the skin as it leaves the vessel. A small puncture is placed in the vein with a sterile 23-25 G needle. Droplets of blood are collected with a capillary or collection tube held at a 45° angle to the skin. Hemostasis is achieved by applying gentle pressure with a gauze sponge or cotton swab over the puncture site for at least 1 minute. Alternatively, a cauterizing agent such as a styptic pencil may be used to achieve hemostasis. The puncture site is inspected visually to ensure adequate hemostasis before returning the rat to its cage.

No more than 1% (10 ml/kg) of the rat's body weight may be collected every two weeks. This amount may be collected in up to 5 collections. When multiple collections are performed, a single vein should be collected from no more than once a day and the right and left veins should be alternated to allow adequate healing of the vessel.

Jugular vein blood collection

Sampling from the jugular vein requires considerable competence to avoid harming the rat. Success with the procedure is largely dependent on proper restraint and positioning of the animal. The neck can be held in hyperextension by fastening a strip of gauze behind the upper incisors and pulling the head back or to the side. Removal of hair from the ventral neck region by shaving or by use of a depilatory will make identification of landmarks easier. The site for venipuncture is just cephalad to the point where the external jugular vein passes between the pectoral muscle and the clavicle. If the needle is inserted through the pectoral muscle, it is stabilized better within the vein.

Blood collection can be conducted with a one- or a two-person technique. Jugular venipuncture in the unanesthetized rat using the two-person technique requires a restraint board, as well as a restrainer and phlebotomist (Figure 43). Essential to the success of this technique are optimal restraint and constant communication between team members. When properly restrained, the rat is in dorsal recumbency, with its' hindlimbs restrained at the hips and forelimbs secured by rope ties so that the limbs are perpendicular to the long axis of the body, and its' head restrained by a device made from a plastic cup, slit down the middle and perforated with breathing holes. The head is pulled in the direction opposite the venipuncture site. Alternatively, with adequate training, the animal can be restrained entirely by hand. Care needs to be taken when applying the restraint to prevent damage to the forelimbs. The phlebotomist inserts a 23-gauge, ¾-inch needle attached to a 1- to 3-ml syringe under the ventral aspect of the clavicle, keeping the syringe parallel to the restraint board, 1 cm lateral to the animal's midline, and advances the needle to a depth of ~1 cm. After the needle has pierced the skin, the phlebotomist exerts gentle negative pressure; if bright-red blood appears (arterial blood), the needle is withdrawn and pressure is applied to the site.

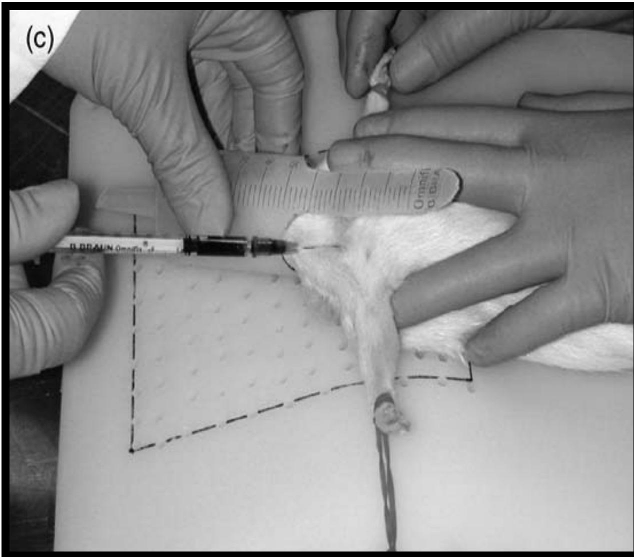


Figure 43

Use of the one-person technique requires extensive training for the restraint hold. For this technique, the non-dominant hand is used for restraint and the dominant hand to shave and collect the blood sample. The phlebotomist uses the thumb and 3rd finger to restraint the arms pulling them back at the elbows (Figure 44a). Then, the 4th and 5th fingers are placed over rat's arm and the index finger should be free to restraint head back (Figure 44b). The index finger is placed on top of the head, running down the back of the head, stabilizing loose skin (Figure 44c). The rat should not be able to move its' head. If the finger is too loose the rat will bend its head down (Figure 44d). Once the rat is secured, the dominant hand can be used to shave and wipe with alcohol the venipuncture area (Figure 44e). The clavicle is palpated at the point of the shoulder (cannot visualize vein) (Figure 44f). The needle is inserted just above the clavicle (Figure 44g). The vein is superficial, thus the needle should be inserted at a slight angle. The blood sample is collected by withdrawing the syringe plunger. Once finished, pressure is applied at the venipuncture site for 1-2 minutes to prevent blood loss and hematoma formation (Figure 44h).

