A

Species-Specific Information — Techniques for Handling, Sexing, Injection, and Blood Collection

A.1 Animal Handling

When handling animals, always remember to approach them in a **confident** and **relaxed** manner. Animals should be handled as **regularly** as possible to help reduce stress and to allow the animals to get used to you.

It is important to undergo **training** if you are going to restrain an animal for a procedure, as some techniques require a lot of practice and you may make a mistake if you are unfamiliar with the methods whilst trying to perform a procedure. Techniques vary from species to species; other factors such as the size, weight, age, and temperament of the animal are considered when selecting the method of restraint.

Handling methods may differ between handlers. For instance, some handlers may be able to lift a 5-kg rabbit with little effort, whereas some others may find it quite heavy to pick up and will therefore probably not be able to restrain it using the same method. There are also different techniques for normal handling and sexing of the animal and for transferring it from one cage to the next, as opposed to restraining or handling sick animals.

Injection and blood collection are the most common procedures that research personnel perform on animals, and these techniques require knowledge of general handling of animals.

A.1.1 General principles for animal handling

- Animals should be approached in a confident and relaxed manner.
- Animals should be handled regularly to help reduce stress and to calm them down when restraining them for procedures to be performed on them.
- Most animals have sharp claws and prefer not to be placed on slippery surfaces, so, where possible, use a cage top (for rodents) or a nonslip cover/liner for benches.
- With practice, most species of animals are easily restrained and handled.
- There is no one correct method of handling or restraining animals, but the general principle is that it should not cause pain

or discomfort to the animal. It should also be comfortable for the handler, especially when the animal is being restrained for an injection, so that the handler is able to concentrate on the injection procedure.

- The methods shown in the species-specific sections are recommended, although some people may feel more comfortable using slightly different ways to restrain the animals, which is also acceptable.

It may be obvious, but one basic tip to remember is to keep your fingers away from the mouth of the animal, especially when performing a procedure such as an injection. Many people, while busy concentrating on positioning the needle, forget that their fingers are within easy reach of the mouth of a mouse or rat and hence get bitten.

A.2 Injections and Blood Collection

As dosing and blood collection of experimental animals are common procedures, it is necessary to look at these in greater detail. Blood sampling is a common procedure that is performed regularly on all species, whether for diagnostic purposes (health monitoring) or as part of the experiment requirements. There are many different methods of compound administration and blood collection, some of which will be described in the species-specific sections.

Below are some considerations to keep in mind before injecting or taking blood, such as the volume that may be safely administered or withdrawn.

A.2.1 Injections

A.2.1.1 *Animal handling*

- The correct restraint technique — manual, mechanical (restrainers), or chemical (anaesthetics) — should be used to minimise stress to animals.
- Good animal handling prevents injury to animals, e.g. vertebral injuries in rabbits.

- Good animal handling also helps personnel to avoid injuries such as bites, scratches, and needlestick injuries.

A.2.1.2 *Administration volumes*

Table A.1 lists the recommended maximum volumes that are considered as good practice for the commonly employed routes in the species covered in this book.

- For nonaqueous injection material, consideration must be given to the time of absorption before redosing.
- No more than two intramuscular sites should be used per day.
- Subcutaneous sites should be limited to two to three sites per day.

A.2.1.3 *Administrative routes*

A.2.1.3.1 *Oral route*

If the experimental protocol requires restriction of the animal's food intake, care must be taken, as large-dose volumes (40 mL/kg)

Table A.1 Recommended maximum volumes.

	Route and Volumes (mL/kg except *mL/site)					
Species	**Oral**	**SC**	**IP**	**IM**	**IV (bolus)**	**IV (slow injection)**
Mouse	10 (50)	10 (40)	20 (80)	0.05* (0.1)*	5	(25)
Rat	10 (40)	5 (10)	10 (20)	0.1* (0.2)*	5	(20)
Rabbit	10 (15)	1 (2)	5 (20)	0.25 (0.5)	2	(10)
Dog	5 (15)	1 (2)	1 (20)	0.25 (0.5)	2.5	(5)
NHP	5 (15)	2 (5)	–(10)	0.25 (0.5)	2	(—)
Mini-pig	10 (15)	1 (2)	1 (20)	0.25 (0.5)	2.5	(5)

Note: SC, subcutaneous; IP, intraperitoneal; IM, intramuscular; IV, intravenous; NHP, nonhuman primate; (—), data not available.
Figures on the left side of the columns are intended as a guide to "good practice" for single or multiple dosing. The second set of figures in parentheses are the possible maximum volumes which, if exceeded, may result in scientific and welfare concerns.

have been shown to overload the stomach capacity and pass immediately into the small bowel.[a] Larger volumes may also reflux into the oesophagus.

A.2.1.3.2 *Parenteral routes*

For substances administered by injection, there are several factors to consider, including the dose volume, stability (before and after administration), pH, viscosity, osmolality, buffering capacity, sterility, and biocompatibility of the formulation. The smallest needle size should always be used, taking into account the dose volume, viscosity of injection material, speed of injection, and animal species.

A.2.1.3.3 *Subcutaneous (SC/SQ/Subcut) injection*

Subcutaneous injection is given under the skin (cutis) and is frequently used. The rate and extent of absorption depend on the formulation. Large volumes can safely be administered using the SC route.

A.2.1.3.4 *Intraperitoneal (IP) injection*

Intraperitoneal injection is not frequently used for multiple-dose studies because of possible complications such as accidental injection into the intestinal tract, causing peritonitis. Drug absorption from the peritoneal cavity after the administration of the compound as a suspension is dependent on the properties of the drug particles and the vehicle, and may be absorbed into both systemic and portal circulations. The largest volumes may be injected relatively safely by experienced individuals using the intraperitoneal route.

A.2.1.3.5 *Intramuscular (IM) injection*

Intramuscular injections may be painful because muscle fibres, which are closely packed together, are distended by the injection

[a] Hejgaard KC *et al.* Assessing welfare of rats undergoing gavaging with varying volumes. Measurements on open field behaviour, temperature, plasma corticosterone and glucose [Abstract]. *Rev Cienc* **23/24**: 16, 1999.

article. Sites need to be chosen to minimise the possibility of nerve damage and pain. If dosing multiple times, a range of sites should be selected.

A.2.1.3.6 *Intravenous (IV) injection*

There are two types of intravenous injection: bolus, where a single large sample is given rapidly; and slow injection, where the article is administered over a period of time.

- Bolus injections require the test substance to be compatible with blood and not too viscous. When large volumes are required to be given, the injection material should be warmed to body temperature. The rate of injection is an important factor in intravenous administration; it is suggested that, for rodents, the rate should not exceed 3 mL/min.

  No detectable changes in haematocrit or heart rate were observed in dogs following rapid intravenous injection of 6 mL/kg saline, but 20 mL/kg was associated with 15% haemodilution and a transient tachycardia (up 46% over 1 min).[b]
- Slow intravenous injections are usually given either because the compound is insoluble or unstable in solution or due to irritancy of a large volume. For slow intravenous injections over the course of 5–10 min, a standard or butterfly needle may be used, or an intravenous cannula may be taped into place or surgically implanted to minimise the stress of repeated injections or prolonged anaesthesia/sedation.

  It has been shown that rats may be given daily intravenous injections of isotonic saline at dosages of up to 80 mL/kg at 1 mL/min for 4 days without any significant signs of distress or pulmonary lesions.[c] However, pulmonary lesions increased in incidence and severity when the duration of treatment was increased to 30 days and the injection was administered at

[b] Zeoli *et al*. A limit rapid intravenous injection volume in dogs [Abstract 284]. *Toxicol Sci* **42**: 58, 1998.

[c] Morton D *et al*. Effects of infusion rates in rats receiving repeated large volumes of saline solution intravenously. *Lab Anim Sci* **47**: 656–659, 1997.

0.25 mL/min, 0.5 mL/min, or 1.0 mL/min.[d] There may well have been adverse effects at an earlier time point, but the pathology had not had time to develop.

A.2.1.3.7 *Intradermal (ID) injection*

Intradermal injection is typically used for the assessment of immune, inflammatory, or sensitisation responses.[e,f] Material may be formulated with an adjuvant to enhance the response, but care must be taken, as quite often this route of administration is painful for the animal (specifically in footpad and eye pinea injections). Volumes of 0.05–0.1 mL can be used, depending on the thickness of skin and the species.

A.2.1.3.8 *Vehicles for administration*

The vehicle or solution that the injection article is placed in needs to be carefully selected. The vehicles should offer optimal exposure without influencing the results obtained for the compound under investigation; they should ideally be biologically inert, and have no effect on the biophysical properties of the compound or any toxic effects on the animals. Simple vehicles used to administer compounds include aqueous isotonic solutions, buffered solutions, cosolvent systems, suspensions, and oils. For nonaqueous injection articles, it is important to consider the time of absorption before redosing.

A.2.1.3.9 *Frequency of needle punctures*

It is important to carry out the minimum number of needle punctures consistent with obtaining good scientific data. The

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[d] Morton *et al*. Histologic lesions associated with intravenous infusions of large volumes of isotonic saline solution in rats for 30 days. *Toxicol Pathol* **25**: 390–394, 1997.

[e] Leenars PPAM. *Adjuvants in Laboratory Animals* (Synopsis of PhD thesis and publications). Ponsen & Looijen BV, Wageningen, The Netherlands, p. 214, 1997.

[f] Leenars PPAM *et al*. Assessment of side-effects induced by injection of different adjuvant/antigen combinations in rabbits and mice. *Lab Anim* **32**: 387–406, 1998.

same puncture site should not be used, i.e. use different points along a vein or different locations on the skin (for subcutaneous injections).

A.2.2 Blood collection

Before you start collecting blood, you need to know the following:

A.2.2.1 *Cardiovascular physiology*

A.2.2.1.1 *Total blood volume*

In all species, the total blood volume is approximately 6%–8% of the total body weight (of lean animals), so, to be safe, we can assume that 6% of body weight = blood.

- 6 mL of blood per 100 g
- 60 mL per blood per kg

A.2.2.1.2 *Safe acute sampling volume*

Acute blood sampling is the one-time removal of a large volume of blood or multiple small samples of blood over a short period of time (24 h).

- 10%–15% of circulating blood volume may be removed once every 3 weeks.
- 1% of body weight can be collected every 3 weeks (or in total over a 24-h period).

A.2.2.1.3 *Chronic sampling*

Chronic blood sampling is the frequent and repeated removal of small quantities of blood over a long period of time.

- For chronic sampling, the rule of thumb is 0.1% of body weight every day for 21 days (e.g. a 30-g mouse can have 0.03 mL of blood collected every day for 21 days).
- The total volume of blood collected by chronic sampling is higher than acute, as the body continuously produces blood to replace that taken.

A.2.2.2 *Anatomy*

It is important that before you start to collect the blood of an animal, you have a good idea of its basic anatomy, such as the location of its heart, veins, and arteries, and how much blood can be collected from each site.

A.2.2.2.1 *Venous access*

For the collection of small volumes of blood (< 0.1 mL), for haematological or chemical estimations requiring only 50–200 μL (1–4 drops), a superficial vein can be punctured, such as the tail vein, saphenous vein, or marginal ear vein.

A.2.2.2.2 *Arteries*

Large volumes of blood can be obtained relatively easily and quickly from the arteries, such as the central ear artery in rabbits, but care must be taken to prevent excessive bleeding.

A.2.2.2.3 *Cardiac puncture*

Cardiac puncture should always be carried out under a general anaesthetic and must be considered a terminal procedure in all species.

A.2.2.2.4 *Cannulation*

Cannulation is important to reduce the discomfort of repeated bleeds. Temporary cannulae such as butterfly needles and over-the-needle cannulae may be used in the short term, whereas surgical implantation of biocompatible cannulae may be required for long-term use. Cannulation allows repeated blood sampling with minimal distress and discomfort to the animal.

- Indwelling catheters need to be flushed with a solution of heparin to reduce the risk of thrombosis (blood clot).
- Discard a sample at least three times the volume of the line before a specimen is obtained for analysis.

A.2.2.3 *Steps involved in blood collection*

- Be prepared! Preparing all necessary equipment is essential before beginning the procedure. Once blood starts to flow, it is very difficult to go and get something you have forgotten.
- Animal preparation — handle the animal before the event to reduce the animal's stress. Bring the animal cage to the procedure room or biosafety cabinet and restrain/sedate the animal, depending on the technique to be used and the species.
- Site preparation — remove the fur if necessary and swab the collection site with alcohol.
- Collect blood.
- Animal and site monitoring — hold a gauze pad on the blood collection site until bleeding stops (haemostasis).

Remember: If you lack the confidence to perform a procedure, inform your colleagues. Training by the animal facility care staff or veterinarian is usually available. Colleagues and principal investigators (PIs) may also be able to assist. Do not perform a procedure you are not confident in or comfortable with, as there is a higher chance of you making a mistake which will add to the animal's discomfort.

A.2.2.4 *Recognition of signs of hypovolaemic shock and anaemia*

If too much blood is taken or if the blood is taken from a particular site too quickly, hypovolaemic shock or anaemia may result. The signs of hypovolaemic shock and anemia are as follows:

A.2.2.4.1 *Hypovolaemic shock*

- Fast and thready pulse
- Pale, dry mucous membranes
- Cold skin and extremities
- Hyperventilation (panting, shortness of breath)
- Subnormal body temperature

A.2.2.4.2 *Anaemia*

- Pale mucous membranes inside mouth and conjunctiva (eye)
- Pale tongue, gums, ears, and footpads
- Capillary refill test (where you pinch the mucous membrane for a moment and then wait for blood to refill) that takes more than 3 seconds
- Exercise intolerance
- Increased respiratory rate at rest (extreme conditions)

A.2.2.5 *Blood collection volumes*

Tables A.2 and A.3 list the recommended sites for blood sampling as well as the total blood volumes and maximum sampling volumes that are considered as good practice for the species covered in this book.

A.2.2.5.1 *Lateral tarsal (saphenous) vein*

Saphenous vein injection is used routinely in a number of small and large animal species. Volumes as large as 5% of the circulating blood volume may be taken. Generally, it does not require the use of an anaesthetic and is therefore particularly suitable for repeated blood sampling, as required in pharmacokinetic studies.

The saphenous vein is on the lateral aspect of the tarsal joint, and is easier to see when the fur is shaved and the area wiped with alcohol. There appear to be no complications reported other than persistent (minor) bleeding, and the method has the advantage that anaesthesia is generally not required.

Table A.2 Recommended sites for blood sampling.

Species	Recommended site
Mouse	Saphenous vein, lateral tail vein
Rat	Saphenous vein, lateral tail vein, sublingual vein
Rabbit	Marginal ear vein, central ear artery, jugular vein
Dog	Cephalic vein, jugular vein, saphenous vein
Macaque	Cephalic vein, saphenous vein, femoral vein
Mini-pig	Cranial vena cava

Table A.3 Total blood volumes and recommended maximum blood sample volumes for species of given body weight.

Species (Weight)	Blood volume (mL)	7.5% (mL)	10% (mL)	15% (mL)	20% (mL)
Mouse (25 g)	1.8	0.1	0.2	0.3	0.4
Rat (250 g)	16	1.2	1.6	2.4	3.2
Rabbit (4 kg)	224	17	22	34	45
Dog (10 kg)	850	64	85	127	170
Macaque (Rhesus) (5 kg)	280	21	28	42	56
Macaque (Cynomolgus) (5 kg)	325	24	32	49	65
Mini-pig (15 kg)	975	73	98	146	195

A.2.2.5.2 *Marginal ear vein/Central ear artery*

Blood sampling from the marginal ear vein is commonly used in rabbits, guinea pigs, and miniature swine. Good restraint is necessary, and the application of a local anaesthetic cream or spray (e.g. xylocaine) 20 to 30 minutes before taking blood helps to prevent pain and distress.

A.2.2.5.3 *Lateral tail vein*

In principle, this route is similar to the lateral tarsal vein, but tends to yield smaller blood volumes (0.1–0.15 mL in mice, up to 2 mL in warmed rats). Blood is removed either by a syringe/needle or by stab puncture of a lateral tail vein. Anaesthesia is usually not required, which makes this route particularly suited for repeated blood sampling. Vasodilation is important to promote bleeding and can be enhanced by placing the animal under a heat lamp or on a warming plate for a few minutes prior to the procedure.

A.2.2.5.4 *Retro-orbital plexus*

Retro-orbital bleeding is quite a commonly used technique, but has been observed to cause adverse effects. Bleeding from

the plexus should always be carried out under general anaesthesia in all species; anaesthesia is a requirement in some national regulations. An interval of 2 weeks between bleeds at the same site should allow damaged tissue to repair in most cases,[g] but this does not mean that the animals do not experience some discomfort during the early stages before healing is complete.

The potential adverse effects of this technique include the following:

- Retro-orbital haemorrhage resulting in haematoma and excessive pressure on the eye, which is painful for the animal;
- Pressure on the eye to stop bleeding, which may result in corneal ulceration, keratitis, pannus formation, rupture of the globe, and micro-ophthalmia;
- Damage to the optic nerve and other intraorbital structures that can lead to deficits in vision and even blindness; and
- Fracture of the fragile bones of the orbit and neural damage by the micropipette and penetration of the eye globe itself.

A.2.2.6 *Equipment*

The following are required for routine blood collection:

- Blood collection tubes — blood can be collected with a regular needle and syringe, with a butterfly needle attached directly into the collection tube.
- Evacuated containers are designed to fill with a predetermined volume of blood by vacuum. The rubber stoppers are colour-coded according to the additive that the tube contains. Various sizes are available. Vacutainers should only be used with large animals or for cardiac puncture in animals the size of a large guinea pig and above.
- Blood should **never** be poured from one tube to another in case the tubes have different additives or coatings.

[g] van Herck H *et al*. Histological changes in the orbital region of rats after orbital puncture. *Lab Anim* **26**: 53–58, 1992.

- Blood from each individual animal should be collected in a new container to ensure that an accurate diagnosis of the individual's blood can be carried out.
- Needles — the gauge number indicates the diameter of the needle: the larger the gauge, the smaller the needle. Needles are available for evacuated systems and for use with a syringe, single draw, or butterfly system. Always use the smallest needle suitable for the technique to minimise distress to the animal.
- Holder/Adapter — this is for use with the evacuated collection system.
- Tourniquet — this is a band or device that applies pressure to the blood vessel to aid blood collection. When using a tourniquet, ensure that it is not too tight and remember to remove it after blood collection.
- Alcohol swab — 70% isopropyl alcohol is generally applied to a small gauze pad, then wiped over the injection/blood collection site to disinfect it.
- Iodine wipes/swabs — these should be used if blood culture is to be drawn.
- Gauze — this should be applied to the blood collection site after withdrawal of the needle.
- Sharps container — needles should be placed in the sharps container **immediately** after use. Needles should **never** be broken, bent, or recapped.

Note: Tubes with additives must be thoroughly mixed to ensure that the additive is evenly distributed in the blood.

A.2.2.6.1 *Needles (see Fig. A.1)*

Needles come in various sizes, referred to as the "gauge" (G). As the gauge size increases, the diameter of the needle decreases. The gauge ranges from 10 to 33; however, in general, only sizes between 18G and 30G are used. Needles from around 18G to 20G are generally used for large animals or to collect large volumes of blood by cardiac puncture in other species; needles from 21G to 27G are most commonly used for all species; and

smaller needles (higher than 27G) are generally not used, unless intradermal injections of small volumes are required in small animals.

The other important factor to note is the length of the needle. Needles come in various lengths, but the length used for injection in laboratory animals usually varies from ½″ to 1 ½″, depending on the location of the injection and the size of the animal.

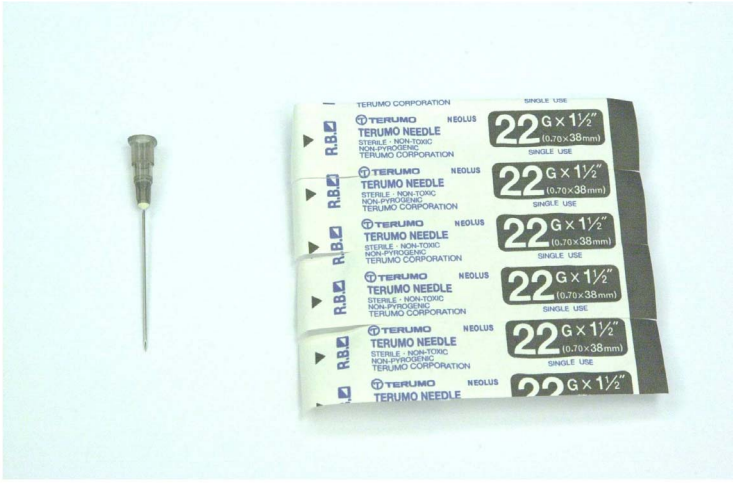
Needles come in different colours, both on the packaging and on the hub of the needle, that correspond to the gauge.

When selecting the size of the needle, you need to consider the following:

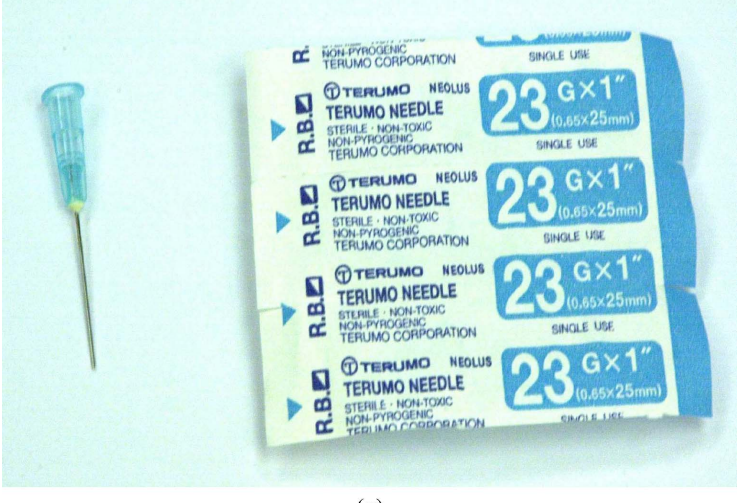
- Size of the animal;
- Injection site;
- Volume of injection article/blood withdrawal — larger volumes tend to require bigger needles; and
- Viscosity of injection article — aqueous solutions will easily flow through high-gauge needles, whereas oil immersions will require a wider needle (lower gauge).

