

## Standard Procedures for Blood Collection

### Recommended Limit Volumes and Recovery Periods (All Species)

**Table 4. Limit volumes and recovery periods**

Single sampling (e.g. toxicity study)		Multiple sampling (e.g. toxicokinetic study)	
% Circulatory blood volume removed	Approximate recovery period	% Circulatory blood volume removed in 24 h	Approximate recovery period
7.5%	1 week	7.5%	1 week
10%	2 weeks	10–15%	2 weeks
15%	4 weeks	20%	3 weeks

Diehl et al 2001

#### **Acute Blood Sampling:**

Animal            Body weight    \* *Recommended single sample*

Mouse 25g                    \* 0.15 ml

Rat    250g                    \* 1.5 ml

Rabbit 2.0 kg                \* 12.0 ml

#### **Chronic Blood Sampling:**

Animal            Body weight    \*\* *Recommended daily sample*

Mouse 25g                    \*\* 0.015 ml (15 micro-litres)

Rat    250g                    \*\* 0.15 ml (150 micro-litres)


Rabbit 2.0 kg                \*\* 1.2 ml

#### **Recommended Mean Circulating Blood Volumes (Diehl et al, 2001)**

Mouse: 72ml/kg

Rat: 64ml/kg

Rabbit: 56ml/kg

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### **Blood Collection (All Species)**

For all blood collection, choose a needle bore that is small enough to fit within the expected blood vessel diameter.

A smaller needle bore will enable easier placement of the needle within the blood vessel, however, there may be a higher risk of blood clotting inside the needle or skin tissue clogging up the needle.


Always use a suitable size syringe for blood collection. Use the appropriate size syringe that will be able to collect and hold the expected volume of blood. A syringe that is too large may not be able to accurately measure the desired blood volume to be collected, it may also produce too much suction during blood collection causing blood vessel collapse.

### **Blood Collection for Rats and Mice**

#### **Tail Vein (Needle and Syringe) (C/D): (Mouse and Rat)**

- 1) Ensure the mouse/rat is mechanically restrained or sedated/anaesthetised.
- 2) The tail may be warmed to dilate the vessel.
- 3) Direct an infra-red lamp, warm water bath, or warming device (eg heating blanket, heating pad) to the tail for 1-2 minutes.
- 4) Swab tail with 70% ethanol.
- 5) The first puncture site should be as far away from the body as possible. Subsequent puncture attempts can be done on the same vein nearer the body.
- 6) If the vein is difficult to locate, gently twist the tail to locate the dark veins.
- 7) If the rat tail veins are difficult to locate, the outer layer of tail scales can be gently scraped off.
- 8) Hold the tail straight with the non-dominant hand and the bevel of the needle facing upwards, insert the needle at a 30-40 degree angle to the horizontal.
- 9) Do not place fingers directly under puncture site.
- 10) Once the needle is in the vein, adjust to a more horizontal angle while sliding the needle in.
- 11) Insert about 30-50% of the needle length into the vein.
- 12) Hold the tail and syringe together with the non-dominant hand.
- 13) Aspirate the syringe slightly to see if any blood appears in the needle hub.
- 14) If no blood appears in the needle hub, stop aspirating, almost pull out the needle from the vein with the needle tip still remaining in the animal, change the insertion angle and try again.
- 15) Continue to gently aspirate the blood until there is sufficient volume.
- 16) Remove the needle in the same angle of insertion and if there is any bleeding at the puncture site, apply pressure with a clean dry guaze swab until bleeding stops.

#### **Tail vein bleed needle only technique (C/D): (Mouse and Rat)**

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Alternatively, only the needle without the syringe can be used to collect blood. Follow the same technique as 6Ai until blood is seen freely flowing into the needle hub. A capillary tube can be used to collect blood from the needle hub. If more blood is required, gently massage the vein from the body towards the needle to encourage more blood flow.

**Tail vein bleed puncture technique (C/D): (Mouse and Rat)**

The vein can also be punctured with a needle or lancet, and the blood collected with a capillary tube.

If more blood is required, gently massage the vein from the body towards the needle to encourage more blood flow.

**Tail vein bleed cut tail tip technique (D): (Mouse and Rat)**

The mouse/rat must be under full general anaesthesia for this method.