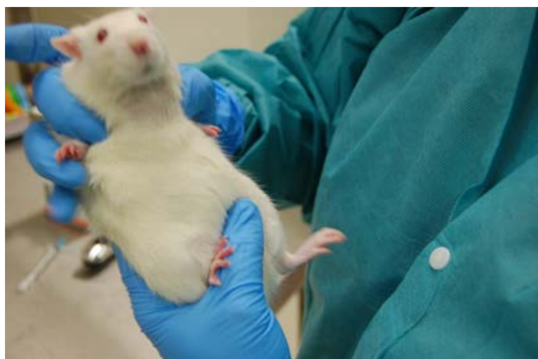


Figure 44a

Figure 44b

Figure 44c **Figure 44d**

Figure 44e **Figure 44f**

Figure 44g **Figure 44h**

Sampling should be carried out aseptically. One tenth to 2 ml (normally 0.1 - 0.3 ml) of blood can be collected per sample and, depending on the sample volume and scientific justification, up to eight samples can be collected during a 24-hour period. The number of needle sticks at each attempt should be a limited to three. If more samples are needed, then surgical cannulation or temporary cannulation of a different blood vessel should be considered. Blood flow should be stopped before the rat is returned to its cage by applying gentle pressure to the blood sampling site for ~30 seconds.

Cardiac puncture (diaphragmatic approach)

Cardiac puncture is performed in rats using the same technique as was described for mice with the following differences. A 1.5 inch 20 G or larger needle attached to a 5-10 ml syringe is

inserted into either notch and directed toward the midline at approximately a 40° angle. Once blood collection is completed, death should be ensured by a secondary method (e.g. waiting for the onset of rigor mortis or by creating a bi-lateral pneumothorax (both sides of the thoracic cavity are opened by cutting across the ribs and sternum with scissors).

HAMSTERS

Orbital venous sinus blood collection

The technique describing blood collection from the mouse's orbital venous sinus should be followed for orbital venous sinus collection in the hamster except the microhematocrit tube should be inserted into the lateral canthus rather than the medial. The technique must be performed under general anesthesia and post bleeding hemostasis is essential to prevent complications.

Cardiac puncture (diaphragmatic approach)

The technique for cardiac puncture from the hamster is identical to that described for the mouse. A 3-5 ml syringe should be used if large blood volumes are desired. This procedure is performed under general anesthesia as a terminal event only. The animal must be sacrificed at the completion of the procedure prior to awakening from anesthesia.

GUINEA PIGS

Orbital venous plexus blood collection

The technique describing blood collection from the rat's orbital venous plexus should be followed for orbital venous plexus collection in the guinea pig. Remember this technique must be performed under general anesthesia and post-bleeding hemostasis is essential to prevent complications.

Saphenous, lingual, or penile veins blood collection

Small volumes of blood may be collected with a needle and syringe from the saphenous, lingual, or penile veins. Contact RARC for training.

Cardiac puncture (diaphragmatic approach)

The technique for cardiac puncture from the guinea pig is identical to that described for rats. This procedure must be performed as a terminal event and general anesthesia is required. The animal must be sacrificed at the completion of the procedure prior to awakening from anesthesia.

RABBITS

Central auricular artery and marginal auricular veins blood collection

The central auricular (ear) artery or marginal auricular veins are a useful site for survival collection of moderate volumes of blood. Vasodilatation should be facilitated by administering the phenothiazine tranquilizer and alpha-adrenergic receptor blocker acepromazine (0.25 - 0.5 ml SC or 0.05 ml IV) approximately 5 - 10 minutes prior to blood collection. Blood collection may also be facilitated by the topical application of a lidocaine–prilocaine local anesthetic cream 1 h prior to sampling. A 21 G or larger gauge butterfly needle is preferred; however, a 1 inch 21 G or larger gauge needle and syringe may also be utilized. The insertion site is disinfected using an

alcohol-soaked swab prior to inserting the needle, bevel up, into the artery (Figure 45). An immediate flashback is observed and blood is allowed to flow out of the open end of the butterfly needle into a suitable container or alternatively blood can be collected directly into a syringe. It is essential to apply pressure to the artery or vein over the insertion site for at least 3 minutes to provide suitable hemostasis. Significant blood loss can occur from the artery if adequate hemostasis is not provided.