(a)

Fig. A.1 Different needle sizes and their functions. (a) 18G 1½″ needles — usually used for cardiac puncture in medium to large animals and for large-volume injection in large animals.

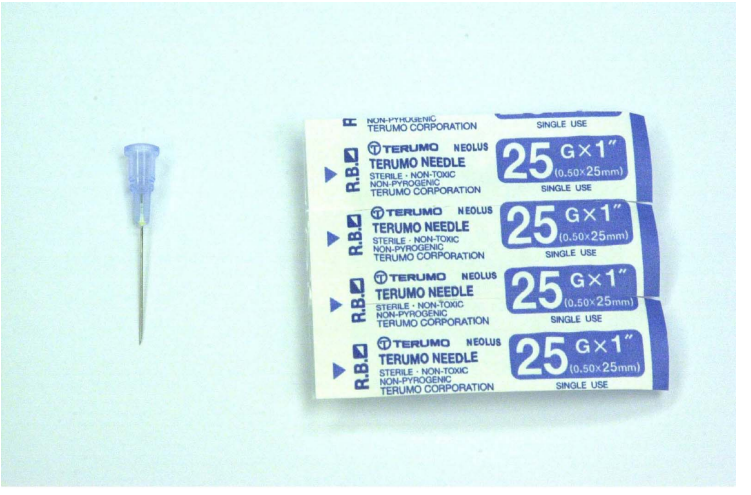


(b)

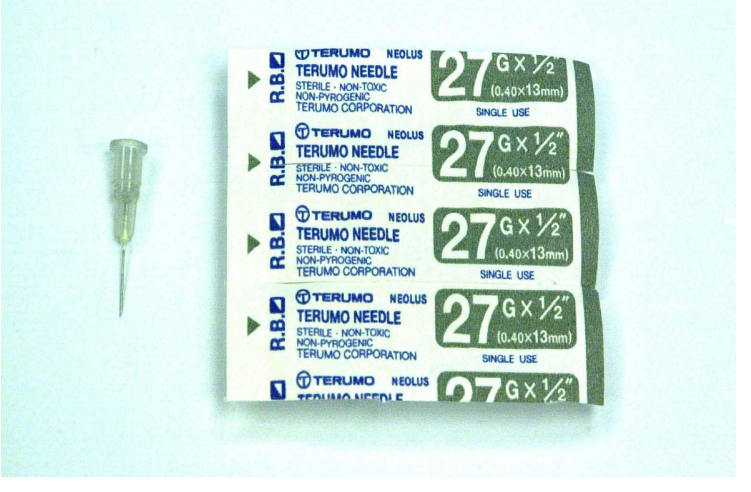


(c)

Fig. A.1 (*Continued*) (b) 22G 1½″ needles — can be used in various species for injection or blood collection, but one needs to be cautious when using these in small animals due to their length. (c) 23G 1″ needles — good for cardiac puncture in rodents and for injection of viscous material.

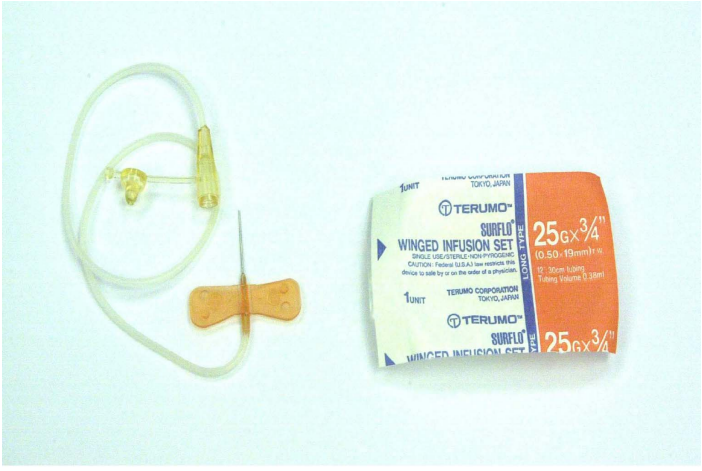


(d)



(e)

Fig. A.1 (*Continued*) (d) 25G 1″ needles — can be used for blood collection or injection in a variety of species. (e) 27G ½″ needles — usually used for injection in small animals only.



(f)

Fig. A.1 (*Continued*) (f) 25G ¾″ "Butterfly" needles — usually used for injection or blood collection in medium to large animals. "Butterfly" needles have the advantage of allowing for some movement during injection/blood collection, resulting in less stress.

A.2.2.7 *Vein selection*

- Palpate and trace the path of the vein with the index finger. Arteries pulsate, are more elastic, and have a thick wall.
- If superficial veins are not readily apparent, you can force blood into the vein by massaging the arm from the wrist to the elbow, tapping the site with the index and second finger, applying a warm, damp washcloth to the site for 5 minutes, or lowering the extremity over the bedside to allow the veins to fill.

A.2.2.7.1 *Preventing haematomas (bruising)*

- Use the major superficial veins.
- Make sure the needle fully penetrates the uppermost wall of the vein. (Partial penetration may allow blood to leak into

the soft tissue surrounding the vein by way of the needle bevel.)
- When using a tourniquet, remember to remove it before removing the needle.
- Apply pressure to the blood collection site.

A.2.2.7.2 *Preventing haemolysis (which can interfere with tests)*

- Mix tubes with anticoagulant additives gently 5–10 times.
- Avoid drawing blood from a haematoma.
- Avoid drawing the plunger back (aspirating) too forcefully, when using a needle and syringe; aspirate slowly and allow the syringe to fill before continuing.
- Avoid "probing" with the needle.

A.2.2.8 *Safety*

- Always wear appropriate personal protective equipment (PPE) (gloves, lab coat, etc.) when handling blood/body fluids.
- Change gloves after handling each animal/cage of animals, or when contaminated.
- Dispose of items in appropriate containers (sharps bins, biohazard bags, etc.).
- Dispose of needles immediately after blood withdrawal. Do not bend, break, recap, or resheath needles to avoid accidental needle puncture or splashing of contents.
- Clean up any blood spills with a suitable disinfectant such as 10% bleach.

A.2.2.8.1 *If you get a needlestick injury*

- Remove your gloves.
- Squeeze the puncture site to promote bleeding.
- Wash the area well with soap and water.
- Record the animal cage number/animal ID (especially for nonhuman primates and biohazard animals).
- Report the incident to your superior/safety officer or doctor for appropriate treatment and follow-up.

A.2.2.9 *Troubleshooting — what to do if no blood is obtained (see Fig. A.2)*

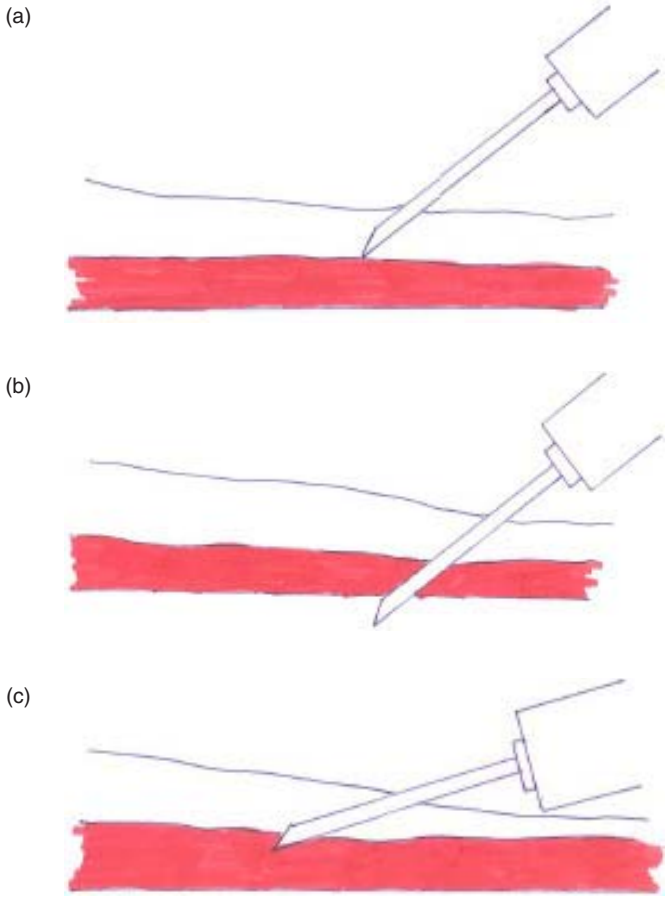


Fig. A.2 What to do if no blood is obtained. (a) Change the position of the needle. Move the needle forward, as it may not be in the lumen of the vein. (b) Try moving the needle backward, as it may have penetrated too far and gone through the vein and out the other side. (c) Adjust the angle of the needle (by rotating), as the bevel of the needle (flat part) may be blocked by the vein wall.

A.2.2.10 *Troubleshooting — what to do if blood stops flowing*

- The needle may have slipped out of the vein; this often happens when collecting large quantities of blood with more than one tube. Reposition the needle.
- The vein may have collapsed; this may be the result of too much aspiration. Remove the needle and insert it higher up on the vein or in an alternative location.

A.2.2.11 *Blood collection tubes*

Blood collection tubes are colour-coded to make it easier for operators to see what additive is in the tube. The following is a list of the tubes and their uses:

Table A.4 Colour codes of blood collection tubes.

Colour	Additive	Action	Uses
Red top	None	Blood clots and the serum is separated by centrifuge	Chemistries, immunology and serology, blood bank
Light green top	Plasma separating tube (PST) with lithium heparin	Anticoagulants with lithium heparin; plasma is separated with PST gel at the bottom of the tube	Chemistries
Purple top	EDTA	Forms calcium salts to remove calcium	Haematology (complete blood count) and blood bank; requires full draw — invert 8 times to prevent clotting and platelet clumping

(*Continued*)

Table A.4 (*Continued*)

Colour	Additive	Action	Uses
Light blue top	Sodium citrate	Forms calcium salts to remove calcium	Coagulation tests; requires full draw
Green top	Sodium heparin or lithium heparin	Inactivates thrombin and thromboplastin	Lithium level — use sodium heparin Ammonia level — use sodium or lithium heparin
Dark blue top	EDTA	Tube is designed to contain no contaminating metals	Trace element testing (zinc, copper, lead, mercury) and toxicology
Light grey top	Sodium fluoride and potassium oxalate	Antiglycolytic agent preserves glucose for up to 5 days	For lithium level use sodium heparin glucoses; requires full draw (may cause haemolysis if short draw)
Yellow-black top	Broth mixture	Preserves viability of microorganisms	Microbiology — aerobes, anaerobes, fungi
Black top	Sodium citrate (buffered)	Forms calcium salts to remove calcium	Westergren sedimentation rate; requires full draw
Orange top	Thrombin	Quickly clots blood	STAT serum chemistries
Light brown top	Sodium heparin	Inactivates thrombin and thromboplastin; contains virtually no lead	Serum lead determination
Pink top	Potassium EDTA	Forms calcium salts	Molecular/Viral load testing

A.3 Mice (*Mus musculus*)

Careful handling and restraint are required to minimise discomfort when injecting any substance into a small animal. Practice should be carried out by first using models or euthanised animals. Always use aseptic techniques.

Mice should be picked up by the base of the tail, close to the body. Pregnant animals and young animals (preweaning) may need to be scooped up with one or both hands. Weaner mice may need to be picked up by the tail, and care should be taken as they are usually very lively and will jump out of the cage at any given opportunity. When transferring weaners, make sure that the cage lid is on the cage; and if it is necessary to leave a space, just push the lid back to make a small gap that allows the mice through yet prevents any escapees.

When handling mice, always observe the animal facility regulations, as many facilities now house mice in individually ventilated cages (IVCs) that should only be opened in a cage-changing station/laminar flow hood to protect the health of the animals (and sometimes the users). Gloves and other PPE will be required for handling animals; again, this may vary depending on the animal facility.

A.3.1 Sentinels

Sentinel animals are usually housed in each rodent room and tested periodically (monthly to biannually for common viruses, bacteria, and parasites). Serology is performed on a more regular basis to test for viruses, and a comprehensive test (including necropsy, serology, virology, parasitology, and histology of selected target tissues) is performed periodically.

Reports of all test results are maintained by the animal facility management/veterinarians and are available upon request. Any positive results need to be discussed with the veterinary staff regarding the possible impact to the animal's health and the research programme, and a course of action can then be decided upon.

A.3.2 Physiologic parameters

Body temperature = 36.5°C–38.0°C
Heart rate = 325–780/min
Respiratory rate = 94–163/min
Tidal volume = 0.09–0.23 mL

Avertin is widely used in mice as it offers good, reliable anaesthesia that is easy to use; operators are able to weigh the mice and give the dose according to the anaesthetic dose chart. Avertin does not provide much analgesia, so pain relief must be administered either at the time of anaesthesia or shortly thereafter. Avertin is made by mixing equal amounts of tribromyl ethyl alcohol and tertiary amyl alcohol (usually to a 2.5% dilution). If avertin is improperly prepared or stored in the light, it will break down into dibromoacetic acid and hydrobromic acid, which can be lethal in 24 hours. **Freshly mixed solutions are strongly recommended for safe use**. The solution can be kept for as long as 4 months if it is stored in the dark at 4°C (usually inside a refrigerator). Often, plastic tubes wrapped in aluminium foil are used to protect the solution from the light. The solution should be tested to ensure that it has a pH > 5.

A.3.3 Volume for injection

The maximum volume to be injected depends on the site of injection and the size of the mouse. Too much fluid too rapidly may cause pulmonary oedema (see Tables A.5 and A.6).

The following are widely accepted standards:

Table A.5 Volume for injection.

	IP	IM	IV	SC
Mouse (Adult)	Up to 2.0 mL	0.05 mL/site	0.1–0.2 mL	0.5 mL (up to 4 sites); 2 mL total
Needle size	27G–30G	27G–30G	26G–27G	25G–27G

Table A.6 Anaesthesia and analgesia (suggested agents and doses).

Agent	Dosage and Route of Administration	
Restraint/Premedication		
Atropine	0.02–0.05 mg/kg	IM
Diazepam (Valium®)	5 mg/kg	IP
Ketamine (Ketaset®, Vetalar®)	22–44 mg/kg	IM
Telazol® (for restraint)	100–160 mg/kg	IM/IP
Carbon dioxide[a] (in O^2 concentration of 10%–50%)	Until onset of anaesthesia	Inhalant
Anaesthesia		
Sodium pentobarbital	50–90 mg/kg	IP
Ketamine[b]	50–200 mg/kg	IP
Avertin (tribromoethanol)	125–250 mg/kg 0.02 mL/g (1.2% solution)	IP
Ketamine/Xylazine:		
Add 7 mg xylazine[c] to 35 mg ketamine	70–80 mg/kg	IM/IP
Or		
Add 1.0 mL xylazine (20 mg/mL) +1.0 mL ketamine (100 mg/mL) +4.6 mL sterile water	0.1 mL/20 g	IM/IP
Halothane (Fluothone®)	—	Inhalant
Isoflurane	—	Inhalant

(*Continued*)

Table A.6 (*Continued*)

Agent	Dosage and Route of Administration	
Analgesia		
Butorphanol tartrate (Torbugesic®)	2.5–5 mg/kg/1–2 h	SC
Buprenorphine (Temgesic®)	2 mg/kg/12 h	SC/IP
Oxymorphone	0.15 mg/kg/4 h	IM
Ketorolac (Toradol®)	0.7–10 mg/kg/24 h	Oral dosing

[a] Take care when using CO_2 for short-acting anaesthesia, as the dose required is close to the lethal dose. Once onset of anaesthesia is confirmed, remove the animal from the chamber immediately.
[b] Suitable for minor surgery procedures only, as it is short-acting.
[c] Xylazine is available in **two strengths** (20 mg/mL and 100 mg/mL). Ensure the correct dose is calculated based on the strength being used.

A.3.4 Mouse handling and sexing — for removal from caging and transport

1. Grasp the mouse near the base of its tail [Fig. A.3(a)].
2. Lift the animal out of the cage and place it in new caging or on a firm surface.
3. Do not suspend the mouse by its tail for a prolonged period of time because of stress on the animal. Support its body weight quickly, especially for pregnant animals.
4. Always double-check the sex of the animal with the cage card [Fig. A.3(b)].

A.3.5 Mouse restraint techniques for technical manipulation

A.3.5.1 *Scruffing*

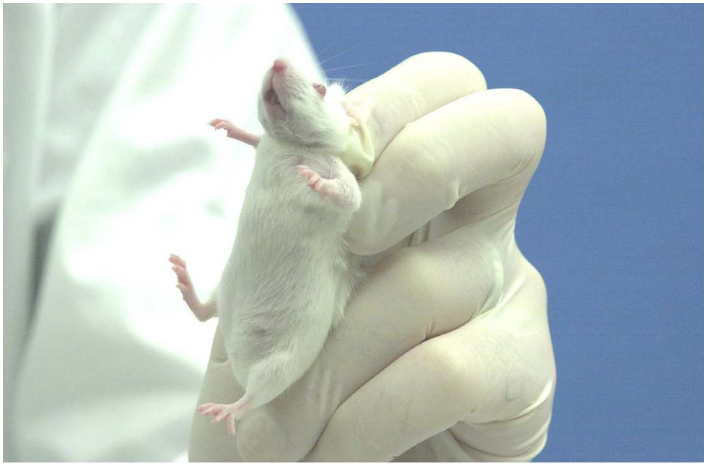
1. Restrain the mouse by grasping near the base of its tail.
2. Place the mouse onto a cage top to take advantage of the mouse gripping the top.
3. Grasp the nape of its neck with the forefinger and thumb of the other hand, gathering the loose skin from around the neck (below the head) and back.

(a)

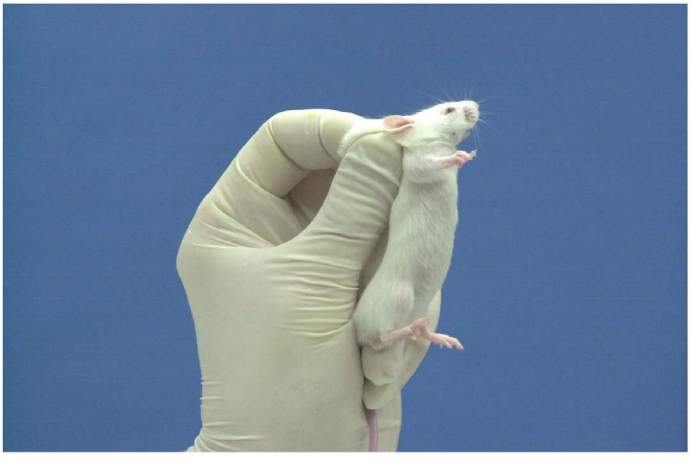
(b)

Fig. A.3 Mouse handling and sexing. (a) Removal from the cage and transport. (b) Identification of the sex of the animal. (Female on the left, male on the right. Notice the distance between the anus and genitals is greater in the male.)

4. Ensure that you gather enough skin to prevent the head from turning, while allowing the animal to breathe normally.
5. Place the tail between your ring and little fingers to secure and control the animal. The tail must be secured to prevent the mouse from moving and loosening the grip.
6. The tail can also be held against the palm of the hand.



(a)



(b)

Fig. A.4 Scruffing — for technical manipulation.

7. The mouse is now ready for technical manipulation (Fig. A.4).
8. Make sure that you feel comfortable holding the mouse in this position for some time because if you are not comfortable, there is a higher risk of failure.
9. Always use the alternative hand to your writing hand for restraining the mouse.

A.3.5.2 *Mechanical restraint (plastic restrainer)*

1. Restrain the mouse by grasping near the base of its tail.
2. Grasp the nape of its neck with the other hand.
3. Place its tail between your fingers to secure and control the animal.
4. Place the mouse's head into the opening of the restraint box.
5. Release your hold on its neck while maintaining the grasp on its tail.
6. Place the securing block in the appropriate slot for necessary restraint.
7. Alternatively, take the mouse by the base of its tail and gently but firmly pull it through into the restrainer, and place the securing block close to its head while allowing it to breathe easily. This technique may vary depending on the design of the plastic restrainer (Fig. A.5).
8. Take care because if the mouse is oversized or if the securing block is too close to the animal, it may prevent the animal from breathing properly, resulting in death.
9. Ensure that animals are only housed in the restraint device long enough to carry out the procedure required and then returned to their cage. Restraining animals for

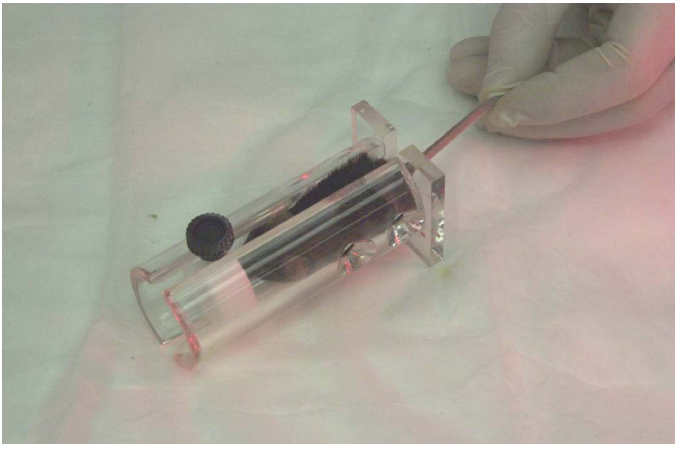


Fig. A.5 Using mechanical restraint (plastic restrainer) for technical manipulation.

extended periods of time will result in additional stress, which may have detrimental effects on the animal and your experiment.

10. Take care when using heated lamps/warming plates with the restraint device, as the animal will not have the ability to escape if the area is too hot and, again, this may have detrimental effects and may even lead to death due to dehydration.

A.3.6 Ear punching for identification

1. Restrain the mouse by scruffing.
2. Place an ear punch in the desired location [Fig. A.6(a)].
3. Firmly and quickly punch its ear to avoid an incomplete cut.
4. Occasionally, the piece of tissue removed will be attached to the ear. This can usually be removed with the help of a pair of forceps.

(a)

(b)

Fig. A.6 Ear punching — for identification.

5. Ear punches are available in various sizes. For mice, a 1-mm or 1.5-mm-diameter ear punch is generally suitable [Fig. A.6(b)].
6. Monitor the animals frequently and inspect those with ear punches, as these can sometimes tear or heal over (if the original hole is too small) and may need to be repeated.
7. There are several different ear punch numbering systems available. Any of these are suitable, but it is important to ensure that they are in conformation with the system being used in your facility. If your facility does not have a standard system for ear punch numbering, make a note on the cage card of the system you are using for future reference.