The tip of the tail is cut with sterile sharp scissors.

The blood is collected from the tail tip.


If more blood is required, gently massage the vein from the body to the tail tip.

When sufficient blood is collected, use a clean dry guaze swab to apply pressure until bleeding stops. If bleeding still does not stop, hemostasis powder such as blood stop powder, potassium permanganate or styptic powder can be applied to the bleeding point.

Subsequent blood withdrawals taken from this site can be done by scraping off the scab from the tail tip.

**Saphenous Vein (Needle and Syringe) (C/D): (Mouse and Rat)**

- 1) The mouse/rat should be sufficiently mechanically restrained or sedated/anaesthetised.
- 2) The fur on the caudo-lateral side of the hindlimb may be shaved for better visualisation of the saphenous vein.
- 3) A tourniquet is applied to the axilla of the hindlimb to enable vessel dilation.
- 4) A rubber band is wrapped around the hindlimb axilla and a locking surgical instrument such as a hemostat is used to hold the rubber band in place.
- 5) Swab vein with 70% ethanol.
- 6) To enhance vessel dilation, the vein can be gently tapped/flicked.
- 7) Extend the hindlimb slightly with the non-dominant hand and the thumb of the non-dominant hand may be placed next to and parallel to the vein to stabilise it.
- 8) Starting distally with the bevel of the needle upwards, insert the needle in the same line as the vein at a 30-40 degree angle to the horizontal.
- 9) Once the needle is in the vein, slide the needle into the vein at a more horizontal angle.
- 10) Continue inserting the needle until at least 30% of the needle length is inside the vein.
- 11) Hold the leg and syringe together with the non-dominant hand.
- 12) Aspirate the syringe slightly to see if blood appears in the needle hub.
- 13) If no blood is seen in the needle hub gently pull out the needle in the same angle of insertion, leaving the needle tip in the animal.

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- 14) Change the angle of injection and try again.
- 15) If blood is seen in the needle hub, continue aspirating for small volumes of blood.
- 16) For larger volumes of blood, release the tourniquet by releasing the locking instrument.
- 17) Continue aspirating gently until the desired volume of blood is collected.
- 18) Gently pull out the needle in the same angle of insertion.
- 19) If there is any bleeding at the puncture site, use a clean dry swab to apply pressure to the bleeding point until bleeding stops.

#### **Saphenous vein Needle only technique (C/D): (Mouse and Rat)**

The same method as 6Bi can be used with a needle only.

The blood can be collected from the needle hub with a capillary tube.

If larger volumes of blood is required, the tourniquet must be released and the vein can be gently massaged from the body towards the needle.


#### **Saphenous vein Puncture technique (C/D): (Mouse and Rat)**

Alternatively, following the steps in 6Bi, a needle or lancet can be used to puncture the vein. A capillary tube can be used to collect the blood or the blood can be dripped directly into a collection vessel.

For larger volumes of blood, release the tourniquet and gently massage the vein from the body towards the puncture site.

#### **Facial Vein (C/D): (Mouse and Rat)**

- 1) The mouse/rat may be restrained manually or sedated/anesthetised.
- 2) The head should be stabilised against a flat horizontal surface with one side of the head facing upwards.
- 3) The dark "dimple" seen on the middle lower jawline of the head is a landmark.
- 4) Trace a point one eye-length caudal to the dimple, and one eye width dorsal from there. This is the approximate puncture site.
- 5) Swab the puncture site with 70% ethanol.
- 6) Using the appropriate lancet size for the size of the animal (follow the manufacturer recommendations), puncture the site.
- 7) Blood should start flowing from the puncture site.
- 8) The blood can be collected with a clean collection vessel.
- 9) When sufficient blood has been collected, use a clean dry gauze swab to apply pressure to the bleeding site until bleeding stops.
- 10) Possible adverse reactions are: Bleeding from the ears, nose or mouth. The lancet may have been too large, gone in too deep, or was used at the wrong site.
- 11) Immediately seek veterinary attention. Euthanasia may have to be performed if bleeding is uncontrollable or the animal goes into shock.
- 12) A needle may be used as a puncture device instead of a lancet, however this is not generally recommended due to the adverse potential side effects.