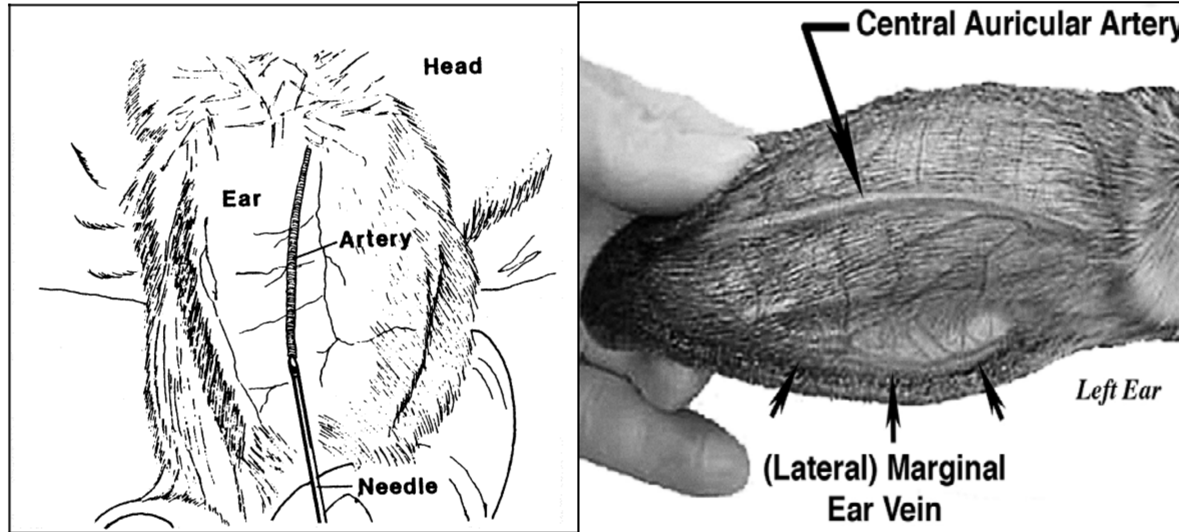


Figure 45

<u>Cardiac puncture (diaphragmatic approach)</u>

Large volumes of blood can be collected directly from the heart of anesthetized rabbits as a terminal event. The technique is similar to that described for mice and rats, however a larger needle and syringe (1.5 inch ≥18 G; ≥20 ml) should be used. Death must be confirmed at the completion of the procedure by administering pentobarbital (120 mg/kg) IV.

FERRETS

Even with optimal handling, peripheral vascular access is difficult in ferrets due to their short appendages, small ears, and thick fur. Accessible blood vessels include the cephalic, jugular, cranial vena cava, and lateral saphenous veins, as well as the ventral tail artery. When a large quantity is required (greater than approximately 1 ml of whole blood), jugular or cranial vena cava sampling is preferred, or cardiac puncture may be performed if a terminal collection (Figure 46). For small sample quantities, including catheter placement, cephalic or lateral saphenous venipuncture is indicated (Figures 47 and 48). Access to these vessels may be achieved when the animal is either sedated or conscious and hair removal may assist with visualization of the vessel. Surgical placement of vascular access ports in the jugular vein and femoral artery has also been described when frequent blood sampling is required. Please contact RARC's Veterinary Services for more details.

Figure 46. Jugular, cranial vena cava (anesthesia required)

Figure 47. Cephalic vein

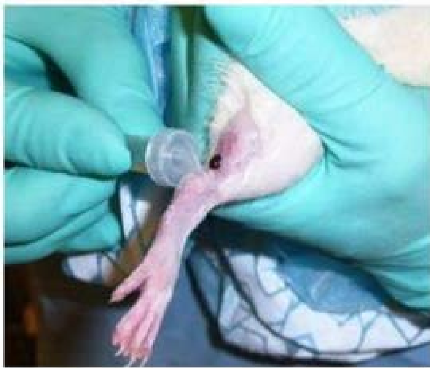


Figure 48. Saphenous vein

Chronic Administration and Sampling

Catheters and Vascular Access Ports: When a study requires repeated parenteral administration or sampling over an extended period of time, a more permanent access system is recommended. Options include the implantation and exteriorization of a chronic catheter, a subcutaneous access port, or an osmotic pump. Chronic vascular access utilizes peripheral vessels (femoral artery/vein, carotid artery or jugular vein) in most species. If catheters are to be used for a long period of time, the technique of cannulation must provide for the protection of the catheter and allow freedom of movement for the animal. The most common method of protecting the cannula is by creating a subcutaneous tunnel from the site of vessel cannulation to the dorsum of the neck using blunt dissection with scissors or trocars through which the canula will pass before exiting. Exiting the