A.3.7 Subcutaneous (SC) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)
- Hypodermic needle (25G–30G)
- Injection article
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the appropriate amount of article to be administered.
2. Remember to use different needles for drawing up the injection article and for injection to prevent contamination of the injection site.
3. Restrain the mouse by scruffing or use an appropriate anaesthesia.
4. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Insert the needle at the base of the skin fold between your thumb and forefinger [Figs. A.7(a) and A.7(b)], keeping the needle straight because if there is an angle to the needle, it may pierce the muscle or go through the skin and into your finger.
6. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.

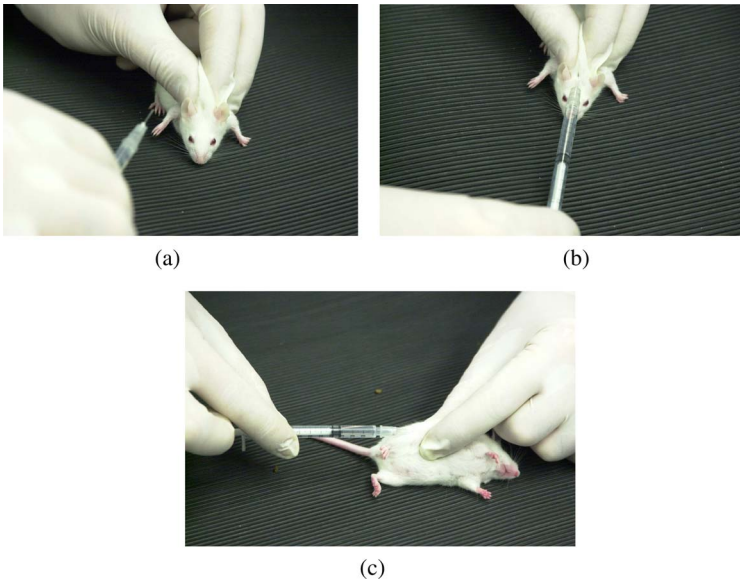


Fig. A.7 Subcutaneous injection.

7. Administer the article in a steady, fluid motion. As you inject, you can feel the injection article creating a bulbous under the skin between your fingers.
8. A safer method is to inject into the flank [Fig. A.7(c)], between the hind leg and the front leg. This is also the preferred location for injecting tumour cells, as there is room for the tumour to grow safely without putting pressure on vital organs/blood vessels.

A.3.8 Intraperitoneal (IP) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)
- Hypodermic needle (25G–30G), ½″
- Injection article
- Isopropyl alcohol
- Gauze

Fig. A.8 Intraperitoneal injection.

1. Fill the syringe with the appropriate amount of article to be administered.
2. Restrain the mouse by scruffing.
3. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Position the animal so that its head is lower than its body to allow any internal organs to move out of the way. Draw an imaginary line horizontally across the top of the hind legs, dividing the abdomen into four "quadrants".
5. Insert the needle into the lower left/right quadrant of the abdomen at a 30° angle (Fig. A.8).
6. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
7. If other fluids are seen in the syringe upon aspiration, such as a yellow/clear colour (indicating puncture of the urinary bladder) or green/brown colour (indicating puncture of the intestines/caecum), discard the needle and syringe and start again.
8. Administer the article in a steady, fluid motion.

A.3.9 Intradermal (ID) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)
- Hypodermic needle (27G–30G)

(*Continued*)

(*Continued*)

- Injection article
- Isopropyl alcohol
- Gauze
- Clippers, or #40 scalpel blade and scalpel blade holder

1. Intradermal injection is not typically carried out in mice, apart from the administration of certain compounds via the footpad or ear pinea.
2. Intradermal injection **must** be performed **under anaesthesia**.
3. Anaesthetise the mouse. Once the mouse is anaesthetised, proceed.
4. When injecting on the back of the mouse, take the scalpel holder and scalpel carefully in one hand and extend the skin between the fingers of the other hand. With the scalpel almost flat against the fur, gently rub the scalpel blade back and forth to remove the hair. This will give a nice, smooth surface and is better than using hair clippers, as it is easier to visualise the skin after injection.
5. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
6. Insert the needle carefully through the dermis at a 30° angle.
7. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
8. Administer the article slowly, with a maximum volume of 50 μL for footpad and ear pinea injection, to 100 μL per injection site for intradermal injections on the back of the animal to avoid tissue trauma. Successful injection results in a small, circular skin welt.

A.3.10 Intravenous (IV) injection utilising lateral tail veins

Materials required:

- Personal protective equipment (PPE)
- Plexiglas restraint box

(*Continued*)

(*Continued*)

- Syringe (1 mL)
- Hypodermic needle (25G–30G)
- Injection article
- Isopropyl alcohol
- Gauze

1. Place the mouse into a plastic restraint device or anaesthetise it.
2. Prepare the tail with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
3. Needle placement should be no closer to the body than half the length of the tail. It is good practice to start as close to the tip of the tail as possible, moving closer to the body if the injection is unsuccessful, as it is not possible to insert at a lower location.
4. Ensure that you can visualise the lateral tail veins. This can be assisted with the use of a heated lamp or by placing the animal in a cage warmer or on top of a warming plate for a few minutes prior to injection.
5. With the tail under tension, insert the needle approximately parallel to the vein (Fig. A.9).
6. Ensure proper needle placement by inserting the needle at least 3 mm into the lumen of the vein. Once in the lumen, the needle should feel smooth and there should be no resistance upon injection.

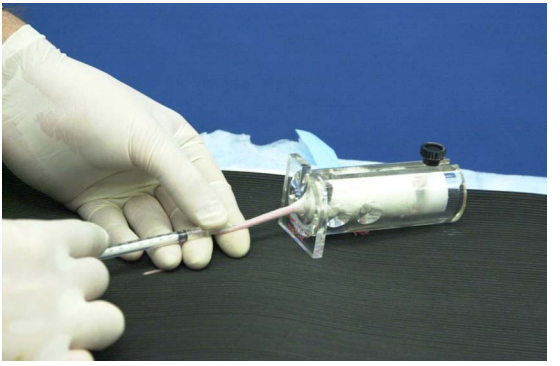


Fig. A.9 Intravenous injection utilising lateral tail veins.

7. Administer the article in a slow, fluid motion to avoid rupture of the vessel. You will be able to visualise a clearing of the lumen as the injection article replaces the blood in the vein.
8. If the solution leaks into the surrounding tissues or forms a bleb, remove the needle and insert again slightly higher on the vein (closer to the body).
9. Upon completion, ensure good haemostasis (i.e. that any bleeding has stopped) before returning the mouse to its cage.

A.3.11 Gavaging of mouse

Materials required:

- Personal protective equipment (PPE)
- Biomedical needles (animal feeding needles 1″–1½″, 20G–22G)
- Syringe (1–3 mL)
- Injection article

1. Select the correct-sized gavage needle, ensuring that there is a metal ball on the end to prevent the tip from being sharp [Fig. A.10(a)]. **Never** use a hypodermic needle for oral gavage.
2. Measure the needle length against the mouse's body; the needle should be no longer than from the nose to the last rib (approximate level of the stomach). If the needle is longer, take care to only insert the appropriate length to prevent damaging the stomach. Shorter gavage needles can be used; but if injecting acidic compounds, ensure that the needle fits adequately into the stomach to prevent damage to the oesophagus.
3. Fill the syringe with the appropriate amount of article to be dosed.
4. Restrain the mouse by scruffing [Fig. A.10(b)].
5. Place the tip of the needle in the mouse's mouth [Fig. A.10(c)].
6. Slide the tip down the back of the mouth, moving it toward the front in one fluid motion.
7. Take your time; any resistance felt indicates improper placement, in which case remove the needle and start again.

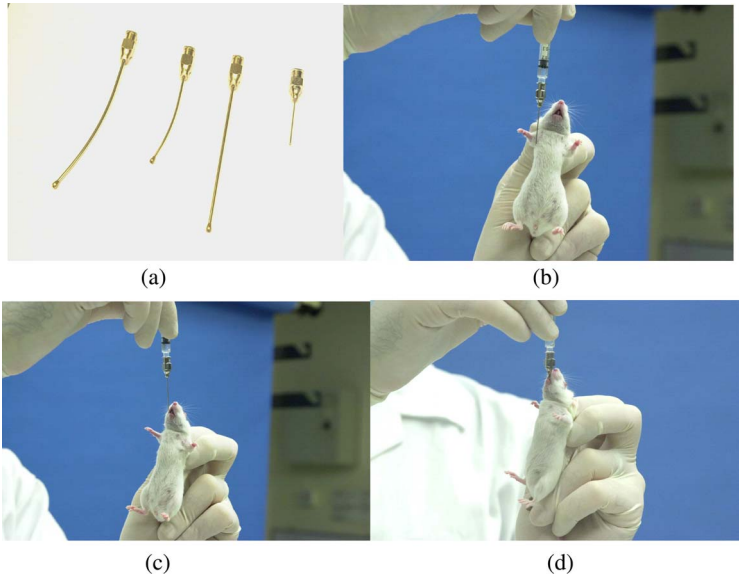


Fig. A.10 Gavaging of mouse.

Do not force the needle, as it may enter the trachea and damage the epiglottis. The needle should slide down into the oesophagus easily.

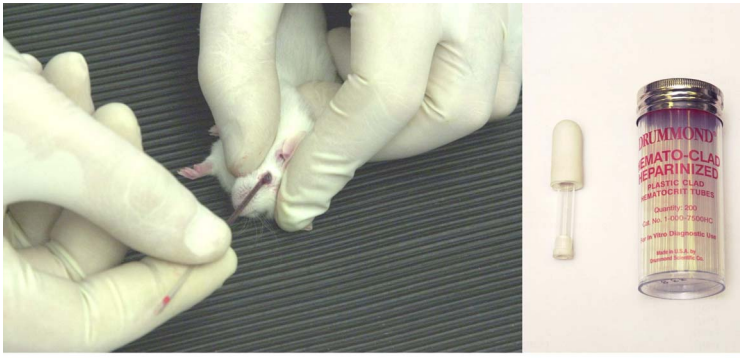
8. Once the needle is properly placed [Fig. A.10(d)], administer the injection article.
9. Remove the needle carefully so as not to damage the oesophagus.
10. If the animal's breathing is laboured, monitor it closely in case the injection article enters the lungs, in which case the animal may need to be euthanised unless it recovers within a few minutes.

A.3.12 Blood withdrawal utilising retro-orbital sinus for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Anaesthetic agent
- Haematocrit tubes or Pasteur pipettes

(*Continued*)



(a) (b)

Fig. A.11 Blood withdrawal utilising retro-orbital sinus for large-volume blood collection.

(*Continued*)

- Collection vessel
- Isopropyl alcohol
- Gauze

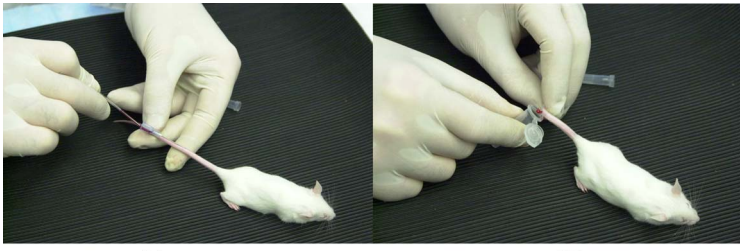
1. Retro-orbital bleeds **must** be performed **under anaesthesia**.
2. Anaesthetise the mouse. After the mouse is anaesthetised, proceed.
3. Place the haematocrit tube or Pasteur pipette at the medial canthus of the eye [Fig. A.11(a)].
4. With a rotating motion, apply gentle pressure to insert the tube through the membrane.
5. Continue rotating the tube on the back of its orbit until blood flows.
6. Collect the blood in an appropriate vessel [Fig. A.11(b)].
7. Upon completion, ensure good haemostasis before returning the animal to the cage by closing the eyelids and placing a gauze pad over the eye until bleeding stops (usually for a few seconds).
8. A pump can be attached to the haematocrit tube to expel blood into a collection tube after completion [Fig. A.11(b)].

A.3.13 Blood withdrawal utilising lateral tail veins for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Plexiglas restrain box or anaesthesia
- Haematocrit tube
- Hypodermic needle (23G–30G)
- Isopropyl alcohol
- Gauze

1. Please note that it is not acceptable to remove part of the tail in order to collect blood only, unless the tissue sample taken is very small (3–5 mm in length) and is required for genotyping.
2. Restrain the mouse using a plastic restraint device or anaesthetise it.
3. Prepare the tail with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Needle placement should be no closer to the body than half the length of the tail.
5. Ensure that you can visualise the lateral tail veins. This can be assisted with the use of a heated lamp or by placing the animal in a cage warmer or on top of a warming plate for a few minutes prior to injection. The lateral tail vein runs along either side of the tail and can be visualised easily in albino mice. In nonalbino strains, it is more important to warm the tail or palpate the vein to find the correct location.
6. With the tail under tension, insert the needle approximately parallel to the vein [Fig. A.12(a)].
7. Ensure proper needle placement by inserting the needle at least 3 mm into the lumen of the vein.
8. Once blood starts to flow into the hub of the needle, place the haematocrit tube into the needle hub or remove the



(a) (b)

Fig. A.12 Blood withdrawal utilising lateral tail veins for small-volume blood collection.

needle to allow the blood to collect directly into a suitable collection tube [Fig. A.12(b)].

9. Blood collection can be assisted by "milking" the vein, by gentle rubbing it to stimulate blood flow.
10. Upon completion, ensure good haemostasis before returning the animal to the cage by placing the gauze pad over the blood collection site and applying pressure until bleeding stops (usually for a few seconds).

A.3.14 Blood withdrawal utilising saphenous veins for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Plexiglas restrain box or anaesthesia
- Haematocrit tube
- Hypodermic needle (23G–30G)
- Isopropyl alcohol
- Gauze
- Clippers, or #40 scalpel blade and scalpel blade holder

1. Restrain the mouse using a plastic restraint device or anaesthetise it.
2. Attach the scalpel blade to the holder.
3. Extend the hind leg and use the scalpel blade or clippers to remove the hair above the heel of the foot until the top of

the leg. When shaving a nonanaesthetised mouse, an assistant may be required.
4. Prepare the skin on the leg with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections). Blood can be collected from either leg.
5. The saphenous vein should be easily visualised on the surface of the leg/thigh.
6. The needle can then be inserted into the vein and removed quickly to puncture the vein to commence bleeding.
7. Using the haematocrit tube, collect the blood from the saphenous vein, applying pressure in a pumping motion to the vein with your fingers to stimulate blood flow.
8. Once the required amount of blood has been collected, flex the foot of the mouse to reduce the flow of blood back to the puncture site.
9. Upon completion, ensure good haemostasis before returning the animal to the cage by placing the gauze pad over the blood collection site and applying pressure until bleeding stops (usually for a few seconds).

A.3.15 Blood withdrawal utilising facial veins for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Haematocrit tube
- Hypodermic needle (18G–25G)
- Isopropyl alcohol
- Gauze

1. This is a relatively new technique, which is gaining more support from scientists who require only 1–10 drops of blood.
2. The facial vein runs just along the bottom of the mandible (jaw) and just at the position of the freckle on the bottom left and right sides of the mouse's face.
3. Restrain the mouse by scruffing. It is important to collect a lot of skin from around the neck, so that the mouse's eyes

start to bulge (just as if under anesthesia and totally relaxed) and the forelegs stick to the sides.
4. Locate the hairless freckle on the side of the jaw.
5. Take the needle and align it so that you are pointing it at the far side of the mouse's face, at the base of the far ear or at the base of the far side of the mouth.
6. The needle can then be inserted into the freckle and removed quickly to puncture the vein to commence bleeding.
7. Using the haematocrit tube, collect the blood from the saphenous vein. Typically, you can get anything from 1 to 10 drops of blood.
8. Once the required amount of blood has been collected, gently dab the site with the gauze and release the mouse back into its cage.
9. Bleeding should stop immediately.

A.3.16 Intracardiac (IC) puncture for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (22G–25G)
- Isopropyl alcohol
- Gauze
- Anaesthesia/CO^2

1. Intracardiac puncture **must** be performed **under anaesthesia** or shortly after euthanasia.
2. Anaesthetise the mouse. After the mouse is anaesthetised, proceed.
3. Prepare the blood collection site with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).

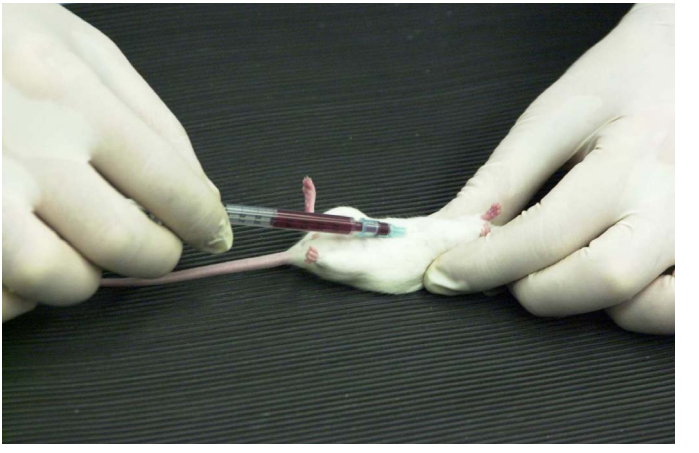


Fig. A.13 Intracardiac puncture for large-volume blood collection.

4. Make sure that you are aware of the location of the heart. If you are not able to locate it, you can place a finger over the chest and feel for the mouse's heartbeat.
5. Insert the needle at the base of the sternum at a 15°–20° angle just lateral of the midline (mouse's left side), and push the needle up into the position of the heart (Fig. A.13).
6. Aspirate the syringe slowly, allowing the blood to collect into the syringe before continuing to aspirate. If the blood flow stops or slows down, rotate the needle and syringe or adjust slightly, as the blood may have clotted (especially in euthanised mice).
7. Do not probe around the chest with the needle as it is very sharp and may cut or damage other tissues, causing internal bleeding.
8. Once the required amount of blood has been collected, the mouse should be euthanised by an appropriate method.
9. Exsanguination (removal of all circulating blood) will in itself cause death if the animal is under anaesthesia at the time of collection, but it is always important to ensure that death has occurred either by monitoring the vital signs or by performing an additional method of euthanasia on the animal as a precaution.

A.4 Rats (*Rattus norvegicus*)

Careful handling and restraint is essential to minimise discomfort for the animal. Practise on euthanised animals. Always use aseptic techniques.

The procedures listed here may be carried out by a single operator. Inexperienced operators may prefer to work in pairs, with one person restraining the rat and another injecting. Very large rats may also be difficult to restrain using only one hand. Rats are intelligent animals and are much more amenable to procedures if they are accustomed to the handler.

A.4.1 Physiologic parameters

Body temperature = 35.9°C–37.5°C
Heart rate = 250–450/min
Respiratory rate = 70–115/min
Tidal volume = 0.6–2.0 mL

Rats are often used for obesity studies; and as such, male rats fed on low-calorie diets usually require higher doses of barbiturates. Avertin has been reported to cause ileus (prevention of the passage of intestinal contents) in rats.

Tables A.7 and A.8 show the maximum volumes to be injected as well as the suggested agents and doses for anaesthesia and analgesia.

Table A.7 Maximum injection volumes per site.

Rat	IV	IP	SC	IM
Volume (mL)	1	5–10	1–2	0.1

Table A.8 Anaesthesia and analgesia (suggested agents and doses).

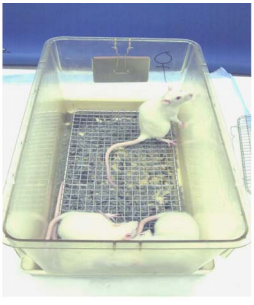
Agent	Dosage and Route of Administration	
Restraint/Premedication		
Atropine	0.04–0.1 mg/kg	SC
Diazepam (Valium®)	0.5–15 mg/kg	IP
Ketamine (Ketaset®, Vetalar®)	22–50 mg/kg	IM
Carbon dioxide[a] (in O^2 concentration of 10%–50%)	Until onset of anaesthesia	Inhalant
Anaesthesia		
Sodium pentobarbital	30–60 mg/kg	IV/IP
Ketamine (10 mg/mL solution)	50–90 mg/kg	IM
	50–100 mg/kg	IP
Ketamine/Xylazine[b]:		
Ketamine	40–80 mg/kg	IM/IP
Xylazine	10 mg/kg	IM/IP
Halothane (Fluothane®)	—	Inhalant
Isoflurane	—	Inhalant
Carbon dioxide)[a]	Until onset of anaesthesia	Inhalant
Telazol®	20–40 mg/kg	IP
	20 mg/kg	IM
Ketamine/Medetomidine:		
Ketamine	60–75 mg/kg	IP
Medetomidine (Domitor®)	0.25–0.5 mg/kg	SC
Analgesia		
Morphine	1.5–6 mg/kg/2–4 h	SC
Butorphanol tartrate (Torbugesic®)	2.5–5 mg/kg/1–2 h	SC
Carprofen	5 mg/kg/12 h	SC
Ketorolac	3–5 mg/kg/12–24 h	Oral dosing
	1 mg/kg/12–24 h	IM
Buprenorphine	0.01–0.05 mg/kg	SC/IP
Reversal Agents		
Yohimbine (reversal agent for xylazine or medetomidine)	1–2 mg/kg	IM/IP

[a] Take care when using CO_2 for short-acting anaesthesia, as the dose required is close to the lethal dose. Once onset of anaesthesia is confirmed, remove the animal from the chamber immediately.

[b] Xylazine is available in **two strengths** (20 mg/mL and 100 mg/mL). Ensure that the correct dose is calculated based on the strength being used.

A.4.2 Rat handling and sexing

1. First, assess the rats in their cage for normal behaviour [Fig. A.14(a)]. The rats should be alert and inquisitive, and will usually stand on their hind legs and move around the cage exploring their environment.
2. Place your hands into the cage, and gently pet and touch the animals. At this point, be careful of touching their faces and of stressing them. Try to calm them and let them sniff you.
3. With firm but gentle pressure, grasp the rat around the thorax with your thumb and forefinger under each of its front legs.
4. Lift the rat out of the cage and place it in a new cage or on a firm surface.
5. For aggressive rats, pick them up by grasping them by the base of the tail, close to the body.