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- 13) For the use of hypodermic needles as a puncture device, the technique should be practiced on freshly euthanised animals and animals under full general anaesthesia until the user is able to consistently safely puncture only the facial vein.

**Cardiac bleed (Dorsal recumbency 45 degree angle) (D): (Mouse and Rat)**

- 1) The mouse/rat must be under full general anaesthesia.
- 2) Check depth of anaesthesia with the toe pinch reflex.
- 3) This is a terminal procedure and the animal must be euthanised after this procedure.
- 4) As much of the circulating blood volume can be collected in this procedure.
- 5) Lay the animal in dorsal recumbency.
- 6) Use the non-dominant hand to stabilise the chest and prevent it from rotating during the procedure.
- 7) Identify the heart location by feeling for the area of strongest heartbeat on the chest.
- 8) The heart lies approximately slightly off centre to the sternum on the left of the animal.
- 9) Also drawing an imaginary line from the elbows and intersecting it with the sternum is the approximate site of the heart.
- 10) Swab the puncture site with 70% ethanol.
- 11) Puncture the chest just under the last rib to the left of the xiphoid process with the needle pointing towards the nose, puncture at a 30-40 degree angle to the horizontal.
- 12) Once the needle is in the animal, slide the needle in at a more horizontal angle.
- 13) When the needle tip is at the approximate site of the heart, gently aspirate the syringe.
- 14) There should be blood seen in the needle hub.
- 15) If no blood is seen in the needle hub, slowly withdraw the needle in the same angle while gently aspirating.
- 16) When blood is seen in the needle hub, stop withdrawing the needle and continue aspirating gently.
- 17) If no blood is seen in the needle hub throughout, almost withdraw the needle, leaving the needle tip still in the animal, change the angle of insertion and try again.
- 18) While aspirating the blood, use gentle pressure to prevent the heart from collapsing.
- 19) While aspirating the blood, if the blood flow stops and a lot of suction pressure is felt in the syringe, stop aspirating and release the plunger. Allow the plunger to normalise itself. Attempt to aspirate again.
- 20) Once sufficient blood has been collected, the animal must be euthanised.

**Cardiac bleed (Dorsal recumbency, 90 degree angle) (D): (Rats and Mice)**

Other steps relevant to cardiac bleed as stated above is also followed.

The animal is laid in dorsal recumbency.


The needle is inserted at a 90 degree angle to the horizontal at the site of the heart.

**Cardiac bleed (Right lateral recumbency 90 degree angle) (D): (Rats and Mice)**

Other steps relevant to cardiac bleed as stated in above is also followed.

the animal is laid in right lateral recumbency.

The needle is inserted at a 90 degree angle to the horizontal at the site of the heart.


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### **Retro-orbital Bleed (D): (Rats and Mice)**

- 1) Eyes should be alternated.
- 2) The mouse/rat should be under full general anaesthesia, check the depth of anaesthesia with the toe pinch reflex before proceeding.
- 3) The animal should be in lateral recumbency.
- 4) Use the non-dominant hand to stabilise the head and to place gentle pressure above and below the eye to make it protrude slightly.
- 5) Insert the tip of the capillary tube at the medial or lateral canthus of the eye, between the eyeball and the eyelid, aiming for the vessel bed under the eye.
- 6) When the tip of the capillary tube has hit the orbital bone, this is felt as some resistance, gently rotate the capillary tube to abrade the vessel bed. More force may be needed for rats.
- 7) Stop rotating the capillary tube when blood starts to rise inside the tube.
- 8) If more blood is required or the blood flow stops, the capillary tube can be gently slightly lifted up to improve blood flow.
- 9) For even more blood, the animal can be carried, holding on to the head and the capillary tube, and the head rotated 180 degrees. The blood flowing out of the capillary tube can be collected in a clean collection vessel.