catheters from the dorsal surface of the neck minimizes the possibility of damage by the animal to the catheter or setup. Catheter materials, e.g., silicone, polyurethane, polyethylene, and PVC, vary in cost and physical attributes such as flexibility, strength, biocompatibility, and thrombogenicity. Stiff catheters and sharp tip edges should be avoided as these can cause irritation to the vessel lining and promote tissue proliferation and thrombi formation.

Subcutaneous vascular access devices (VAPs; Figure 17) consist of a rigid reservoir (plastic or stainless steel) and a silicone septum buried beneath the skin so that it may be repeatedly punctured by a needle. Such devices give access to vascular or other structures to allow injections or withdrawal of blood without the risk of animal-induced damage or infections associated with catheters that perforate the skin. Accessing the port requires the use of a specialized non-coring or Huber needle that decreases damage to the septum.

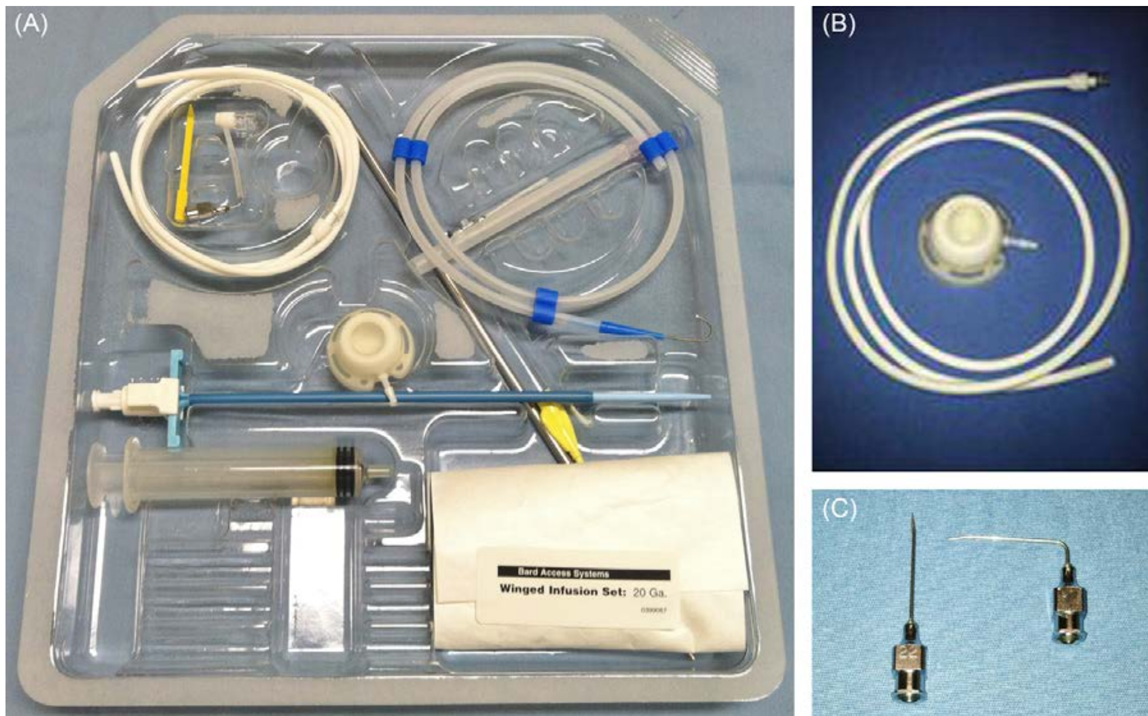


Figure 17. (A) Standard vascular access port kit. (B) Vascular access port. (C) Huber needles.