(a)

(b) (c)

Fig. A.14 Rat handling and sexing. (a) Rats in cage. (b) Female rat and (c) male rat.

6. **Do not** suspend the rat by its tail or its upper body for a prolonged time period. Support its body weight quickly, either on the cage top or on the arm of your lab coat.
7. Do not let the rats hold on to the cage top whilst you attempt to handle them, as they are strong and can easily pull away, resulting in injuries.
8. Check the sex of the rats and ensure that the cage card information is correct [Figs. A.14(b) and A.14(c)].

A.4.3 Rat restraint techniques for technical manipulation

A.4.3.1 *Manual restraint*

1. With firm yet gentle pressure, grasp the rat around the thorax with your thumb and middle finger under each of its front legs (Fig. A.15).
2. With your free index finger still under its leg, grasp the loose skin on the nape of its neck.
3. Take care not to squeeze the rat or apply too much pressure to its diaphragm, as this may result in injury and suffocation.
4. Do not attempt to scruff rats unless you are very experienced, as rats, unlike mice, object strongly and vocally to being scruffed unless they are handled frequently.

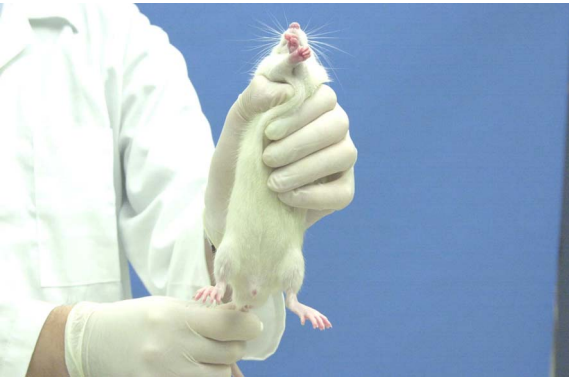


Fig. A.15 Rat restraint technique for technical manipulation.

5. Extend the tail to keep the back straight, preventing the rat from turning around.
6. The animal is now ready for technical manipulation.
7. If you encounter an aggressive rat, you can wear a cloth glove or place a small hand towel around your hand when restraining.
8. Take care when using metal chain gloves, as the rat's claws can get caught in the links, resulting in injuries to the rat.

A.4.3.2 *Mechanical restraint*

Materials required:

- Personal protective equipment (PPE)
- Plexiglas restraint box

1. With firm but gentle pressure, grasp the rat around the thorax with your thumb and forefinger under each of its front legs.
2. Place the animal's tail between your fingers to secure and control it.
3. Place the rat's head in the opening of the restraint box.
4. Release your hold on its body, while maintaining your grasp on its tail.

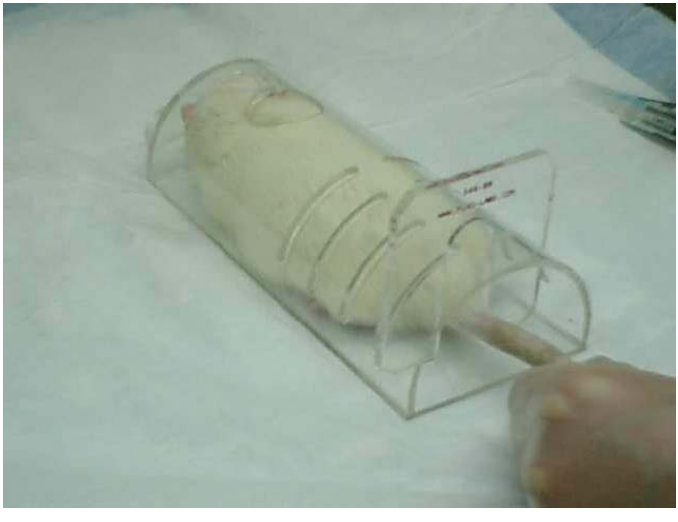


Fig. A.16 Mechanical restraint for technical manipulation.

5. Place the securing block in the appropriate slot for necessary restraint (Fig. A.16).
6. Alternatively, take the rat by the base of its tail and gently but firmly pull it through into the restrainer, and place the securing block close to the head while allowing the rat to breathe easily. This technique may vary depending on the design of the plastic restrainer (Fig. A.16).
7. Take care because if the rat is oversized or if the securing block is too close to the animal, it may prevent the animal from breathing properly, resulting in death.
8. Ensure that animals are only housed in the restraint device long enough to carry out the procedure required and then returned to their cage. Restraining animals for extended periods of time will result in additional stress, which may have detrimental effects on the animal and your experiment.
9. Take care when using heated lamps/warming plates with the restraint device, as the animal will not have the ability to escape if the area is too hot and, again, this may have detrimental effects and may even lead to death due to dehydration.

A.4.4 Ear punching for identification

Materials required:

- Personal protective equipment (PPE)
- Ear punch

1. Restrain the rat (refer to the restraint technique).
2. Place ear punch in the desired location.
3. Firmly and quickly punch the ear to avoid an incomplete cut (Fig. A.17).
4. Occasionally, the piece of tissue removed will be attached to the ear. This can usually be removed with the help of a pair of forceps.
5. Ear punches are available in various sizes. For rats, a 1-mm- or 1.5-mm-diameter ear punch is generally suitable.
6. Monitor the animals frequently and inspect those with ear punches, as these can sometimes tear or heal over (if the original hole is too small) and may need to be repeated.

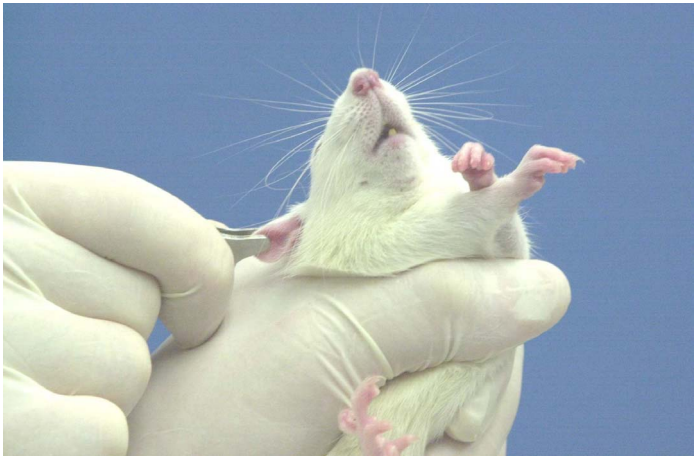


Fig. A.17 Ear punching for identification.

7. There are several different ear punch numbering systems available. Any of these are suitable, but it is important to ensure that they are in conformation with the system being used in your facility. If your facility does not have a standard system for ear punch numbering, make a note on the cage card of the system you are using.

A.4.5 Intramuscular (IM) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)
- Hypodermic needle (22G–30G)
- Injection article
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the appropriate amount of article to be administered.
2. Remember to use different needles for drawing up the injection article and for injection to prevent contamination of the injection site.

3. Restrain the rat (refer to the restraint technique) or anaesthetise it.
4. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Insert the needle into the caudal thigh (at the top back of the hind leg) or quadricep muscles (behind the femur) (Fig. A.18).
6. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
7. Administer the article in a steady, fluid motion. **Do not** administer rapidly, as this may cause tissue trauma.
8. Note that only small quantities (maximum 0.1 mL) should be administered intramuscularly to prevent tissue trauma and discomfort.

A.4.6 Subcutaneous (SC) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–2 mL)
- Hypodermic needle (22G–30G)
- Injection article

(*Continued*)

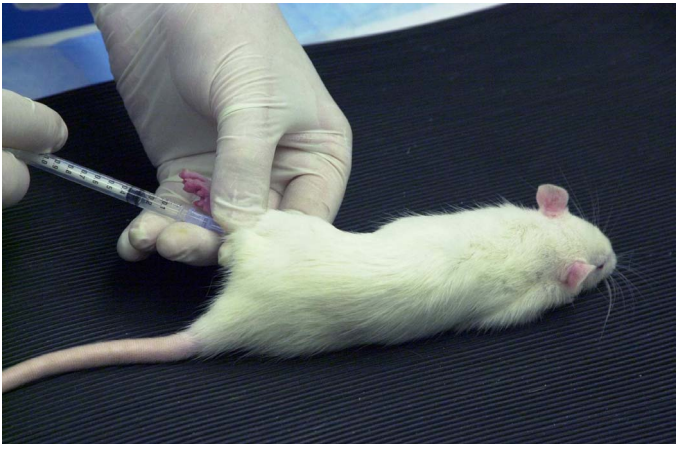
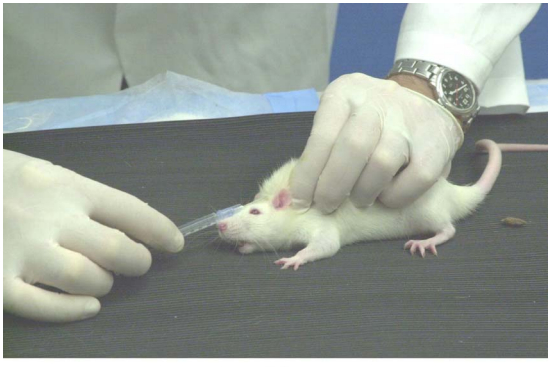


Fig. A.18 Intramuscular injection.

(*Continued*)

- Isopropyl alcohol
- Gauze

1. Fill the syringe with the appropriate amount of article to be administered.
2. Remember to use different needles for drawing up the injection article and for injection to prevent contamination of the injection site.
3. Restrain the rat by scruffing; using the base of your palm, pin the rat down onto a smooth surface [Fig. A.19(a)].
4. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).



(a)

(b)

Fig. A.19 Subcutaneous injection.

5. Insert the needle at the base of the skin fold between your thumb and forefinger, keeping the needle straight because if there is an angle to the needle, it may pierce the muscle or go through the skin and into your finger.
6. Aspirate the syringe to ensure proper placement. Any sign of blood indicates improper placement, in which case the needle needs to be repositioned.
7. Administer the article in a steady, fluid motion. As you inject, you can feel the injection article creating a bulbous under the skin between your fingers.
8. A safer method is to inject into the flank [Fig. A.19(b)], between the hind leg and the front leg. This is also the preferred location for injecting tumour cells, as there is room for the tumour to grow safely without putting pressure on vital organs/blood vessels.

A.4.7 Intraperitoneal (IP) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (25–30G), ½″–1″
- Injection article
- Isopropyl alcohol
- Gauze

1. It is recommended that two persons carry out this procedure — one person can restrain the rat, whilst the other injects — unless the operator has sufficient handling skills to restrain the rat comfortably with one hand.
2. Fill the syringe with the appropriate amount of article to be administered.
3. Restrain the rat by using the restraint technique or by scruffing if a single operator is injecting.
4. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Position the animal so that its head is lower than its body to allow any internal organs to move out of the way. Draw an

imaginary line horizontally across the top of the hind legs, dividing the abdomen into four "quadrants".
6. Insert the needle into the lower right quadrant (on the bottom left) of the rat's abdomen at a 30° angle.
7. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
8. If other fluids are seen in the syringe upon aspiration, such as a yellow/clear colour (indicating puncture of the urinary bladder) or a green/brown colour (indicating puncture of the intestines/caecum), discard the needle and syringe and start again.
9. Administer the article in a steady, fluid motion.

A.4.8 Intradermal (ID) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)
- Hypodermic needle (22G–25G)
- Anaesthetic
- Isopropyl alcohol
- Gauze
- Clippers, or #40 scalpel blade and scalpel blade holder

1. Intradermal injection **must** be carried out **under anaesthesia**.
2. It is not a common procedure on rats, but it may be performed if small volumes are injected.
3. Anaesthetise the rat. Once the rat is anaesthetised, proceed.
4. When injecting on the back of the rat, take the scalpel holder and scalpel carefully in one hand and extend the skin between the fingers of the other hand. With the scalpel almost flat against the fur, gently rub the scalpel blade back and forth to remove the hair. This will give a nice, smooth surface and is better than using hair clippers, as it is easier to visualise the skin after injection.

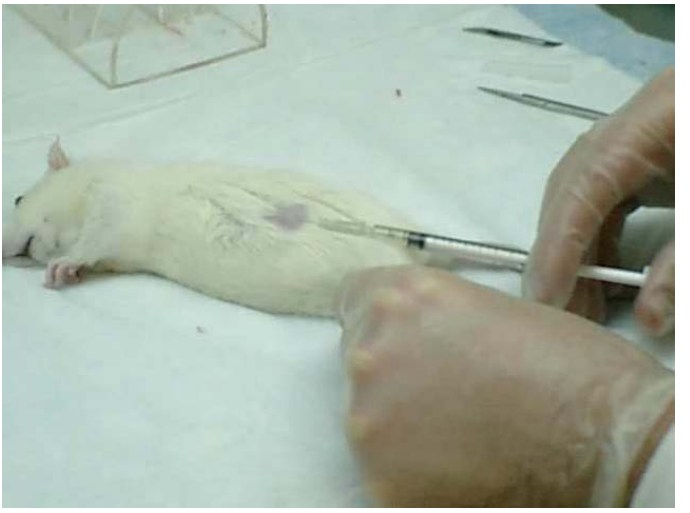


Fig. A.20 Intradermal injection.

5. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
6. Insert the needle carefully through the dermis at a 30° angle (Fig. A.20).
7. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
8. Administer the article slowly with a maximum volume of 100 µL per injection site for intradermal injections on the back of the animal to avoid tissue trauma. Successful injection results in a small, circular skin welt.

A.4.9 Intravenous (IV) injection utilising lateral tail veins

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)

(*Continued*)

(*Continued*)

- Restraint box
- Hypodermic needle (25G–30G)
- Isopropyl alcohol
- Gauze
- Injection article

1. Place the rat into a plastic restraint device or anaesthetise it.
2. Prepare the tail with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
3. Needle placement should be no closer to the body than half the length of the tail. It is good practice to start as close to the tip of the tail as possible, moving closer to the body if the injection is unsuccessful, as it is not possible to insert at a lower location.
4. Ensure that you can visualise the lateral tail veins. This can be assisted with the use of a heated lamp or by placing the animals in a cage warmer or on top of a warming plate for a few minutes prior to injection.
5. With the tail under tension, insert the needle approximately parallel to the vein (Fig. A.21).
6. Ensure proper needle placement by inserting the needle at least 3 mm into the lumen of the vein. Once in the lumen,

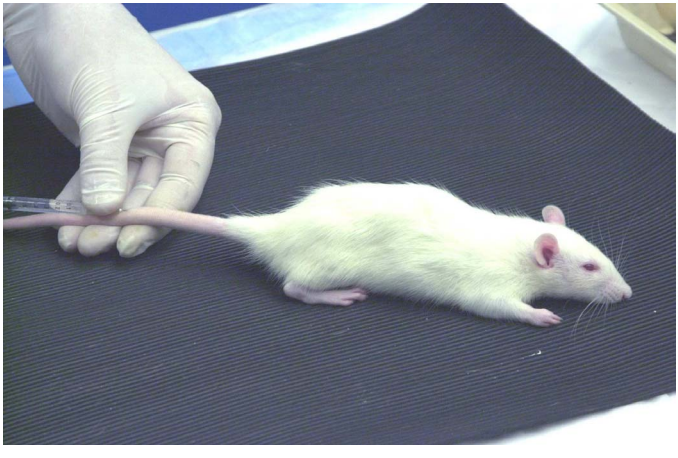


Fig. A.21 Intravenous injection utilising lateral tail veins.

the needle should feel smooth and there should be no resistance upon injection.
7. Administer the article in a slow, fluid motion to avoid rupture of the vessel. You will be able to visualise a clearing of the lumen as the injection article replaces the blood in the vein.
8. If the solution leaks into the surrounding tissues or forms a bleb, remove the needle and insert again slightly higher on the vein (closer to the body).
9. Upon completion, ensure good haemostasis (i.e. that any bleeding has stopped) before returning the animal to its cage.

A.4.10 Gavaging of rat

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Biomedical needles (animal feeding; 2″–3″, 16G–18G)
- Injection article

1. Select the correct-sized gavage needle, ensuring that there is a metal ball on the end to prevent the tip from being sharp. **Never** use a hypodermic needle for oral gavage.
2. Measure the needle length against the mouse's body; the needle should be no longer than from the nose to the last rib (approximate level of the stomach). If the needle is longer, take care to only insert the appropriate length to prevent damaging the stomach. Shorter gavage needles can be used; but if injecting acidic compounds, ensure that the needle fits adequately into the stomach to prevent damage to the oesophagus.
3. It is recommended that two persons carry out this procedure. One person can restrain the rat, whilst the other inserts the gavage needle. Experienced operators may be able to restrain the rat with one hand whilst gavaging with the other, but care must be taken.
4. Fill the syringe with the appropriate amount of article to be dosed.
5. Restrain the rat using the restraint technique or by scruffing (if single operator).

6. Place the tip of the needle into the rat's mouth.
7. Slide the tip down the back of the mouth, moving it toward the front in one fluid motion.
8. Take your time; any resistance felt indicates improper placement, in which case remove the needle and start again. **Do not** force the needle, as it may enter the trachea and damage the epiglottis. The needle should slide down into the oesophagus easily.
9. Once the needle is properly placed, administer the injection article.
10. Remove the needle carefully so as not to damage the oesophagus.
11. If the animal's breathing is laboured, monitor it closely in case the injection article enters the lungs, in which case the animal may need to be euthanised unless it recovers within a few minutes.

A.4.11 Blood withdrawal utilising orbital sinus for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Anaesthetic
- Haematocrit tubes or Pasteur pipettes
- Collection vessel
- Gauze

1. Retro-orbital bleeds **must** be performed **under anaesthesia**.
2. Anaesthetise the rat. After the rat is anaesthetised, proceed.
3. Place the haematocrit tube or Pasteur pipette at the medial canthus of the eye (Fig. A.22).
4. With a rotating motion, apply gentle pressure to insert the tube through the membrane.
5. Continue rotating the tube on the back of its orbit until blood flows.
6. Note that the membrane on rats is harder to pierce through than mice, so additional pressure and rotations are required.
7. Collect the blood in an appropriate vessel.

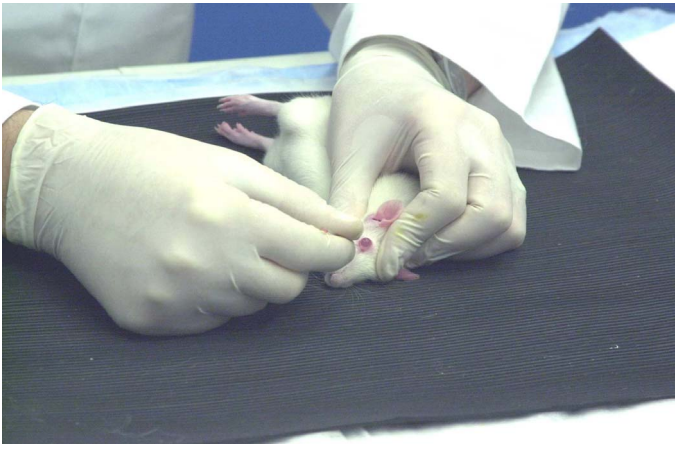


Fig. A.22 Blood withdrawal utilising orbital sinus for large-volume blood collection.

8. Upon completion, ensure good haemostasis before returning the animal to the cage by closing the eyelids and placing the gauze pad over the eye until bleeding stops (usually for a few seconds).
9. A pump can be attached to the haematocrit tube to expel blood into a collection tube after completion.

A.4.12 Blood withdrawal utilising lateral tail veins for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Restraint box or anaesthesia
- Hypodermic needle (25G–30G)
- Isopropyl alcohol
- Gauze

1. Please note that it is not acceptable to remove part of the tail in order to collect blood only, unless the tissue sample taken is very small (3–5 mm in length) and is required for genotyping.

2. Restrain the rat using a plastic restraint device or anaesthetise it.
3. Prepare the tail with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Needle placement should be no closer to the body than half the length of the tail.
5. Ensure that you can visualise the lateral tail veins. This can be assisted with the use of a heated lamp or by placing the animals in a cage warmer or on top of a warming plate for a few minutes prior to injection. The lateral tail vein runs along either side of the tail and can be visualised easily in albino rats. In nonalbino strains, it is more important to warm the tail or palpate the vein to find the correct location.
6. With the tail under tension, insert the needle approximately parallel to the vein.
7. Ensure proper needle placement by inserting the needle at least 3 mm into the lumen of the vein.
8. Once blood starts to flow into the hub of the needle, place the haematocrit tube into the needle hub or remove the needle to allow the blood to collect directly into a suitable collection tube (Fig. A.23).

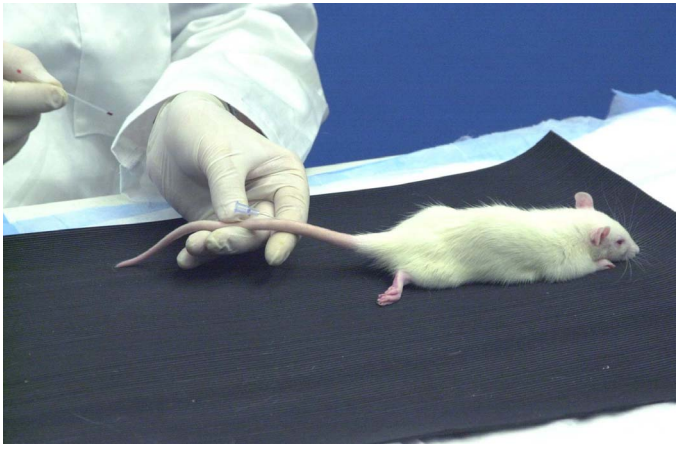


Fig. A.23 Blood withdrawal utilising lateral tail veins for small-volume blood collection.

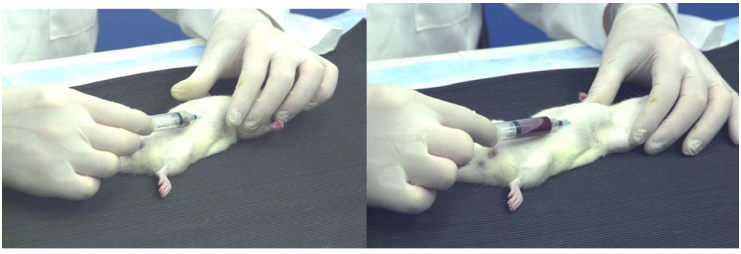
9. Blood collection can be assisted by "milking" the vein, by gentle rubbing to stimulate blood flow.
10. Upon completion, ensure good haemostasis before returning the animal to the cage by placing the gauze pad over the blood collection site and applying pressure until bleeding stops (usually for a few seconds).