### **Submental Bleeding (C/D) (Mouse and Rat) (Regan et al, 2016)**


- 1) Ensure that the mouse/rat is scruffed near the ears so that the head is extended and straightened. Ensure that any loose skin is drawn back to expose the submental region. Ensure that breathing and blood flow is not restricted while doing this. If breathing difficulties or constricted blood flow is noted, release the scruffing hold and re-scruff.
- 2) Locate the landmark, which is the hair/fur whorl in the centre of the mandible.
- 3) The approximate area for puncture is at the 11 and 1 o'clock position from the central fur whorl just under the bony prominence of the mandible.
- 4) Swab the puncture site with 70% ethanol.
- 5) Using appropriate sized lancet for the size of the animal (follow the manufacturer recommendation), puncture the site pictured in the attached article (Regan et al, 2016). The lancet size is usually 4-5 mm for mice.
- 6) A hypodermic needle may be used as a puncture device instead of a lancet, however this is not generally recommended due to the adverse potential side effects.
- 7) For the use of hypodermic needles as a puncture device, the technique should be practiced on freshly euthanised animals and animals under full general anaesthesia until the user is able to consistently and safely puncture only the blood vessels in the submental region.
- 8) The blood should start flowing from the puncture site if the blood vessels in the submental region have been correctly punctured.
- 9) The blood can be collected by directing the blood flow into a clean collection vessel.

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- 10) When sufficient blood has been collected, use a clean dry guaze swab to apply pressure to the bleeding site until bleeding stops.
- 11) Bleeding usually spontaneously stops when animal is released from being scruffed.
- 12) Possible adverse reactions are: Bleeding into the oral cavity, problems breathing etc. The lancet may have been too large, gone in too deep, or was used at the wrong site.
- 13) Immediately seek veterinary attention. Euthanasia may have to be performed if bleeding is uncontrollable or the animal goes into shock.

**Cardiac bleed (visualisation of the heart through the diaphragm): (Mouse and Rat)**

- 1) Relevant steps of 6Di are followed.
- 2) Surgical prep of the upper abdomen and lower chest may be done to minimise contamination of the sample.
- 3) Alternatively the area is sprayed down with 70% ethanol to wet down the hair and minimise hair flying around.
- 4) Using surgical scissors or scalpel blade, and forceps, make a midline skin incision from the mid-abdomen to the last rib.
- 5) If required, the skin incision may be extended to the pubis for better visualisation.
- 6) The abdominal muscle is picked up with forceps and lifted up and away from the abdominal organs, the same length of the skin incision is made to the abdominal muscle on the midline.
- 7) The abdomen may be retracted with surgical instruments or ALM retractors.
- 8) The heart is visualised beating through the diaphragm and the cardiac bleed as per 6Di is performed.
- 9) Be careful of rupturing the diaphragm.
- 10) Once the diaphragm is ruptured the animal will begin to die very quickly.
- 11) The animal must be euthanised after this procedure.

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## **Blood Collection for Rabbits**

### **Central Ear Artery**

- 1) The rabbit must be anaesthetised.
- 2) Swab the area with 70% ethanol.
- 3) Use a butterfly needle connected to a syringe.
- 4) Insert the needle with the bevel facing upwared into the central ear artery pointing towards the head.
- 5) Aspirate the syringe slightly to see if any blood appears in the needle hub.
- 6) If no blood appears in the needle hub, stop aspirating, almost pull out the needle from the vein with the needle tip still remaining in the animal, change the insertion angle and try again.
- 7) Continue to gently aspirate the blood until there is sufficient volume.
- 8) Remove the needle in the same angle of insertion and if there is any bleeding at the puncture site, apply pressure with a clean dry guaze swab until bleeding stops.

### **Cardiac Bleeding**

- 1) For greater volume of blood, cardiac bleeding is recommended.
- 2) This procedure must be done under anesthesia.
- 3) Position the animal in lateral recumbency, where its right side lies on the table and the left side exposed.
- 4) Locate the heart between the 3<sup>rd</sup> and 4<sup>th</sup> intercostal space or at the point of the elbow.
- 5) Also feel for the location with the strongest heartbeat.
- 6) Disinfect the area with 70% ethanol solution.
- 7) For large volumes of blood, the syringe will need to be removed from the needle and another syringe attached to the needle while the needle is still in the heart.
- 8) Insert the needle between 3<sup>rd</sup> and 4<sup>th</sup> intercostal space.
- 9) Proper placement was obtained when needle and syringe move with the heartbeat, or if blood comes out as the syringe is aspirated.
- 10) Aspirate the required amount of blood.
- 11) For large volumes of blood, the syringe will need to be removed from the needle and another syringe attached to the needle while the needle is still in the heart.
- 12) The rabbit must be euthanized after this procedure.