Appropriate aseptic catheter maintenance techniques must be followed to prevent infection. Strict asepsis is necessary to prevent infection once these devices are in place and care must be taken to properly prepare the catheter or skin site prior to injection or sampling. When using a VAP clipping the area free of hair and aseptic preparation of the site is recommended prior to access. Sterile supplies, including gloves to handle materials, needles, and solutions, should be used during the procedure. In conjunction with proper catheter handling, the instillation of antibiotic and enzyme solutions into the catheter has been shown to be an effective way to prevent catheter-related sepsis. Anticoagulants such as heparin, sodium citrate, sodium EDTA, and taurolidine citrate can be used as locking solutions; high-concentrate (50%) dextrose solution may be added to inhibit bacterial growth. Protection of both exteriorized catheters and subcutaneously implanted devices including VAPs can be done using conventional bandaging and dressing, but close observation of the animal may be necessary to prevent damage to the site. Canvas and/or nylon vests and tight-fitted 'undershirts' that protect implanted catheters are commercially available for a variety of species and are often preferred.

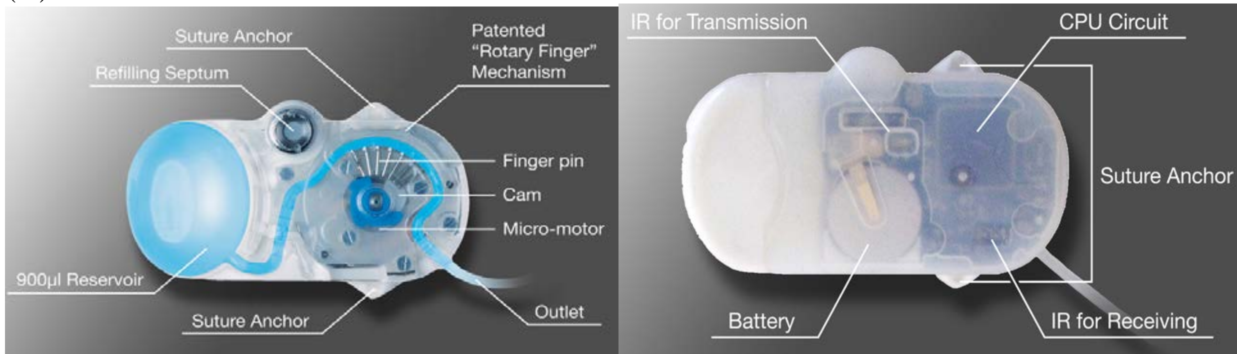
Implantable Pumps: Implantable pumps are a convenient and cost-effective method for continuous drug delivery to laboratory animals. Pumps allow for a continuous infusion versus the typical peak to trough exposure profiles of bolus injections. These pumps provide significant animal welfare

benefits over repeat dosing by other routes, where restraint may be required, thus also reducing animal handling artifacts. With the attachment of specialized catheters, specific administration sites may be targeted. Advantages of implantable pumps include: ensuring around-the-clock exposure to test agents at predictable levels; permitting continuous administration of short half-life proteins and peptides; dosing for extended periods; eliminating the need for nighttime or weekend dosing; and, reducing handling stress to laboratory animals. They are also cost-effective, small enough for use in mice or young rats, and allow for targeted delivery of agents to virtually any tissue. There are two principal types of implantable pumps, osmotic and motorized.

Osmotic minipumps (Alzet® osmotic pump, ALZA Corporation, Palo Alto, CA) are implantable pumps that deliver a specified volume of fluid over a defined period (Figure 18A). These pumps consist of a flexible, impermeable reservoir chamber surrounded by a sealed layer containing an osmotic agent, all surrounded by a semipermeable membrane. When surgically implanted into an aqueous environment, e.g., subcutaneous or intraperitoneal, the osmotic agent (highly concentrated salt) imbibes water at a rate determined by the semipermeable membrane. The imbibed water generates hydrostatic pressure, which compresses the flexible reservoir chamber to produce a constant outflow of the material in the reservoir. Once the pump's lifespan has been reached, i.e., the duration provided by manufacturer, it has to be surgically removed if animals are to survive as these pumps continue to imbibe water and will rupture releasing the concentrated salt solution resulting in local irritation and swelling of tissues around the pump. A limiting factor in the use of osmotic pumps is the size of the pump relative to that of the animal in which they are to be implanted. Small animals can only be implanted with small pumps, which limits the volume as well as the infusion rate and duration. Infusion durations can last up to 6 weeks, depending on the rate of infusion and pump size. Osmotic pumps cannot be refilled, but pumps can be replaced to extend the duration.