A.4.13 Intracardiac (IC) puncture for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (21G–25G)
- Isopropyl alcohol
- Gauze
- Anaesthesia/CO_2

1. Intracardiac puncture **must** be performed **under anaesthesia** or shortly after euthanasia.
2. Anaesthetise the rat. After the rat is anaesthetised, proceed.
3. Prepare the blood collection site with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Make sure that you are aware of the location of the heart. If you are not able to locate it, you can place a finger over the chest and feel for the rat's heartbeat.
5. Insert the needle at the base of the sternum at a 20°–30° angle just lateral of the midline (rat's left side) and push the needle up into the position of the heart [Fig. A.24(a)].
6. Aspirate the syringe slowly, allowing the blood to collect into the syringe before continuing to aspirate. If the blood flow stops or slows down, rotate the needle and syringe or adjust slightly, as the blood may have clotted (especially in euthanised rats) [Fig. A.24(b)].
7. Do not probe around the chest with the needle as it is very sharp and may cut or damage other tissues, causing internal bleeding.



(a) (b)

Fig. A.24 Intracardiac puncture for large-volume blood collection.

8. Once the required amount of blood has been collected, the rat should be euthanised by an appropriate method.
9. Exsanguination (removal of all circulating blood) will in itself cause death if the animal is under anaesthesia at the time of collection, but it is always important to ensure that death has occurred either by monitoring the vital signs or by performing an additional method of euthanasia on the animal as a precaution.

A.5 Guinea Pigs (*Cavia porcellus*)

Guinea pigs have a mild disposition and are generally easy to handle. Care must be taken when approaching guinea pigs, as they are nervous animals and are easily startled. Approach them slowly and gently, and try not to make sudden movements or loud noises.

A.5.1 Physiologic parameters

Body temperature = 37.2°C–39.5°C
Heart rate = 230–380/min
Respiratory rate = 42–104/min
Tidal volume = 2.3–5.3 mL/kg

Guinea pigs have a large caecum that can act as a reservoir for anaesthetics. Depending on the drug solubility, the caecum can alter the pharmacologic effect.

Induction of anaesthesia using volatile anaesthetics (e.g. halothane and isoflurane) should be used with caution due to initial breath holding when animals are first exposed to the gas vapours. Repeated exposure to halothane can cause hepatotoxicity. Isoflurane is a safer inhalant anaesthetic to use.

Self-mutilation has been reported in guinea pigs after ketamine administration.

Table A.9 shows suggested agents and doses for anaesthesia and analgesia.

Table A.9 Anaesthesia and analgesia (suggested agents and doses).

Agent	Dosage and Route of Administration	
Restraint/Preanaesthesia		
Atropine	0.05 mg/kg	SC
Diazepam (Valium®)	2.5–5.0 mg/kg	IP/IM
Acetylpromazine	5–10 mg/kg	IM/SC/IV
Ketamine (Ketaset®, Vetalar®)	22–30 mg/kg	IM
Anaesthesia		
Sodium pentobarbital	15–40 mg/kg	IP
Sodium thiopental	20 mg/kg	IV
Ketamine	40–50 mg/kg	IM
Ketamine/Xylazine[a]:		
Xylazine +	5–13 mg/kg	SC
Ketamine	44 mg/kg	SC
Halothane (Fluothane®)	—	Inhalant
Isoflurane	—	Inhalant

(*Continued*)

Table A.9 (*Continued*)

Analgesia		
Buprenorphine	0.05 mg/kg/8–12 h	SC
Morphine	10 mg/kg/2–4 h	SC/IM
Aspirin	86 mg/kg	Oral dosing
Butorphanol tartrate (Torbugesic®)	0.25–0.4 mg/kg	IV/SC
Reversal Agent		
Atipemazole (Antisedan®)	1 mg/kg	IM/IV/SC/IP

[a] Xylazine is available in **two strengths** (20 mg/mL and 100 mg/mL). Ensure that the correct dose is calculated based on the strength being used.

A.5.2 Guinea pig handling and sexing

1. First, assess the guinea pigs in their cage/pen for normal behaviour.
2. With firm but gentle pressure, grasp the guinea pig around the thorax, placing its front leg between your index and middle finger for added support (Fig. A.25).
3. Check the sex of the guinea pigs by applying gentle pressure above the genitalia. The penis of the male will protrude, making sexing easier. Ensure that the cage card information is correct (Fig. A.25).

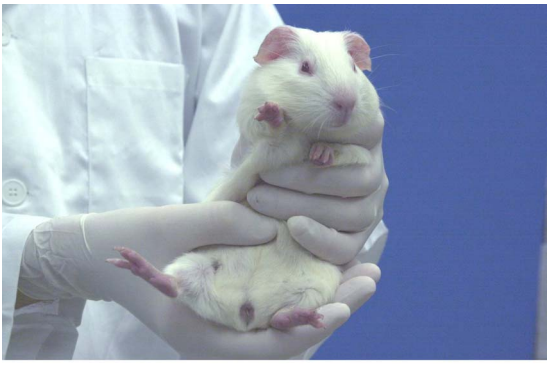
A.5.3 Guinea pig restraint technique for technical manipulation

Guinea pigs are quite docile animals. Adequate restraint is usually achieved by placing the animal on a table top and supporting it with one hand at the head and the other hand around the rump. It may be necessary for an assistant to help hold the guinea pig in place whilst the other person performs the procedures. Alternatively, the guinea pig can be anaesthetised.

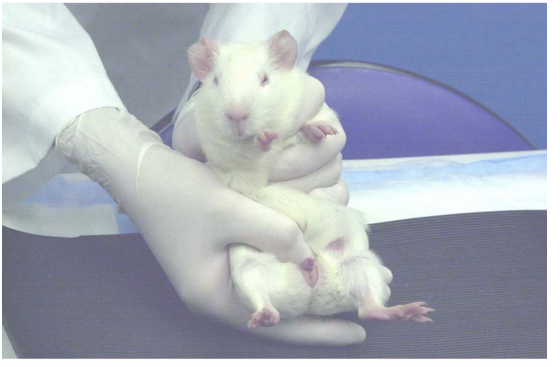
A.5.4 Ear punching for identification

Materials required:

- Personal protective equipment (PPE)
- Ear punch



(a)



(b)

Fig. A.25 Guinea pig handling and sexing. (a) Female guinea pig; (b) male guinea pig.

1. Restrain the guinea pig (refer to the restraint technique).
2. Place the ear punch in the desired location.
3. Firmly and quickly punch the ear to avoid an incomplete cut.
4. Occasionally, the piece of tissue removed will be attached to the ear. This can usually be removed with the help of a pair of forceps.
5. Ear punches are available in various sizes. For guinea pigs, a 1.5-mm-diameter ear punch is generally suitable.
6. Monitor the animals frequently and inspect those with ear punches, as these can sometimes tear or heal over (if the original hole is too small) and may need to be repeated.
7. There are several different ear punch numbering systems available. Any of these are suitable, but it is important to

ensure that they are in conformation with the system being used in your facility. If your facility does not have a standard system for ear punch numbering, make a note on the cage card of the system you are using for future reference.

A.5.5 Intramuscular (IM) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)
- Hypodermic needle (22G–30G)
- Injection article
- Isopropyl alcohol
- Gauze

1. Intramuscular injections may be performed with the help of an assistant.
2. Withdraw the appropriate amount of solution to be administered.
3. Restrain the guinea pig (refer to the restraint technique) or anaesthetise it.
4. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Insert the needle into the caudal thigh, quadriceps, or lumbar (back) muscles.
6. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
7. Administer the article in a steady, fluid motion. **Do not** administer rapidly, as this may cause tissue trauma.

A.5.6 Subcutaneous (SC) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–2 mL)

(*Continued*)

(*Continued*)

- Hypodermic needle (22G–30G)
- Injection article
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the appropriate amount of article to be administered.
2. Restrain the guinea pig (refer to the restraint technique).
3. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Insert the needle at the base of the skin fold between your thumb and forefinger, keeping the needle straight because if there is an angle to the needle, it may pierce the muscle or go through the skin and into your finger.
5. Aspirate the syringe to ensure proper placement. Any sign of blood indicates improper placement, in which case the needle needs to be repositioned. As you inject, you can feel the injection article creating a bulbous under the skin between your fingers.
6. Administer the article in a steady, fluid motion.

A.5.7 Intraperitoneal (IP) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (25G–30G)
- Injection article
- Isopropyl alcohol
- Gauze

1. It is recommended that two persons carry out this procedure. One person can restrain the guinea pig, whilst the other injects.
2. Fill the syringe with the appropriate amount of article to be administered.
3. Restrain the guinea pig.

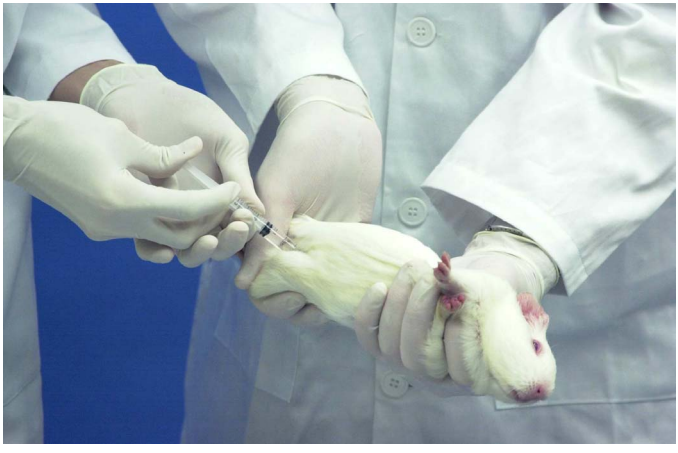


Fig. A.26 Intraperitoneal injection.

4. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Position the animal so that its head is lower than its body to allow any internal organs to move out of the way. Draw an imaginary line horizontally across the top of the hind legs, dividing the abdomen into four "quadrants".
6. Insert the needle into the lower left/right quadrant of the abdomen at a 30° angle (Fig. A.26).
7. Aspirate the syringe to ensure proper placement (Fig. A.26). Any sign of blood indicates improper placement, in which case the needle needs to be repositioned.
8. If other fluids are seen in the syringe upon aspiration, such as a yellow/clear colour (indicating puncture of the urinary bladder) or a green/brown colour (indicating puncture of the intestines/caecum), discard the needle and syringe and start again.
9. Administer the article in a steady, fluid motion.

A.5.8 Intradermal (ID) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)

(*Continued*)

(*Continued*)

- Hypodermic needle (22G–25G)
- Anaesthetic
- Isopropyl alcohol
- Gauze
- Clippers, or #40 blade and scalpel blade holder

1. Intradermal injection **must** be done **under anaesthesia**.
2. Anaesthetise the guinea pig. After the guinea pig is anaesthetised, proceed.
3. When injecting on the back of the guinea pig, take the scalpel holder and scalpel carefully in one hand and extend the skin between the fingers of the other hand. With the scalpel almost flat against the fur, gently rub the scalpel blade back and forth to remove the hair. This will give a nice, smooth surface and is better than using hair clippers, as it is easier to visualise the skin after injection.
4. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Insert the needle carefully through the dermis at a 30° angle (Fig. A.27).

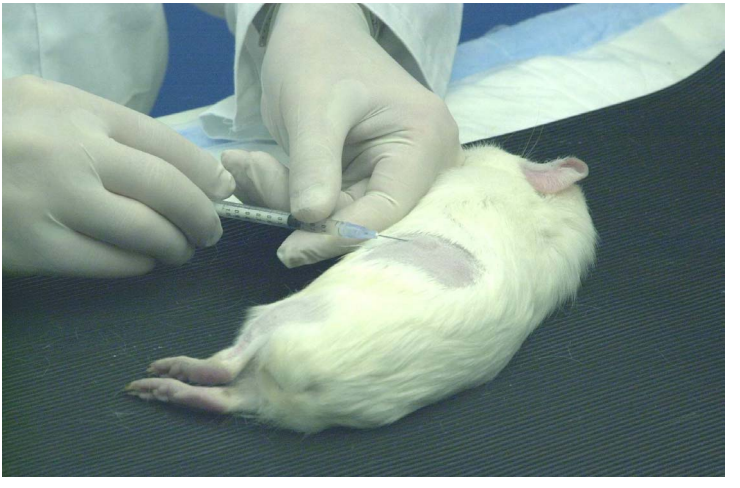


Fig. A.27 Intradermal injection.

6. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
7. Administer the article slowly with a maximum volume of 100 μL per injection site to avoid tissue trauma. Successful injection results in a small, circular skin welt.

A.5.9 Intravenous (IV) injection utilising saphenous or cephalic veins

Materials required:

- Personal protective equipment (PPE)
- Syringe (1 mL)
- Restraint box
- Hypodermic needle (25G–30G)
- Isopropyl alcohol
- Gauze
- Injection article
- Clippers, or #40 blade and scalpel blade holder

1. Intravenous injections to guinea pigs are difficult, as the guinea pig does not have a tail and has very small ear veins.
2. The saphenous veins (on the hind leg, just above the heel) or cephalic veins (on the foreleg) need to be used.
3. It is recommended to anaesthetise the guinea pig before starting.
4. Remove the hair around the vein using hair clippers or a scalpel blade.
5. Prepare the vein with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
6. A tourniquet may be applied to the vein to assist with dilation, or an assistant can apply pressure to the vein.
7. Insert the needle into the skin approximately parallel to the vein.
8. Release the pressure/tourniquet.
9. Ensure proper placement by inserting the needle at least 3 mm into the lumen of the vein.

10. Administer the article in a steady, fluid motion to avoid rupture of the vessel.
11. Upon completion, ensure good haemostasis (i.e. that any bleeding has stopped) before returning the animal to its cage.

Note: Using a heated lamp may enhance the person's ability to view the vein.

A.5.10 Gavaging of guinea pig

Gavaging of guinea pigs is not recommended, as they keep food in their mouths that can easily be forced into the trachea by mistake. If gavaging is essential, use a cotton bud to remove food stored in cheek pouches before proceeding. The gavaging technique is the same as for other rodent species (mice and rats).

A.5.11 Blood withdrawal utilising marginal ear veins for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Hypodermic needle (25G–30G)
- Isopropyl alcohol
- Gauze

1. Restrain the guinea pig or anaesthetise it.
2. Prepare the vein with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
3. Ensure proper needle placement by inserting the needle at least 3 mm into the lumen of the vein.
4. Once blood starts to flow into the hub of the needle, place the haematocrit tube into the needle hub or remove the needle to allow the blood to collect directly into a suitable collection tube.
5. Blood collection can be assisted by "milking" the vein, by gentle rubbing to stimulate blood flow.

6. Upon completion, ensure good haemostasis before returning the animal to the cage by placing the gauze pad over the blood collection site and applying pressure until bleeding stops (usually for a few seconds).
7. To increase blood flow, use a heated lamp. Care must be taken that the animal does not get too hot.

A.5.12 Intracardiac (IC) puncture for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (21G–25G)
- Isopropyl alcohol
- Gauze
- Anaesthetic/CO_2

1. Intracardiac puncture **must** be performed **under anaesthesia**.
2. Anaesthetise the guinea pig. After the guinea pig is anaesthetised, proceed.
3. Prepare the blood collection site with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Make sure that you are aware of the location of the heart. If you are not able to locate it, place a finger over the chest and feel for the guinea pig's heartbeat.
5. Insert the needle at the base of the sternum at a 20°–30° angle just lateral of the midline (guinea pig's left side) and push the needle up into the position of the heart.
6. Aspirate the syringe slowly, allowing the blood to collect into the syringe before continuing to aspirate. If the blood flow stops or slows down, rotate the needle and syringe or adjust slightly, as the blood may have clotted (especially in euthanised guinea pigs).
7. Do not probe around the chest with the needle as it is very sharp and may cut or damage other tissues, causing internal bleeding.

8. Once the required amount of blood has been collected, the guinea pig should be euthanised by an appropriate method.
9. Exsanguination (removal of all circulating blood) will in itself cause death if the animal is under anaesthesia at the time of collection, but it is always important to ensure that death has occurred either by monitoring the vital signs or by performing an additional method of euthanasia on the animal as a precaution.

A.6 Rabbits (*Oryctolagus cuniculus*)

A.6.1 Physiologic parameters

Body temperature = 38°C–39.6°C
Heart rate = 130–325/min
Respiratory rate = 32–60/min
Tidal volume = 4–6 mL/kg

Many rabbits have serum atropinesterase, which causes reduced response to atropine. Glycopyrrolate, another anticholinergic, can be used instead of atropine. Rabbits have a large caecum that can act as a reservoir for anaesthetics. Depending on the drug solubility, the caecum can alter the pharmacologic effect. Induction of anaesthesia using volatile anaesthetics (e.g. halothane and isoflurane) should be done with caution due to initial breath holding when animals are first exposed to irritating gas vapours.

Self-mutilation has been reported in rabbits after IM ketamine administration. Dilution of ketamine with saline will limit this side-effect.

Table A.10 shows the suggested agents and doses for anaesthesia and analgesia.

Table A.10 Anaesthesia and analgesia (suggested agents and doses).

Agent	Dosage and Route of Administration	
Restraint/Preanaesthesia		
Ketamine (Ketaset®, Vetalar®)	15–50 mg/kg	IM
Acetylpromazine	1.0–10 mg/kg	IM/SC/IV
Ketamine/Acetylpromazine (10:1)	15–50 mg/kg	IM
Diazepam (Valium®)	5–10 mg/kg	IV/IM
Glycopyrrolate	0.005–0.011 mg/kg	IM
Butorphanol/Acetylpromazine:		
Butorphanol tartrate (Torbugesic®)	1 mg/kg	SC
Acetylpromazine	1 mg/kg	SC
Anesthesia		
Sodium pentobarbital (3% solution given slowly to effect)	15–40 mg/kg	IV
Ketamine/Xylazine+/ Acepromazine:		
Xylazine[a]	5–10 mg/kg	IM
Ketamine	35–50 mg/kg	IM
Acepromazine	0.75 mg/kg	IM
Ketamine/Midazolam:		
Ketamine	25 mg/kg	IM
Midazolam	1 mg/kg	IM
Ketamine/Diazepam:		
Ketamine	15–50 mg/kg	IM
Diazepam	5–10 mg/kg	IM
Ketamine/Acepromazine/ Butorphanol:		
Ketamine	35 mg/kg	SC
Acepromazine	0.75 mg/kg	SC
Butorphanol tartrate (Torbugesic®)	0.1 mg/kg	SC
Halothane (Fluothane®)	—	Inhalant
Isoflurane	—	Inhalant
Analgesia		
Morphine	5 mg/kg/2–4 h	SC/IM
Acetylsalicytic acid (Aspirin)	500 mg/kg	Oral dosing

(*Continued*)

Table A.10 (*Continued*)

Agent	Dosage and Route of Administration	
Buprenorphine	0.02-0.1 mg/kg/8–12 h	SC
Butorphanol tartrate	0.1–1.5 mg/kg/4 h	IV
(Torbugesic®)	1.0–7.5 mg/kg/4 h	IM/SC
Flunixin meglumine (Banamine®)	1.1 mg/kg/12 h	IM/SC
Carprofen	1.5 mg/kg/12 h	Oral dosing
Ketoprofen	3 mg/kg/12 h	IM
Reversal Agent		
Yohimbine (reversal agent for xylazine or medetomidine)	0.2 mg/kg	IV

[a] Xylazine is available in **two strengths** (20 mg/mL and 100 mg/mL). Ensure that the correct dose is calculated based on the strength being used.

A.6.2 Rabbit handling and sexing

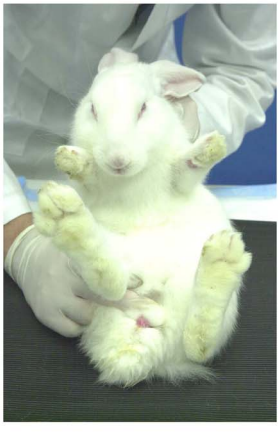
1. Always check the condition of the rabbit before removing it from the cage [Fig. A.28(a)].
2. Grasp the rabbit firmly by the nape of its neck. Place one hand on the rump of the rabbit and lift it from the cage.
3. Support its hind legs with the opposite hand. Tuck its head between its arm and body.
4. Check the sex of the rabbit by applying gentle pressure above the genitalia. The penis of the male will protrude, making sexing easier. Ensure that the sex of the rabbit matches what is written on the cage card [Fig. A.28(b)].

A.6.3 Rabbit restraint technique for technical manipulation

Materials required:

- Personal protective equipment (PPE)
- Restraint box/towel (Fig. A.29)

(a)



(b)

Fig. A.28 Rabbit handling and sexing. (a) Male rabbit; (b) female rabbit.

1. Grasp the rabbit firmly by the nape of its neck. Place one hand on the rump of the rabbit and lift it from the cage.
2. For manual restraint, an assistant can hold the nape of the rabbit's neck and place a gentle but firm hand on the back of the rabbit. Whilst in this normal seated position, the rabbit may be shaved for injections or given IM injections with relative ease.
3. If you are working alone, you should use a plastic restrainer, cat bag, or towel wrap (Fig. A.29), as it is safer for both you and the rabbit.

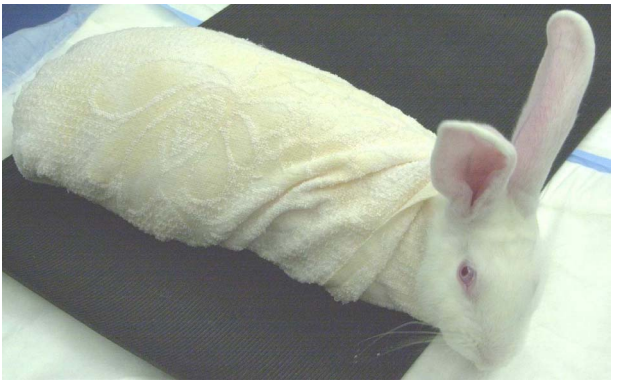


Fig. A.29 Restraint towel for technical manipulation.

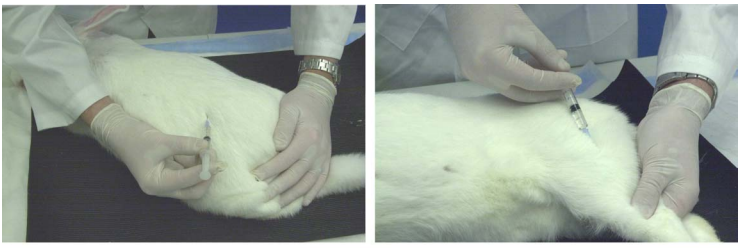
4. Take care when using plastic restrainers as rabbits can get quite stressed when they are placed inside and will occasionally panic, which may result in spinal injuries.
5. Cat bags are commercially available zipper bags, made of a durable material, that prevents the claws of the rabbit (or cat) from scratching through. The head and ears of the rabbit can protrude through the opening, and the rest of the animal is secure within the bag. Please note that these bags can only be used for short-term restraint, as the rabbit will overheat if restrained for prolonged periods.
6. The towel wrap is by far the easiest technique and does not require any special equipment, just a regular towel or drape.
7. Open the towel onto a nonslip surface. Place the rabbit in the centre of the towel and fold one side of the towel over, ensuring that the rabbit's head and ears are not covered, but that the feet and body are covered. The other side of the towel can then be folded in and under, and the back folded under as well, so that the rabbit's weight is on top of the towel, preventing it from wriggling free. The rabbit can comfortably stay in this restraint long enough to be given an injection, or to have its blood drawn or teeth trimmed.

A.6.4 Intramuscular (IM) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (22G–30G)
- Injection article
- Isopropyl alcohol
- Gauze

1. Withdraw the appropriate amount of solution to be administered.
2. Restrain the rabbit (refer to the restraint technique).
3. Prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Insert the needle into the lumbar muscles [Fig. A.30(a)], caudal thigh muscles [Fig. A.30(b)], or quadriceps muscles.
5. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
6. Administer the article in a steady, fluid motion. **Do not** administer rapidly, as this may cause tissue trauma.
7. Caution must be taken to avoid the spine when injecting into the lumbar muscles, and to avoid the sciatic (ischiatic) nerve when injecting the leg.
8. The rabbit may flinch when you are injecting into its lumbar muscles, so it may be necessary to hold the rabbit close to your body to provide added support.



(a) (b)

Fig. A.30 Intramuscular injection.

A.6.5 Subcutaneous (SC) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (22G–25G)
- Injection article
- Isopropyl alcohol
- Gauze
- Clipper

1. Fill the syringe with the appropriate amount of article to be administered.
2. Restrain the rabbit (refer to the restraint technique). Place the animal on a firm surface.
3. Shave the fur with hair clippers, and then prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
4. Insert the needle at the base of the skin fold between your thumb and forefinger (Fig. A.31), keeping the needle straight because if there is an angle to the needle, it may pierce the muscle or go through the skin and into your finger.
5. Aspirate the syringe to ensure proper placement. Any sign of blood indicates improper placement, in which case the

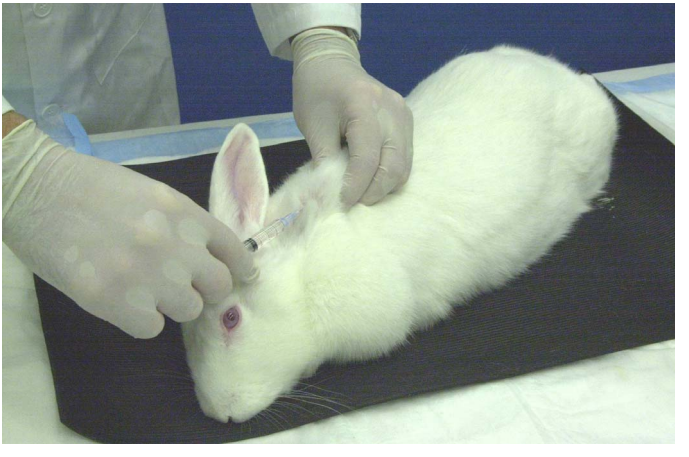


Fig. A.31 Subcutaneous injection.

needle needs to be repositioned. As you inject, you can feel the injection article creating a bulbous under the skin between your fingers.
6. Administer the article in a steady, fluid motion.

A.6.6 Intraperitoneal (IP) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (18G–23G)
- Injection article
- Isopropyl alcohol
- Gauze
- Clippers

1. Fill the syringe with the appropriate amount of article to be administered.
2. Restrain the rabbit (refer to the restraint technique). Place the animal in a ventral recumbent position.
3. It is best to work in pairs, so that one person can restrain the rabbit whilst the other injects.
4. Shave the fur with hair clippers, and then prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Insert the needle into the lower left/right quadrant of the abdomen at a 30° angle.
6. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
7. Administer the article in a steady, fluid motion.

A.6.7 Intradermal (ID) injection

Materials required:

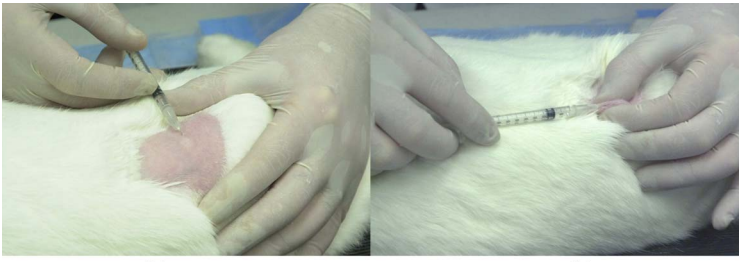
- Personal protective equipment (PPE)
- Syringe (1–3 mL)

(*Continued*)

(*Continued*)

- Hypodermic needle (25G–30G)
- Injection article
- Isopropyl alcohol
- Gauze
- Clippers
- #40 blade
- Scalpel blade holder

1. Fill the syringe with the appropriate amount of article to be administered.
2. Restrain the rabbit (refer to the restraint technique). Place the animal in a ventral recumbent position (two-person technique) or anaesthetise it.
3. Shave the fur on the back with hair clippers, and then prepare the area with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections). The scalpel blade can be used to get a closer shave, after removing most of the hair from the site.
4. Insert the needle between the layers of skin on the back at a 20° angle [Fig. A.32(a)] by stretching or pinching the skin and then injecting into it [Fig. A.32(b)].
5. Aspirate the syringe to ensure proper placement. Any sign of blood in the syringe indicates improper placement, in which case the needle needs to be repositioned.
6. Administer the article slowly with a maximum volume of 250 μL per injection site to avoid tissue trauma. Successful injection results in a small, circular skin welt.



(a) (b)

Fig. A.32 Intradermal injection.

A.6.8 Intravenous (IV) injection utilising marginal ear vein

Materials required:

- Personal protective equipment (PPE)
- Syringe (1–3 mL)
- Hypodermic needle (22G–30G)
- Injection article
- Isopropyl alcohol
- Gauze
- Restraint device
- #40 blade
- Scalpel blade holder

1. Fill the syringe with the appropriate amount of article to be administered.
2. Restrain the rabbit (refer to the restraint technique).
3. Take the scalpel holder and the scalpel carefully in one hand and extend the rabbit's skin between the fingers of the other hand. With the scalpel almost flat against the fur, gently rub the scalpel blade back and forth to remove the hair.
4. Prepare the ear with an alcohol swab to disinfect the skin (this should be routinely done before all injections/blood collections).
5. Insert the needle into the marginal ear vein at a 20° angle (Fig. A.33).
6. Aspirate the syringe to ensure proper placement.
7. As soon as blood appears in the hub of the syringe, administer the article in a slow, fluid motion.
8. Upon completion, ensure good haemostasis before returning the animal to its cage.

A.6.9 Gavaging of rabbit

Materials required:

- Personal protective equipment (PPE)
- Restraint box

(*Continued*)

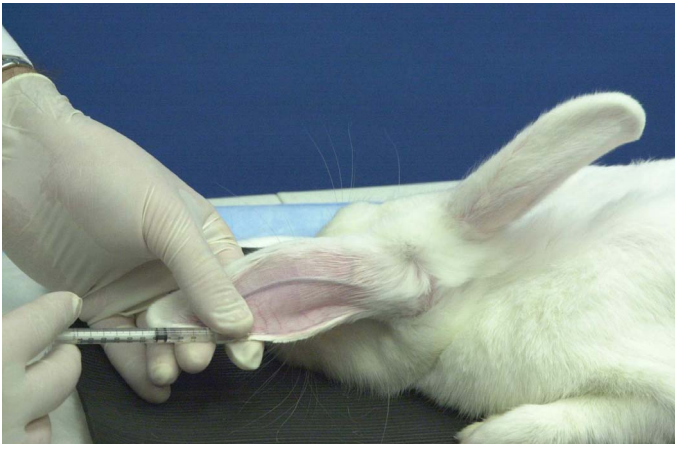


Fig. A.33 Intravenous injection utilising marginal ear vein.

(*Continued*)

- Feeding tube (8–16 French)
- Syringes (3–10 mL)
- Injection article

1. Fill the syringe with the appropriate amount of article to be dosed.
2. Restrain the rabbit (refer to the restraint technique).
3. Secure the animal's mouth in an open position by placing your thumb and forefinger behind its incisors, or by using a plastic tube of the correct size.
4. Measure and mark on the tubing the amount needed to reach the rabbit's stomach.
5. Insert the tubing until resistance is released by swallowing reflex. At this time, introduce the tubing into the oesophagus. Ensure correct placement, i.e. you are not in the trachea, by inserting the tip of the tube into water and watching for bubbles.
6. Insert the remaining length of the tubing required to reach the stomach.
7. Once the tubing is properly placed, administer the article.
8. Flush the tubing with water afterwards to ensure that all of the article to be dosed has left the tube.
9. Clamp off the tubing and remove it from the rabbit's mouth, ensuring no article is inhaled.

A.6.10 Blood withdrawal utilising auricular (central ear) artery and marginal ear vein for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Restraint box
- Syringe (5–60 mL)
- Hypodermic needle or paediatric scalp vein needle (butterfly; 21G–22G)
- Isopropyl alcohol
- Gauze

1. Restrain the rabbit (refer to the restraint technique).
2. Prepare the area with an alcohol swab. Raise the artery (located in the centre of the external ear) by rubbing across the external ear with gentle, quick motions.
3. With the ear under tension, insert the needle approximately parallel to the central artery.
4. Needle placement should be no closer to the base of the ear than the midpoint.
5. Ensure proper placement by inserting the needle at least 10 mm into the lumen of the artery.
6. Aspirate the syringe slowly to avoid artery constriction.
7. Let the blood flow freely into your collection tube.
8. Upon completion, ensure good haemostasis before returning the animal to its cage.
9. Alternatively, small quantities of blood can be withdrawn from the vein by inserting a 27G or 25G needle without a syringe and collecting the blood from the hub of the needle (Fig. A.34).
10. When collecting blood from the artery, ensure that the blood flow has stopped before returning the animal to its cage. This can be achieved by applying pressure to the gauze covering the artery for a few minutes. Take care when using additional items, such as paper clips and clamps, as these may damage the ear.

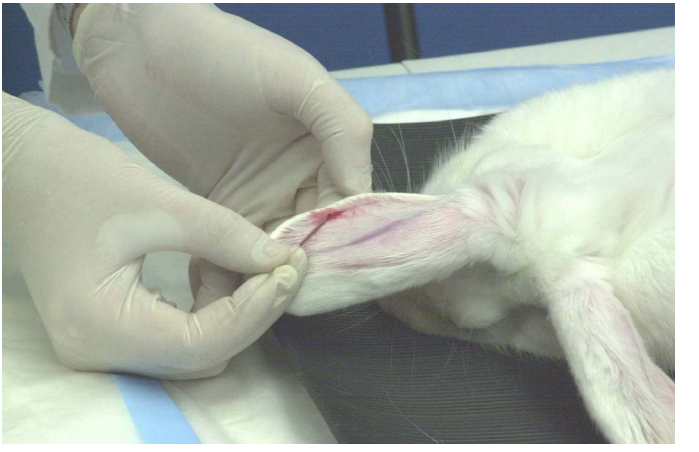


Fig. A.34 Blood withdrawal utilising auricular (central ear) artery and marginal ear vein for large-volume blood collection.

11. Additional blood flow can be assisted with the use of a heated lamp prior to collection.
12. Small volumes of blood can be collected from the marginal ear vein on the exterior of the ear positioned furthest from the head.
13. It is difficult to withdraw blood from the marginal ear vein using a needle and syringe, as the blood pressure is very low. Hence, blood collection directly from the needle hub into the haematocrit tube using just a needle is the preferred method.

A.6.11 Intracardiac (IC) puncture for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe (5–60 mL) or evacuated container
- Hypodermic needle (20G–25G)
- Anaesthetic

(*Continued*)

(*Continued*)

- Isopropyl alcohol
- Gauze

1. Intracardiac puncture **must** be performed **under anaesthesia**.
2. Anaesthetise the rabbit by intramuscular injection (refer to the intramuscular injection technique). Restrain the rabbit in dorsal recumbency.
3. Prepare the injection site with an alcohol swab to disinfect the skin (this should be routinely done before all injections/ blood collections).
4. After the rabbit is anaesthetised, insert the needle at the base of the sternum at a 30°–45° angle just lateral of the midline (rabbit's left side).
5. Alternatively, place the rabbit on its right side, check for a heartbeat to locate the heart, and enter between the third and fourth ribs at the point of the elbow (Fig. A.35).
6. Aspirate the syringe slowly until blood flows.
7. If no blood flows, remove the needle and start again.
8. Care must be taken not to "probe around" inside the chest.
9. It is strongly recommended that this procedure be followed by euthanasia.

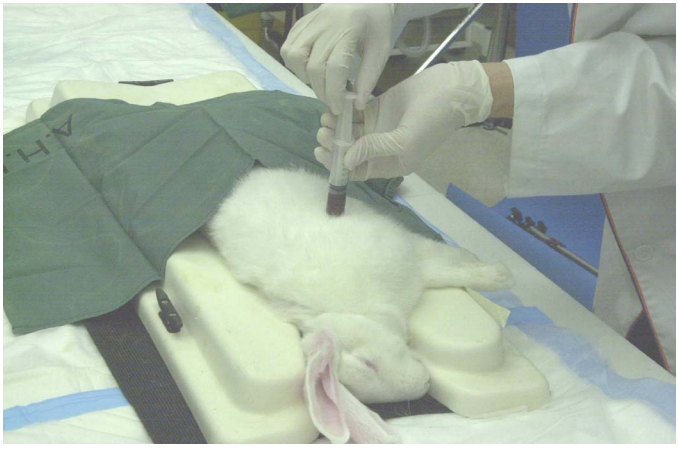


Fig. A.35 Intracardiac puncture for large-volume blood collection.

A.7 Dogs (*Canis familiaris*)

Dogs should be purchased from licensed laboratory breeders and dealers who provide health records, vaccination for major diseases, deworming, and a minimum 1-month conditioning period. Upon arrival, a physical examination should be performed on each dog and a faecal sample checked for endoparasites; if found positive, the animal will need to be treated.

A dog should always be carried with proper support. Physical restraint (in lateral recumbency or in a sitting position) is used when performing nonpainful procedures such as blood collection and injection. Anaesthesia is generally used for all other procedures. You can restrain a dog for examination by placing an arm around the dog's chest; you then use the other arm to restrain the dog's head or leg, depending on the procedure being performed.

Leashes should be used to handle dogs whenever possible. Aggressive or intractable dogs may need to be muzzled. Always bear in mind that proper restraint is necessary to prevent movement that may result in accidental injury to the dog or handler.

Dogs are social animals, and as such require frequent positive human interaction or interaction with other dogs. You may need to spend extra time with a shy or fearful dog in order to make it feel more comfortable. Moving slowly and speaking gently to it will help to prevent it from being alarmed.

Commonly used blood collection sites in dogs are cephalic, saphenous, femoral, and jugular veins. Cardiac puncture for blood collection must be performed as a terminal procedure, and

the animal must be under general anaesthesia. Regardless of the method of collection used, ensure that complete haemostasis has been achieved (using gauze and direct pressure) prior to returning the animal to its cage.

It is recommended that at least two persons carry out a procedure — one to restrain the dog, and the other to perform the injection or blood withdrawal.

A.7.1 Physiologic parameters

Body temperature = 39°C
Heart rate = 100–130/min
Respiratory rate = 22/min
Tidal volume = 250 mL

Ketamine should not be used alone in dogs, as it may cause seizures in some cases. Ketamine should be used in combination with a tranquiliser.

Nonsteroidal anti-inflammatory drugs (NSAIDs) should be used with caution in dogs. Acetaminophen and ibuprofen are contraindicating. Aspirin must be dosed very carefully.

Combinations of narcotics and nonsteroidal agents are commonly used (see Tables A.11 and A.12).

Table A.11 Volume for injection.

Dog	IV	IP	SC	IM
Volume (mL) (slowly)	10–15	200–500	100–200	2–5

Table A.12 Anaesthesia and analgesia (suggested agents and doses).

Agent	Dosage and Route of Administration	
Restraint/Premedication		
Atropine	0.02–0.05 mg/kg	IM/SC/IV
Glycopyrrolate	0.01–0.02 mg/kg	IM/SC

(*Continued*)

Table A.12 (*Continued*)

Agent	Dosage and Route of Administration	
Acetylpromazine	0.055–0.11 mg/kg	IM/SC/IV
	0.55–2.2 mg/kg	Oral dosing
Diazepam (Valium®)	1–5 mg/kg	IM
	0.2–0.6 mg/kg	IV
Medetomidine	0.1–0.8 mg/kg	IM/SC/IV
Xylazine[a,b]	1.0–2.0 mg/kg	IM/SC
Anaesthesia		
Sodium pentobarbital	30 mg/kg	IV
Thiopental sodium	10–35 mg/kg	IV
Ketamine/Xylazine[a,b]:		
Ketamine	5–10 mg/kg	IM
Xylazine[a]	1–2 mg/kg	IM
Ketamine/Diazepam (2:1)[c]:		
Ketamine	5.5 mg/kg	IV
Diazepam	0.33 mg/kg	IV
Ketamine/Medetomidine[b]:		
Ketamine	2.5–7.5 mg/kg	IM
Medetomidine (Domitor®)	0.04 mg/kg	IM
Ketamine/Midazolam[c]:		
Ketamine	5–10 mg/kg	IV
Midazolam	0.28–0.5 mg/kg	IV
Propofol[c]	5.0–7.5 mg/kg	IV
Halothane (Fluothane®)	—	Inhalant
Isoflurane	—	Inhalant
Halothane/Nitrous oxide (50% O_2 + 50% N_2O)	—	Inhalant
Analgesia		
Morphine	0.5–5 mg/kg/2–4 h	SC/IM
Acetylsalicytic acid (Aspirin)	2.5 mg/kg/8 h	Oral dosing
Flunixin meglumine (Banamine®)	0.5–2.2 mg/kg daily	IM/IV
Butorphanol tartrate (Torbugesic®)	0.055–0.11 mg/kg/6–12 h	SC
	0.55 mg/kg/6–12 h	Oral dosing
Buprenorphine	0.01–0.02 mg/kg/12 h	SC/IM
Carprofen (Rimadyl®)	4 mg/kg/24 h	SC/IV
	1–2 mg/kg/12 h	Oral dosing
Ketoprofen	1–2 mg/kg/24 h	SC/IM/IV/Oral dosing

(*Continued*)

Table A.12 (*Continued*)

Agent	Dosage and Route of Administration	
Reversal Agents		
Yohimbine (reverses xylazine)	0.1 mg/kg	IV
Atipamezole (Antisedan®)	0.05 mg/kg	IM
Naloxone (reverses opioids)	0.005–0.02 mg/kg	IV

[a] Xylazine is available in **two strengths** (20 mg/mL, 100 mg/mL). Ensure that the dose calculated is based on the strength being used.
[b] Premedication with atropine or glycopyrrolate is suggested to avoid bradycardia and cardiac arrhythmias with these agents.
[c] Poor analgesia. Only suitable for minor nonpainful procedures.

A.7.2 Dog handling and sexing

1. Restrain the dog (refer to the restraint technique).
2. Check the external genitalia of the dog to identify its sex based on the following criteria:

   - In male dogs, the penis and anus are clearly farther apart than the anus and vulva of the female.
   - In males, the penis can be palpated through the skin due to the presence of an os penis.
   - The external scrotal sac and testicles are visible in older males.
   - The vulva is present in females just below their anal openings.
   - Females in oestrus have swollen vulvas and bloody discharge.

3. Ensure that the information on the cage card is correct.

A.7.3 Dog restraint technique for technical manipulation

Dogs can be restrained on the floor or on a nonslip table top. A dog should always be handled with a gentle but firm grip. Proper personal protective equipment (PPE) must be worn before handling the dog.

1. Restraining a standing dog

   - Put one arm under the neck of the dog and the other behind its rear legs or under its abdomen.
   - Pull the dog's head toward your shoulder for more control.

2. Restraining a sitting dog

   - Put one arm under the neck of the dog and the other around the dog's hind quarters or under its abdomen.
   - Pull the dog's head snugly towards your shoulder.

3. Restraining a dog lying in sternal recumbency

   - Put one arm under the neck of the dog and the other over its back.
   - Lean slightly over the dog.
   - Pull the dog's head toward your shoulder for more control.

4. Restraining a dog in lateral recumbency (unassisted)

   - While the dog is standing, place one arm around the front of the animal, holding its leg on the opposite side from where you are standing.
   - Put your other arm around the dog's hindquarters, holding its leg on the opposite side from where you are standing.
   - Pull the dog snugly to your body.
   - Lift the dog up and gently lay it on its side.
   - Hold the dog's legs (closest to the table), placing your elbow across the dog's hips and neck.

5. Restraining a dog in lateral recumbency (assisted)

   - While the dog is standing, the first person places his/her arms around the front of the animal, holding its front legs.
   - The second person then places his/her arms around the dog's hindquarters, holding its rear legs.
   - Together, the two handlers gently lift the dog up and lay it on its side.
   - Both the dog's front and rear legs must be restrained, while you use your elbows to restrain its hips and neck.

6. Leash
    - A leash (with or without a collar) is used to lead the dog to another cage, room, or carrier.
7. Muzzle
    - Muzzles are used to restrain aggressive dogs.
    - Muzzles must not be left on a dog that is unattended.
8. Chemical restraint
    - Tranquilisers or anaesthetic agents may be given either alone or in combination with physical restraint.