An alternative to the osmotic pump is the iPRECIO® MicroInfusion Pump (Data Sciences International, St. Paul, MN), which is an implantable, refillable, micro-infusion pump. This pump can be surgically implanted in the subcutaneous space of rodents and larger animals (Figure 18B). The pump is both programmable and refillable, with a reservoir port with a resealable septum that can be used to add or remove the substance being administered during the course of the study. This motorized pump, as with an osmotic pump, can be connected to a catheter for administration of agents to various body cavities, tissues, or into the vasculature.

(A)



(B)

Figure 18. (A) Components of the ALZET osmotic pump. (B) Top and bottom view of iPRECIO® SMP-200 pump.

Table I
Recommended Needle Sizes and Maximum Injection Volumes for Various Parenteral Techniques

	Subcutaneous		Intramuscular		Intravenous		Intraperitoneal		Intradermal	
	NS (g)	Vol. (ml)	NS (g)	Vol. (ml)	NS (g)	Vol. (ml)	NS (g)	Vol. (ml)	NS (g)	Vol. (ml)
Mouse	≥23	≤1	≥25	≤0.05	≥25	≤0.5	≥23	≤2	≥26	≤0.1
Rat	≥22	≤5	≥23	≤0.2	≥25	≤2	≥23	≤5	≥26	≤0.1
Hamster	≥22	≤4	≥23	≤0.15	≥25	≤1	≥23	≤3	≥25	≤0.05
Guinea Pig	≥22	≤5	≥23	≤0.3	≥25	≤5	≥23	≤5	≥25	≤0.1
Rabbit	≥22	≤30	≥23	≤1.5	≥24	≤10	NR	NR	≥25	≤0.1
Ferret	≥23	≤10	≥23	≤ 0.5	≥22	≤5	≥23	≤20	≥23	≤0.1

NS=Needle Size; Vol. =Volume; (ml) =Milliliter; (g) =gauge (large gauge - smaller needle); NR=Not Recommended

Table II
Maximum Blood Collection Volumes for Survival and Terminal Bleeds

	Total Blood Volume	Maximum Blood Collection Survival[1]	Maximum Blood Collection Terminal[2]
Mouse	78 ml/kg	8-12 ml/kg	24 ml/kg
Rat	67 ml/kg	7-10 ml/kg	20 ml/kg
Hamster	74 ml/kg	8-11 ml/kg	23 ml/kg
Guinea Pig	69 ml/kg	7-10 ml/kg	20 ml/kg
Rabbit	59 ml/kg	6-9 ml/kg	18 ml/kg
Ferret	75 ml/kg	8-11ml/kg	60 ml/kg

[1]Maximum volume that should not be exceeded during any two-week period.

[2]Estimated maximum amount of blood that can be collected as a terminal bleed.

Figure Credits

Harkness JE and Wagner JE, *The Biology and Medicine of Rabbits & Rodents*, Figures 1, 3, 9
American Association for Laboratory Animal Science, Figures 2, 4, 6-8, 10, 11, 19, 20, 23, 31, 35, 36
https://www.braintreesci.com/prodinfo.asp?number=DC, Figure 5
https://norecopa.no/films-and-slide-shows/rabbit, Figure 12
University of Washington Training Series, Figures 13, 15, 24, 25, 2-30, 32
https://www.youtube.com/watch?v=Us9FKffOtUs, Figure 14a
https://www.medidose.com/EPS-NeedleSafeII.aspx, Figure 16
Fox et al., 2015, *Laboratory Animal Medicine*, Figure 17, 18
EnCCoMPass, Pre-Approved Techniques, Figure 21, 22
Yardeni, Eckhaus, Morris, Huizing, Hoogstraten-Miller, 2011, *Lab Animal*, Figure 26, 27
www.nature.com, Figure 33
Suckow MA, Stevens KA, Wilson RP. The Laboratory Rabbit, Hamster, and Other Rodents, Figure 35 Regan, RD, Fenyk-Melody JE, Tran SM, Chen G, Stocking KL, 2018, JAALAS 55(5), pp.570-576, Figure 40
https://images.app.goo.gl/ApXba6vdtKQUV7pA9 and Waynforth HB and Flecknell P, *Experimental and Surgical Techniques in the Rat*, 2nd ed., Figure 43
Martin H, Blood Collection Rodents presentation, Figure 44
https://www.researchgate.net/publication/281506772_Hematology_of_laboratory_rabbits/figures?lo=1, Figure 45
Quesenberry, KE and Carpenter, JW, 2004, *Ferrets, Rabbits, and Rodents,* Figures 46-48