A.7.4 Identification methods

Materials required:

- Personal protective equipment (PPE)
- Needle and ink for tattooing, or microchip transponder and reader, or metal tag for collar

1. Properly restrain the dog (refer to the restraint technique).
2. Quickly use any of the below-mentioned techniques to identify the dog:
    - **Tattooing**: Using a needle and ink, tattoo permanent numbers and/or letters on a suitable location on the animal, such as its upper rear leg (better visibility).
    - **Microchipping**: Insert the chip subcutaneously under the skin at the back of the neck with the use of an applicator.
    - **Collar tag**: Attach a tag with a unique identification method to the dog's collar. However, collar tags should be used in addition to another form of identification.

A.7.5 Intramuscular (IM) injection

Several sites can be used for intramuscular injections in dogs, including the quadriceps, triceps, lumbar musculature, and hamstring group. **Avoid hitting the sciatic or caudal nerve when injecting into the hamstring muscle group by directing**

the needle backward. Needle sizes for intramuscular injections range from 22G to 25G, and small volumes (2–5 mL) can be injected by this route.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the dog.
2. Properly restrain the dog in standing/sternal/lateral recumbency (refer to the restraint technique). Depending on the temperament of the dog, you may or may not need a handler for intramuscular injection.
3. Swab the site with alcohol to wet the hair coat to ensure intramuscular needle placement.
4. Insert the needle into the muscles of any of the abovementioned sites.
5. Aspirate the syringe to ensure proper placement.
6. Any sign of blood in the syringe indicates improper placement. Reinsert the needle at a different site.
7. If no blood is aspirated, administer the substance.
8. Withdraw the needle, and massage the injection site to facilitate dispersion of the injected substance and to relieve any discomfort.

A.7.6 Subcutaneous (SC) injection

Subcutaneous injections can be given anywhere over the dorsal cervical, thoracic, or lumbar regions, the loose skin over the shoulders and neck being an ideal site. For large volumes, inject 10–20 mL/kg at each site. Needle sizes for subcutaneous injections range from 22G to 25G, depending on animal size and the viscosity and volume of the fluid being injected.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the dog.
2. Properly restrain the dog in standing/lateral recumbency (refer to the restraint technique). Depending on the temperament of the dog, you may or may not need a handler for subcutaneous injection.
3. Swab the site with alcohol to better define the skin surface (optional).
4. Grasp a loose fold of skin and insert the needle under the skin, parallel to the long axis of the skin fold.
5. Aspirate the syringe to ensure proper placement before injecting.
6. Air or blood in the syringe indicates improper placement. Withdraw and reposition the needle.
7. After proper placement is achieved, administer the substance as rapidly as it can be ejected from the syringe.
8. For large volumes, use a flexible delivery system (e.g. infusion set) instead of a needle rigidly attached to a syringe.

Note: Do not use this route in severely dehydrated animals.

A.7.7 Intraperitoneal (IP) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (18G–22G)
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the dog.
2. Restrain the dog using suitable anaesthesia/sedative.
3. Swab the site with alcohol.
4. Insert the needle into the abdominal cavity in the lower right quadrant, avoiding the abdominal organs. The needle should be directed towards the animal's head at an angle of 15°–20°.
5. Aspirate the syringe to ensure proper placement before injecting.
6. If any material is aspirated, the syringe should be removed and disposed of, and the needle repositioned.
7. Administer the substance in a steady motion.

A.7.8 Intradermal (ID) injection

Intradermal injections are commonly given for skin testing and for local blocks. Intradermal injections should be given over the dorsal thoracic or lumbar region. Multiple sites (up to 10) and 20G–25G needles can be used.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the dog.
2. Properly anaesthetise the dog.
3. Clip the fur so that the injection site can be clearly observed. Swab the site with alcohol.
4. Insert the needle bevel up into the skin at approximately a 15°–20° angle.
5. Aspirate the syringe to ensure proper placement before injecting.
6. If any blood or fluid is aspirated, reposition the needle.
7. Administer the substance slowly, creating a small bleb that typically takes several minutes to resolve. Immediate

dissolution of the bleb indicates that the substance has been injected subcutaneously.

A.7.9 Intravenous (IV) injection utilising cephalic vein

Needle sizes for intravenous injections range from 20G to 25G. Before administering the substance, make sure that there are no air bubbles in the syringe.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze
- Electric clippers

1. Fill the syringe with the exact amount of the solution to be administered before handling the dog.
2. Properly restrain the dog (refer to the restraint technique). A handler is required for this injection.
3. The handler extends one of the dog's front legs.
4. Shave the extended leg two inches in length below the elbow on the anterior side, and swab the site with alcohol.
5. Ask the handler to apply slight pressure on the blood vessel using his/her thumb.
6. Insert the needle into the cephalic vein.
7. Aspirate the syringe to ensure proper placement (blood should appear in the syringe).
8. Ask the handler to release his/her hold on the blood vessel before injecting the solution.
9. Administer the injection in a slow, steady flow.
10. Achieve haemostasis using the gauze and direct pressure before returning the dog to its cage.

A.7.10 Intravenous (IV) injection utilising saphenous vein

Needle sizes for intravenous injections range from 20G to 25G. Before administering the substance, make sure that there are no air bubbles in the syringe.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze
- Electric clippers

1. Fill the syringe with the exact amount of the solution to be administered before handling the dog.
2. Properly restrain the dog (refer to the restraint technique). A handler is required for this injection.
3. The handler extends one of the dog's rear legs.
4. Shave the lateral aspect of the extended rear leg to expose the saphenous vein, and swab the site with alcohol.
5. Ask the handler to apply slight pressure to the blood vessel using his/her thumb.
6. Insert the needle into the saphenous vein.
7. Aspirate the syringe to ensure proper placement (blood should appear in the syringe).
8. Ask the handler to release his/her hold on the blood vessel before injecting the solution.
9. Administer the injection in a slow, steady flow.
10. Achieve haemostasis using the gauze and direct pressure before returning the dog to its cage.

A.7.11 Gavaging of dog/Gastric intubation

Gastric intubation is generally performed using a large-bore gastric tube. The diameter of the tube should be approximately the same size as an endotracheal tube used in the same animal. Gavaging is more effective when performed on an anaesthetised or sedated animal.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Gastric tube
- Substance to be injected

1. Fill the syringe with the appropriate amount of substance to be instilled.
2. Properly restrain the dog. If awake, the animal is restrained in sternal recumbency with its head in a neutral position. Anaesthesia is needed if the purpose is to remove toxic contents.
3. Measure the length of the tube from the tip of the nose to the ninth intercostal space.
4. Put a tape to mark the proper length.
5. Place a speculum between the dental arcades.
6. Introduce the tube into the oral cavity, ensuring that the head is neither extended nor flexed.
7. Insert the tube up to the previously measured length (tape mark).
8. Administer the substance.
9. Carefully monitor the animal during its recovery from anaesthesia, making sure that it does not vomit and aspirate residual gavage solution.

A.7.12 Blood withdrawal utilising cephalic vein for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe

(*Continued*)

(*Continued*)

- Hypodermic needle (20G–25G)
- Collection tube
- Isopropyl alcohol
- Gauze
- Electric clippers

1. Properly restrain the dog (refer to the restraint technique). Usually, only physical restraint is required to collect blood. A handler is required for this technique.
2. The handler extends one of the dog's front legs.
3. Shave the extended leg two inches in length below the elbow on the anterior side, and swab the site with alcohol.
4. Ask the handler to apply slight pressure on the blood vessel using his/her thumb.
5. Insert the needle into the cephalic vein.
6. Collect the desired amount of blood (0.5 mL).
7. Before removing the needle, ask the handler to release his/her hold on the blood vessel.
8. Achieve haemostasis using the gauze and direct pressure before returning the dog to its cage.

Note: Always use aseptic techniques including clipping of hair around the sampling site.

A.7.13 Blood withdrawal utilising saphenous vein

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (20G–25G)
- Collection tube
- Isopropyl alcohol
- Gauze
- Electric clippers

1. Properly restrain the dog (refer to the restraint technique). Usually, only physical restraint is required to collect blood. A handler is required for this technique.

2. The handler extends one of the dog's rear legs.
3. Shave the lateral aspect of the extended rear leg to expose the saphenous vein, and swab the site with alcohol.
4. Ask the handler to apply slight pressure on the blood vessel using his/her thumb.
5. Insert the needle into the saphenous vein.
6. Collect the desired amount of blood (2–5 mL/sample).
7. Before removing the needle, ask the handler to release his/her hold on the blood vessel.
8. Achieve haemostasis using the gauze and direct pressure before returning the dog to its cage.

Note: Always use aseptic techniques including clipping of hair around the sampling site.

A.7.14 Blood withdrawal utilising jugular vein for small- and large-volume blood collection

The jugular vein is superficial and easily accessible, so sampling from the jugular vein is quick and simple.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (20G–25G)
- Collection tube
- Isopropyl alcohol
- Gauze
- Electric clippers

1. Properly restrain the dog in sternal recumbency. This technique can usually be carried out with only the use of physical restraint to collect blood. A handler is required to restrain the dog, using the manual restraint technique described earlier, whilst the operator removes the hair and performs the technique.
2. Shave the lateral aspect of one side of the ventral neck to expose the jugular vein, and swab the site with alcohol.

3. Using your thumb, apply pressure on the lower neck region to exclude the blood vessel in the jugular furrow.
4. Insert the needle into the jugular vein.
5. Collect the desired amount of blood (2–20 mL/sample).
6. Before removing the needle, release your hold on the blood vessel.
7. Achieve haemostasis using the gauze and direct pressure before returning the dog to its cage.

Note: Always use aseptic techniques including clipping of hair around the sampling site.

A.7.15 Intracardiac (IC) puncture for terminal collection of large blood volumes

Materials required:

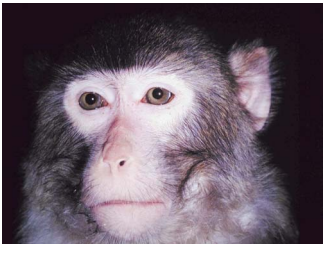
- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (16G or wider, preferably 1.5″ long)
- Collection tube
- Isopropyl alcohol
- Gauze
- Anaesthetic

1. Deep surgical anaesthesia is necessary for intracardiac puncture, unless the animal is already dead.
2. Swab the site with alcohol.
3. Palpate the xyphoid process at the caudal aspect of the sternum. A notch is present on both sides of this process.
4. Insert the needle into either notch and direct it toward the heart.
5. Aspirate the syringe slowly once it has been inserted beneath the skin.
6. Blood will start to flow into the syringe once the needle penetrates the heart.

7. Collect blood preferably from the right or left ventricle (100–900 mL, depending on the size of the dog and whether the heart is beating).
8. Verify the animal's death at the end of the bleed.

Note: Intracardiac puncture should be performed only as a **terminal procedure**; the animal is not allowed to recover from anaesthesia following the puncture. An alternate euthanasia method is recommended after the blood withdrawal.

A.8 Nonhuman Primates (NHPs)



Many problems are encountered while handling and restraining nonhuman primates (NHPs). The use of proper restraint devices and techniques allows safe handling of these animals, and minimises stress and alterations in their physiological parameters. Always ask for help if you are not confident in handling/restraining the animals, and ensure the use of aseptic techniques for procedures. Prior to working with NHPs, you must be familiarised with the procedures to follow in the event of a bite or scratch.

NHPs carry a variety of zoonotic diseases, some of which can cause fatal diseases in humans (e.g. simian herpes B virus, *Mycobacterium tuberculosis*), so proper safeguards should be taken by all personnel involved. It is important to note that, in many cases, the transmission of disease can go in both directions. Therefore, the use of protective clothing protects not only you, but also the animals.

The use of proper personal protective equipment (PPE) will help reduce zoonotic and physical trauma risks. Minimally, the following PPE must be worn while handling and restraining NHPs:

- Disposable latex or nitrile gloves (double)
- Scrubs
- Gown (long-sleeved)
- Properly fitting face mask (N95)
- Face shield
- Nonslippery closed-toe shoes with shoe covers
- Hair cover

The procedures listed here should be carried out quickly and by experienced personnel. Inexperienced operators should never work alone. Make sure you are well trained and experienced before handling conscious primates. Always keep in mind that NHPs are quite aggressive animals; therefore, chemical restraint (ketamine) is generally preferred over physical restraint.

The blood volumes of NHPs vary, but are generally around 8% of body weight. The maximum safe volume for a single collection is 6–10 mL/kg. Common blood collection sites in NHPs include the cephalic, jugular, saphenous, and femoral veins.

A.8.1 Physiologic parameters

Macaque	**Baboon**
Body temperature = 37°C–39°C	Body temperature = 39°C
Heart rate = 120–180/min	Heart rate = 150/min
Respiratory rate = 32–50/min	Respiratory rate = 35/min
Tidal volume = 21 mL	Tidal volume = 50 mL

The dosage and frequency of the administration of all analgesic agents must be tailored to the animal, procedure, and magnitude of pain present. Combinations of narcotics and nonsteroidal agents are commonly used (see Tables A.13 and A.14).

Table A.13 Volume for injection of (a) small NHP and (b) large NHP.

(a)

NHP (small)	IV	IP	SC	IM
Volume (mL) (slowly)	0.5	10–15	5–10	0.3–05

(b)

NHP (large)	IV	IP	SC	IM
Volume (mL) (slowly)	10–20	50–100	10–30	1–3

Table A.14 Anaesthesia and analgesia (suggested agents and doses).

Agent	Dosage and Route of Administration	
Restraint/Premedication		
Atropine	0.02–0.05 mg/kg	IM/SC
Glycopyrrolate	0.005–0.01 mg/kg	IM/SC
Diazepam (Valium®)	0.5–1.0 mg/kg	IM
Xylazine[a]	0.5–2.0 mg/kg	IM
Anaesthesia		
Sodium pentobarbital	20–30 mg/kg	IV
Sodium thiopental (2.5%)	15–20 mg/kg	IV
Ketamine/Xylazine[a,b]:		
Ketamine	7–10 mg/kg	IM
Xylazine[a]	0.25–2.0 mg/kg	IM
Ketamine/Diazepam[c]:		
Ketamine	15 mg/kg	IV
Diazepam (Valium®)	1 mg/kg	IV
Ketamine/Midazolam[c]:		
Ketamine	15 mg/kg	IV
Midazolam	0.5–0.15 mg/kg	IV
Telazol®	4.0–6.0 mg/kg	IM
Halothane (Fluothane®)	—	Inhalant
Isoflurane	—	Inhalant
Analgesia		
Morphine	1–2 mg/kg/4 h	IM/SC
Oxymorphone	0.15 mg/kg/4–6 h	IM
Buprenorphine	0.01–0.03 mg/kg/8–12 h	IM/SC
Acetylsalicytic acid (Aspirin)	10–20 mg/kg/6h	Oral dosing

(*Continued*)

Table A.14 (*Continued*)

Agent	Dosage and Route of Administration	
Acetaminophen	10 mg/kg/8 h	Oral dosing
Flunixin meglumine (Banamine®)	0.5 mg/kg daily	IM
Butorphanol tartrate (Torbugesic®)	0.025 mg/kg/3–6 h	IM
Naproxen	10 mg/kg/12 h	Oral dosing
Ketorolac	15–30 mg/kg	IM
Reversal Agents		
Yohimbine (reverses xylazine)	0.05 mg/kg	IV
Naloxone (reverses opioids)	0.1–0.2 mg/kg as needed	IV

[a] Xylazine is available in **two strengths** (20 mg/mL, 100 mg/mL). Ensure that the dose calculated is based on the strength being used.
[b] Premedication with atropine or glycopyrrolate is suggested to avoid bradycardia and cardiac arrhythmias with these agents.
[c] Poor analgesia. Only suitable for minor nonpainful procedures.

A.8.2 NHP handling and sexing

1. NHPs are handled only with a catchpole and collar, or while chemically restrained. Only experienced/trained personnel should handle NHPs. If a NHP gets loose, a net or blowdart may be used to catch the animal. Never handle a NHP alone.
2. NHPs should be habituated to restraint devices and human presence prior to the commencement of the experimental protocol.
3. Check the external genitalia of a NHP to identify its sex.
   - Scrotum and testicles are clearly visible in males.
   - Penis can be palpated through the skin in males.
   - Vulva is present in females (clitoris and labial folds).
4. Ensure that the cage card information is correct.

A.8.3 NHP physical restraint

Physical restraint should only be attempted by trained, experienced personnel, and it should be both effective and as gentle as possible. Various restraint devices used for NHPs include

cages, nets, chutes and transfer boxes, stocks and restraint tubes, pole and collars, restraint chairs, tether and vest, etc.

For frequent handling, animals may be pole-and-collar-trained; for frequent blood collection, tether systems are recommended. NHPs can be temporarily restrained in a squeeze-back cage to facilitate veinpuncture, injection, topical application of drugs, close-up examination, capture, and other procedures (Fig. A.36).

A.8.4 Manual restraint of a caged, conscious NHP

This technique should only be used with small New World primates.

1. Ask the assistant to release the lock on the pull bar of the NHP's cage.
2. The assistant then immobilises the NHP using the squeeze back of the cage.
3. Introduce a gloved hand into the cage through the bars or by slightly opening the cage door.

Fig. A.36 A squeeze-back cage (notice the use of appropriate PPE).

4. Grasp firmly the forearm of the NHP with your opposite hand (grasp the animal's right hand with your left hand and vice versa).
5. Extending the animal's arm, grasp its upper arm with your free hand so that you have the NHP's upper right arm in your right hand or upper left arm in your left hand.
6. Ask the assistant to "release".
7. Pull the NHP from the cage in a pre-agreed direction as the assistant releases the cage back. This allows the assistant to move in the opposite direction and around the primate.
8. Grasp the NHP's free upper arm with your free hand. Now you have control of both arms of the NHP, with the animal facing **away** from your body. Remember to keep the NHP's legs away from your legs; otherwise, it might grasp your legs and pull itself close enough to bite you.
9. The assistant can now grasp the NHP's rear legs, while you restrain the animal between the both of you for technical manipulation (sample collection, drug administration, and physical examination).

A.8.5 NHP chemical immobilisation

Chemical restraint is preferred over physical restraint when handling NHPs, and is more suitable for safe handling. Chemical restraint is advised prior to any direct contact with NHPs.

1. Make sure that all of the necessary equipment and reagents for the procedure are ready prior to restraint.
2. Immobilise the NHP by following one of the options below for administering chemical agents:

   - IM injection with a hand-held syringe for animals in a squeeze-back cage or physically restrained (e.g. ketamine; 10 mg/kg bwt).
   - Pole syringe for animals confined to a small area such as a cage, chute, or transfer box.
   - Dart systems for delivering chemical agents from a distance in aggressive animals.
   - Blowdart/Pipe for short-range delivery of chemical agents.

3. Keep the amount of chemical restraint and its duration to the minimum necessary to complete the procedure. Revive the animal soon after the completion of the procedure.
4. After the procedure, return the animal to the same cage in which it was initially housed.
5. Observe the behaviour, appetite, hydration status, urine, and faeces of the animal following recovery from chemical immobilisation.

Note: For prolonged immobilisation, endotracheal intubation is recommended, followed by gaseous anaesthetic.

A.8.6 Identification methods

A.8.6.1 *Tattoo*

Tattoo is the most common method of identification in NHPs, but there is a risk of fading, so periodic renewal may be required. Tattoos are easier and faster to read in comparison to other methods.

Materials required:

- Personal protective equipment (PPE)
- Tattoo ink and needle

1. Restrain the NHP (refer to the restraint technique).
2. Properly sedate the animal.
3. Find a location where the tattoo may be easily read without excessive handling of the animal (typically, the inner thigh or inner arm).
4. Apply the tattoo legibly (with numbers or letters), according to the recorded sequence.

A.8.6.2 *Microchip*

Microchip identification is probably the best available method for permanent identification of NHPs. Microchips are permanent and tamper-proof, but are costly to use.

Materials required:

- Personal protective equipment (PPE)
- Microchip
- Transponder
- Surgical instruments

1. Restrain the NHP (refer to the restraint technique).
2. Properly sedate the animal.
3. Choose the location for implant (interscapular skin, behind the ear, at the elbow or wrist).
4. Quickly insert the microchip subcutaneously using a specially designed hypodermic needle (usually supplied ready-loaded with a microchip).
5. Using a scanner, check that the coded digits are reflected.

A.8.7 Intramuscular (IM) injection

The best sites for intramuscular injection are the anterior aspect of the rear leg muscles (quadriceps), the caudal aspect of the arm muscles (triceps), and the muscles of the hip and lower back.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (21G–25G)
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the NHP.
2. Properly restrain the NHP by means of a squeeze-back cage (refer to the restraint technique).
3. Swab the site with alcohol to wet the hair coat to ensure intramuscular needle placement.
4. Palpate a large muscle group and carefully insert the needle into the muscle.

5. Aspirate by applying slight negative pressure to the plunger to ensure proper needle placement.
6. Any sign of blood in the syringe indicates improper placement. Reinsert the needle at a different site.
7. If no blood is aspirated, administer the substance.
8. Withdraw the needle, and massage the injection site to facilitate dispersion of the injected substance and to relieve any discomfort.

A.8.8 Subcutaneous (SC) injection

Subcutaneous injections are best administered under the skin between the shoulders or in the flank area, although delivery of the substance subcutaneously is slightly more difficult to ensure. The most common use of the subcutaneous route is for replacement fluid therapy in cases where intravenous administration is not critical.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (21G–25G)
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the NHP.
2. Properly restrain the NHP by means of a squeeze-back cage (refer to the restraint technique).
3. Swab the site with alcohol to better define the skin surface.
4. Grasp the skin between your thumb and forefinger, and retract from the underlying skin.
5. Penetrate the skin with the needle at approximately a 15° angle to the injection site.
6. Aspirate the syringe to ensure proper placement before injecting.

7. Air or blood in the syringe indicates improper placement. Withdraw and reposition the needle.
8. After proper placement is achieved, administer the substance rapidly. A small bleb should appear as the dosing progresses.
9. Compress the needle exit site for approximately 30 seconds after dosing to prevent leakage of the administered substance.

Note: This procedure is not advisable for use in severely dehydrated animals.

A.8.9 Intraperitoneal (IP) injection

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (18G–22G)
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the NHP.
2. Properly anaesthetise the NHP, keeping its head at a lower level than the rest of the body to move the viscera forward. A handler is required to perform this technique.
3. Swab the site with alcohol.
4. Insert the needle into the abdominal cavity in the lower quadrant, avoiding the abdominal organs. The needle should be directed towards the NHP's head at an angle of 15°–20°.
5. Aspirate the syringe to ensure proper placement before injecting.
6. If any material is aspirated, the syringe should be removed and disposed of, and the needle repositioned.
7. Administer the substance in a smooth and steady motion. Large volumes can be given by this route.

A.8.10 Intradermal (ID) injection

Intradermal injection is commonly given into the dermis of the eyelid for tuberculin testing.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (22G–27G)
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the NHP.
2. Properly anaesthetise the NHP.
3. Swab the site with alcohol after clipping the fur from the injection site, if required.
4. Insert the needle bevel up into the skin at approximately a 5°–10° angle. Once the hole of the bevel is under the skin, do not move the needle any further.
5. Aspirate the syringe to ensure proper placement before injecting.
6. If any blood or fluid is aspirated, reposition the needle.
7. Administer the substance slowly, creating a small bleb that typically takes several minutes to resolve. Immediate dissolution of the bleb indicates that the substance has been injected subcutaneously.

A.8.11 Intravenous (IV) injection utilising saphenous vein

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (20G–24G)
- Substance to be injected

(*Continued*)

(*Continued*)

- Isopropyl alcohol
- Gauze
- Electric clippers

1. Fill the syringe with the exact amount of the solution to be administered before handling the NHP.
2. Properly restrain the NHP (refer to the restraint technique). A handler is required to perform this technique.
3. Extend one of the NHP's rear legs.
4. Shave the lateral aspect of the extended rear leg to expose the saphenous vein, and swab the site with alcohol.
5. Insert the needle into the saphenous vein.
6. Aspirate the syringe to ensure proper placement (blood will start to draw back into the syringe).
7. Administer the injection in a slow, steady flow. Watch out for perivascular fluid accumulation. If fluid accumulates, stop the injection and remove the catheter, and restart the procedure.
8. Achieve haemostasis using the gauze and direct pressure to the injection site. Confirm by flexing and extending the NHP's leg several times before returning the animal to its cage.

Note: Always use aseptic techniques including clipping of hair around the sampling site.

A.8.12 Gavaging of NHP for delivery of intragastric medication

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Gastric tube
- Substance to be injected

1. Fill the syringe with the appropriate amount of substance to be instilled.

2. Properly restrain the NHP in a restraint chair (refer to the restraint technique). A handler is required to perform this technique.
3. Attach an infant-feeding tube to the syringe.
4. Lubricate the tube with a small amount of lubricant jelly.
5. Measure the length of the tube to be inserted by holding it next to the NHP and measuring from the nose to the last rib.
6. Put a tape to mark the proper length.
7. Insert the tube into the ventral-medial corner of one of the NHP's nostrils.
8. Gently push the tube up to the previously measured length (tape mark). Watch the throat to ensure that the NHP is swallowing.
9. Aspirate. If stomach contents or detectable vacuum is noted in the tube, administer 1 mL of the substance.
10. Aspirate again to confirm proper placement (stomach contents in the tube) and administer the remaining substance.
11. Slowly remove the tube.

A.8.13 Blood withdrawal utilising cephalic vein for small-volume blood collection

Handling procedures for NHPs often trigger anxiety and fear, which may lead to deviations in the animals' normal physiological functions. Training the animals to cooperate during vein puncture can help to avoid distress responses associated with the conventional involuntary blood collection procedures.

Training NHPs to cooperate during vein puncture can help in the refinement of research protocols by eliminating significant cortisol responses. These benefits are also extended to animal care staff by reducing their chances of being bitten or scratched.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (20G–25G)

(*Continued*)

(*Continued*)

- Collection tube
- Isopropyl alcohol
- Gauze
- Electric clippers

1. Properly restrain the NHP (refer to the restraint technique). A handler is required for this technique.
2. Extend either the front or hind leg of the NHP to access the cephalic vein on the anterior side.
3. Shave the extended leg, and swab the site with alcohol.
4. Ask the handler to hold off the blood vessel using his/her thumb.
5. Insert the needle into the cephalic vein at an acute angle.
6. Create negative pressure by slightly withdrawing the syringe plunger as soon as the needle passes under the skin.
7. Advance the needle until blood is aspirated in the syringe barrel, indicating proper placement.
8. Ask the handler to release his/her hold on the blood vessel.
9. Collect the desired amount of blood (1–2 mL).
10. Achieve haemostasis using the gauze and direct pressure to the injection site. Confirm by flexing and extending the NHP's leg several times.
11. Check for evidence of swelling or haematoma before returning the animal to its cage.

Note: Always use aseptic techniques including clipping of hair around the sampling site.

A.8.14 Blood withdrawal utilising saphenous vein for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (20G–25G)

(*Continued*)

(*Continued*)

- Collection tube
- Isopropyl alcohol
- Gauze
- Electric clippers

1. Properly restrain the NHP (refer to the restraint technique). A handler is required for this technique.
2. Extend the NHP's hind leg to access the saphenous vein on the posterior side.
3. Shave the extended leg, and swab the site with alcohol.
4. Ask the handler to hold off the blood vessel using his/her thumb.
5. Insert the needle into the saphenous vein at an acute angle.
6. Create negative pressure by slightly withdrawing the syringe plunger as soon as the needle passes under the skin.
7. Advance the needle until blood is aspirated in the syringe barrel, indicating proper placement.
8. Ask the handler to release his/her hold on the blood vessel.
9. Collect the desired amount of blood (1–2 mL).
10. Achieve haemostasis using the gauze and direct pressure to the injection site. Confirm by flexing and extending the NHP's leg several times.
11. Check for evidence of swelling or haematoma before returning the animal to its cage.

Note: Always use aseptic techniques including clipping of hair around the sampling site.

A.8.15 Blood withdrawal utilising femoral vein for large-volume blood collection

Bleeding from the femoral vein can be quite difficult, as the femoral vein is not normally visible.

Materials required:

- Personal protective equipment (PPE)
- Syringe

(*Continued*)

(*Continued*)

- Hypodermic needle
- Collection tube
- Isopropyl alcohol
- Gauze

1. Properly restrain the NHP (refer to the restraint technique). A handler is required for this technique.
2. Extend one of the NHP's rear legs and access the femoral vein in the upper inner thigh. This can sometimes be aided by using a tourniquet to dilate the vein and rolling the vein over the femur, so that it is prevented from moving.
3. Swab the site with alcohol, and locate the blood vessel by palpating the pulse of the femoral artery.
4. Insert the needle medially to the pulse at an acute angle.
5. Create negative pressure by slightly withdrawing the syringe plunger as soon as the needle passes under the skin.
6. Aspiration of dark blood into the syringe barrel indicates proper placement.
7. Collect the desired amount of blood.
8. Achieve haemostasis using the gauze and direct pressure to the injection site. Confirm by flexing and extending the NHP's leg several times.
9. Check for evidence of swelling or haematoma before returning the animal to its cage.

A.8.16 Intracardiac (IC) puncture for terminal collection of large blood volumes

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (18G–21G, preferably 2″ long)
- Collection tube
- Isopropyl alcohol
- Gauze
- Anaesthetic

1. Deep surgical anaesthesia is necessary for intracardiac puncture.
2. Place the unconscious animal in dorsal or lateral recumbency and palpate the heart.
3. Swab the site with alcohol.
4. Insert the needle either at the intercostal space between the fourth and sixth ribs (at a 90° angle) or alternatively next to the xyphoid process of the sternum (at a 45° angle), directing it toward the heart.
5. Aspirate the syringe slowly once it has been inserted beneath the skin.
6. Reflux of blood is apparent once the needle penetrates the heart.
7. Collect blood preferably from the right or left ventricle.
8. Verify the animal's death at the end of the bleed.

Note: Intracardiac puncture should be performed only as a **terminal procedure**, and the animal is not allowed to recover from anaesthesia following the puncture. An alternative euthanasia method is recommended after the blood withdrawal.

A.9 Miniature Swine

Miniature swine are increasingly being used in research, as their appropriate size and temperament make them much better suited to the laboratory (due to space restrictions) and easier to work with. Miniature swine also have organs and tissues that are similar in size to those of humans (compared with adult farm breeds), making them more suitable for many surgical research protocols.

Miniature swine generally have a milder disposition than farm breeds, which — along with their smaller size — makes them easier to handle and restrain. These animals are also good for long-term studies, as they do not become as large as domestic swine.

A.9.1 Safety

Swine are generally large animals with low centres of gravity; this, along with their strength, makes them quite hazardous to individuals entering a pen or enclosed space. Miniature swine, though smaller than regular domestic breeds, can still weigh up to 80–100 kg and are capable of inflicting injury.

Personal protective equipment (PPE) should be worn when working with swine, including gloves, water-resistant shoe covers or boots (preferably with safety toes to prevent damage to feet), and long-sleeved apparel (such as lab coats).

Most miniature swine breeds are docile, but some may become aggressive and may bite or charge, potentially inflicting serious injury. **Never work alone when dealing with large or aggressive animals**.

When lifting animals or restraining them, ensure that you use proper lifting or restraining equipment to prevent injury (especially to the back).

Pigs can be noisy, especially during feeding times and when they are being restrained. Prolonged exposure to such high levels of noise can cause irreversible ear damage; therefore, it is important to use protective devices such as earplugs or mufflers when working around swine.

A.9.2 Catheterisation

When it is necessary to take frequent blood samples from pigs or to give frequent injections, a good alternative is to insert a permanent venous catheter. This may be placed in the external jugular vein. The catheter is inserted between the shoulders, under general anaesthesia, and is tunneled under the skin to the neck using a metal rod, where it is inserted into the jugular vein. It is then possible to take blood samples without causing pain or distress to the animal.

The port is positioned between the shoulders to prevent the animal from biting at it, and may be easily accessed for injection or blood withdrawal. After injection or blood withdrawal, the catheter is rinsed with a heparin solution to prevent blood from clotting inside, which would block the catheter.

A.9.3 Miniature swine handling and sexing

1. First, assess the swine in their pen for normal behaviour.
2. Pigs may be herded from one area to another with the aid of a pig board, which is usually made of plastic and prevents the pigs from escaping.
3. Using the pig board, guide a pig to the side of the pen, where it may then be picked up or examined. Note that pigs will generally squeal at a very high level when being handled, unless they are accustomed to regular handling.
4. Smaller pigs may be picked up by their hind legs, but take care that you grip firmly but gently at the thigh, so as not to cause pain or injury.
5. Small pigs may also be picked up under the thorax with one hand below the head and keeping it close to the body.
6. Larger animals may be herded into a suitable trolley for manoeuver, or with a squeeze-back, to restrain the animal for injection.
7. Male pigs can easily be identified by their external genitalia, and females by the vulva below the anus.

A.9.4 Miniature swine restraint technique for technical manipulation

1. Swine may be restrained manually or with the aid of a restraint device, such as a squeeze-back trolley or a Panepinto sling (Fig. A.37).
2. Panepinto slings are very useful as they immobilise the animal, preventing it from moving during procedures.
3. When using a restraint device such as the Panepinto sling or squeeze-back trolley, it is good practice to acclimatise the animal to it beforehand in order to prevent the animal from getting stressed.

Fig. A.37 Panepinto sling for restraint.

4. Aggressive animals may be given a suitable sedative or tranquiliser to restrain them for injection and blood collection. In rare cases where it is not possible to inject an aggressive animal with an anaesthetic, it is also possible to herd the pig into an enclosed area, such as a large plastic bin, and directly pipe in a low concentration of anaesthetic gas to relax the animal suitably enough to inject with an appropriate agent.

A.9.5 Identification methods

Materials required:

- Personal protective equipment (PPE)
- Microchip transponder and reader, or plastic coloured tag for ear tagging

1. Properly restrain the pig (refer to the restraint technique).
2. Quickly use either of the below-mentioned techniques to identify the pig:

    - **Microchipping**: Insert the chip subcutaneously under the skin at the back of the neck with the use of an applicator.

- **Ear tag**: Take an appropriately coloured ear tag, corresponding to the sow or boar colour, and place it inside the applicator. The applicator should be placed behind the ear with the tag in front. Hold the ear still with one hand and quickly and firmly squeeze the applicator so that the metal disk at the back adheres to the ear tag, keeping it in place.

A.9.6 Intramuscular (IM) injection

Several sites can be used for intramuscular injections in pigs, but the rump at the top of the buttocks remains the most common as there is a large muscle mass there, although other locations such as the quadriceps, triceps, lumbar musculature, and hamstring group may also be used. **Avoid hitting the sciatic or caudal nerve when injecting into the hamstring muscle group by directing the needle backward.** Needle sizes for intramuscular injections range from 22G to 25G. Small volumes (2–5 mL) can be injected by this route.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the pig.
2. Properly restrain the pig, either in a standing position or using a butterfly needle and syringe for animals that are enclosed in a trolley or a Panepinto sling (refer to the restraint technique).
3. Swab the site with alcohol.
4. Insert the needle into the muscles of any of the abovementioned sites.
5. Aspirate the syringe to ensure proper placement.
6. Any sign of blood in the syringe indicates improper placement. Reinsert the needle at a different site.

7. If no blood is aspirated, administer the substance.
8. Withdraw the needle, and massage the injection site to facilitate dispersion of the injected substance and to relieve any discomfort.

A.9.7 Subcutaneous (SC) injection

Subcutaneous injections are generally difficult to administer in pigs, as they do not have loose skin that can easily be grasped. If subcutaneous injection is required, the most likely site would be behind the neck. Needle sizes for subcutaneous injections range from 22G to 25G, depending on the animal size and the viscosity and volume of the fluid being injected.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the pig.
2. Properly restrain the pig (refer to the restraint technique).
3. Swab the site with alcohol.
4. Grasp a loose fold of skin and insert the needle under the skin, parallel to the long axis of the skin fold.
5. Aspirate the syringe to ensure proper placement before injecting.
6. Air or blood in the syringe indicates improper placement. Withdraw and reposition the needle.
7. After proper placement is achieved, administer the substance as rapidly as it can be ejected from the syringe.

A.9.8 Intraperitoneal (IP) injection

Intraperitoneal injection is not generally given to pigs, unless they are under general anaesthesia.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (18G–22G)
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the pig.
2. Restrain the pig using suitable anaesthesia/sedative.
3. Swab the site with alcohol.
4. Insert the needle into the abdominal cavity in the lower right quadrant, avoiding the abdominal organs. The needle should be directed towards the animal's head at an angle of 15°–20°.
5. Aspirate the syringe to ensure proper placement before injecting.
6. If any material is aspirated, the syringe should be removed and disposed of, and the needle repositioned.
7. Administer the substance in a steady motion.

A.9.9 Intradermal (ID) injection

Intradermal injections are commonly given for skin testing and for local blocks. Intradermal injections should be given over the dorsal thoracic or lumbar region. Multiple sites (up to 10) and 20G–25G needles can be used.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the substance to be administered before handling the pig.
2. Properly anaesthetise the pig, or use an appropriate sedative or tranquiliser.
3. Insert the needle bevel up into the skin at approximately a 15°–20° angle.
4. Aspirate the syringe to ensure proper placement before injecting.
5. If any blood or fluid is aspirated, reposition the needle.
6. Administer the substance slowly, creating a small bleb that typically takes several minutes to resolve. Immediate dissolution of the bleb indicates that the substance has been injected subcutaneously.

A.9.10 Intravenous (IV) injection utilising cephalic vein

Needle sizes for intravenous injections range from 20G to 25G. Before administering the substance, make sure that there are no air bubbles in the syringe.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the solution to be administered before handling the pig.
2. Properly restrain the pig (refer to the restraint technique). A handler is required for this injection.
3. The handler extends one of the pig's front legs.
4. Swab the site with alcohol.
5. Ask the handler to apply slight pressure on the blood vessel using his/her thumb.
6. Insert the needle into the cephalic vein.

7. Aspirate the syringe to ensure proper placement (blood should appear in the syringe).
8. Ask the handler to release his/her hold on the blood vessel before injecting the solution.
9. Administer the injection in a slow, steady flow.
10. Achieve haemostasis using the gauze and direct pressure before returning the pig to its pen.

A.9.11 Intravenous (IV) injection stilising saphenous vein

Needle sizes for intravenous injections range from 20G to 25G. Before administering the substance, make sure that there are no air bubbles in the syringe.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the solution to be administered before handling the pig.
2. Properly restrain the pig (refer to the restraint technique). A handler is required for this injection.
3. The handler extends one of the pig's rear legs.
4. Swab the site with alcohol.
5. Ask the handler to apply slight pressure to the blood vessel using his/her thumb.
6. Insert the needle into the saphenous vein.
7. Aspirate the syringe to ensure proper placement (blood should appear in the syringe).
8. Ask the handler to release his/her hold on the blood vessel before injecting the solution.
9. Administer the injection in a slow, steady flow.
10. Achieve haemostasis using the gauze and direct pressure before returning the pig to its pen.

A.9.12 Intravenous (IV) injection utilising ear vein

Needle sizes for intravenous injections range from 20G to 25G. Before administering the substance, make sure that there are no air bubbles in the syringe.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the solution to be administered before handling the pig.
2. Properly restrain the pig (refer to the restraint technique). A handler is required for this injection.
3. Swab the site with alcohol.
4. The needle and the pig's ear are fixed between the operator's thumb and forefinger to assist in dilating the vein.
5. Insert the needle into the ear vein.
6. Aspirate the syringe to ensure proper placement (blood should appear in the syringe).
7. Ask the handler to release his/her hold on the blood vessel before injecting the solution.
8. Administer the injection in a slow, steady flow.
9. Achieve haemostasis using the gauze and direct pressure before returning the pig to its pen.

A.9.13 Intravenous (IV) injection utilising cranial vena cava

Needle sizes for intravenous injections range from 20G to 25G. Before administering the substance, make sure that there are no air bubbles in the syringe.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle
- Substance to be injected
- Isopropyl alcohol
- Gauze

1. Fill the syringe with the exact amount of the solution to be administered before handling the pig.
2. Properly restrain the pig (refer to the restraint technique). A handler is required for this injection.
3. Swab the site with alcohol.
4. In order to avoid injury to the vagus nerve, the needle is inserted into the right side of the neck, lateral to the manubrium sterni, and directed at a 30°–45° angle toward the left shoulder.
5. A popping sensation will be felt by the sampler when the needle enters the vein, and then blood can be readily withdrawn.
6. Aspirate the syringe to ensure proper placement (blood should appear in the syringe).
7. Ask the handler to release his/her hold on the blood vessel before injecting the solution.
8. Administer the injection in a slow, steady flow.
9. Achieve haemostasis using the gauze and direct pressure before returning the pig to its pen.

A.9.14 Gavaging of miniature swine

Gastric intubation is generally performed using a large-bore gastric tube. The diameter of the tube should be approximately the same size as an endotracheal tube used in the same animal. Gavaging is more effective when performed in an anaesthetised or sedated animal.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Gastric tube/"Balling" tube (Fig. A.38)
- Substance to be injected

1. Fill the syringe with the appropriate amount of substance to be instilled.
2. Properly restrain the pig (refer to the restraint technique)
3. Measure the length of the tube from the tip of the nose to the ninth intercostal space.
4. Put a tape to mark the proper length.
5. Place a speculum between the dental arcades.
6. Introduce the tube into the oral cavity, ensuring that the head is neither extended nor flexed.
7. Insert the tube up to the previously measured length (tape mark).
8. Administer the substance.
9. Carefully monitor the animal during its recovery from anaesthesia, making sure that it does not vomit and aspirate residual gavage solution.
10. The "balling" tube may be used as an alternative in which the dosing article is put into a pellet form so that when the tube is inserted into the mouth, the plunger may be pressed,

(a) (b)

Fig. A.38 Gavaging of miniature swine. (a) Balling tube with capsule (for administration); (b) balling tube in use.

forcing the pellet down the throat and administering the article (Fig. A.38).

A.9.15 Blood withdrawal utilising jugular vein for large-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (20G–25G)
- Collection tube
- Isopropyl alcohol
- Gauze

1. Properly restrain the pig (refer to the restraint technique). Usually, only physical restraint is required to collect blood. A handler is required for this technique.
2. Swab the site with alcohol.
3. The animal must be held with its neck stretched upwards.
4. The needle should be directed caudodorsally, in this case perpendicular to the skin.
5. The correct puncture site is in the deepest point of the jugular groove formed between the medial sternocephalic and lateral brachiocephalic muscles.
6. Right-handed operators will usually find it easier to use the animal's right jugular vein.
7. The blood sample should be taken from the right external jugular vein, with the assistant holding the needle holder with his/her left hand while at the same time pressing it gently against the pig's neck.
8. Collect the desired amount of blood.
9. Before removing the needle, ask the handler to release his/her hold on the blood vessel.
10. Achieve haemostasis using the gauze and direct pressure before returning the pig to its pen.

A.9.16 Blood withdrawal utilising milk vein for small-volume blood collection

The milk vein (the subcutanous abdominal vein) is easily visible lateral to the teats on smaller pigs. The vein can often be palpated as a groove in the muscle.

Materials required:

- Personal protective equipment (PPE)
- Syringe
- Hypodermic needle (20G–25G)
- Collection tube
- Isopropyl alcohol
- Gauze

1. Properly restrain the pig (refer to the restraint technique). Usually, anaesthesia/sedative restraint is required.
2. Swab the site with alcohol.
3. The needle is inserted where the vein is most visible.
4. The vein is palpated, and the skin is punctured at the point where the vein is felt most clearly.
5. Insert the needle caudally.
6. Collect the desired amount of blood.
7. Before removing the needle, apply pressure to the blood vessel.
8. Achieve haemostasis using the gauze and direct pressure before returning the pig to its pen.

A.9.17 Blood withdrawal utilising tail vein for small-volume blood collection

Materials required:

- Personal protective equipment (PPE)
- Syringe

(*Continued*)

(*Continued*)

- Hypodermic needle (20G–25G)
- Collection tube
- Isopropyl alcohol
- Gauze

1. Properly restrain the pig (refer to the restraint technique). Usually, anaesthesia/sedative restraint is required.
2. Swab the site with alcohol.
3. The medial caudal vein lies in a groove under the tail, next to the artery.
4. The operator raises the tail with one hand and punctures the vein with the other.
5. The puncture site is at the first freely movable tail joint. This is around the fifth tail vertebra.
6. In adult pigs, the needle should be inserted at an angle of 45° to the skin.
7. In smaller pigs, it is recommended to hold the tail nearly horizontally and to stick the needle in nearly parallel to the skin.
8. Collect the desired amount of blood.
9. Before removing the needle, apply pressure to the blood vessel.
10. Achieve haemostasis using the gauze and direct pressure before returning the pig to its pen.