Institutional Animal Care and Use Committee		UNTHSC
Title: Blood Collection Guidelines		
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A. BACKGROUND INFORMATION

a. To avoid complications such as hemorrhagic shock and to minimize pain and distress to the animals, blood collection procedures must be performed within recommended guidelines.
b. The information in this Standard Operating Procedure (SOP) is intended to assist researchers to choose the most appropriate technique for blood sampling in a humane and efficient way.
c. Blood samples may be needed for analysis of:
    i. Biochemical, metabolic, toxicological or immunological parameters.
    ii. Examination or culture of micro-organisms
    iii. Production of antibodies

B. RESPONSIBILITIES

a. It is the responsibility of the investigator to:
    i. Choose the best method of blood collection for the volume needed and the welfare of the animal.
    ii. Describe the method of blood collection in the animal use protocol (including the purpose, the route, duration and frequency of sampling, and when the sample is taken, for example as a terminal procedure).
    iii. Be properly trained or assure that staff performing blood collection is properly trained in blood collection techniques. If the investigator or their staff need training, the investigator shall contact DLAM staff.
    iv. To follow the blood collection guidelines in this SOP.
b. It is the responsibility of the Institutional Animal Care and Use Committee (IACUC) to assure that investigators and staff are following this SOP.

C. PROCEDURES

a. Venipuncture
    i. Blood can be collected from all species using venipuncture methods. Below is a list of the most common methods and the species they can be used on. If the method or species that you will use is not listed, please consult with the Veterinarian.
    ii. Superficial Temporal Vein, or Mandibular Vein (adult mice only)
        1. This is a humane, efficient, and economical way to bleed laboratory mice.

2. A lancet (Goldenrod Animal Lancet found at http://www.medipoint.com/html/animal_lancets.html) or a needle can be used.
3. No anesthesia is required.
4. Multiple blood draws are possible using this method (from the same mouse multiple times in a day or daily, depending on the volume taken).
5. Large volumes can be taken.

iii. Retro-Orbital Sinus/Plexus (mice, rats, hamsters)

1. The animal must be anesthetized with general anesthesia.
2. This procedure must be performed by skilled personnel.
3. A minimum of 10 days should be allowed for tissue repair before repeat sampling of the same orbit.
4. Care must be taken to ensure adequate hemostasis following the procedure.
5. The use of sterile capillary tubes and pipettes will prevent infection and potential long-term damage to the eye. Assure that the edges of the capillary tubes are smooth to decrease the likelihood of damage to the eye.
6. This method is considered to be painful. Topical ophthalmic anesthetic may provide pain relief after the procedure. Justification to use this method must be included in the protocol.
7. Large volumes can be collected.

iv. Saphenous Vein (mice, rats, hamsters, guinea pigs)

1. Anesthesia is not required.
2. After the skin is shaved, the vein is punctured percutaneously and blood is passively collected as it pools on the skin or with a capillary tube.
3. Moderate to large volumes can be collected.

v. Tail Vein (mice, rats)

1. Anesthesia is not required.
2. The animal should be effectively restrained.
3. Can be done my cannulating the blood vessel (with a needle) or by superficially nicking the vessel.
4. With tail nicking, the clot/scab can be gently removed for repeated small samples (example: for glucose measurements).
5. Warming the tail with a heat lamp or warm water bath will aid with blood collection.
6. Pressure must be applied to the bleeding site to stop the flow of blood and/or silver nitrate may be applied to stop the bleeding.
7. A small volume can be collected in mice and a moderate volume can be collected in rats.

vi. Marginal Ear Vein (rabbit)

1. The rabbit should be placed in a restrainer.
2. The marginal ear vein can be cut to collect blood in a tube or a needle can be used.

3. The ear can be warmed by hand before blood collection.
4. Local anesthetic cream can be applied 30 minutes before sampling.
5. Blood flow should be stopped before placing the animal back into its home cage.
6. Small to moderate amounts of blood can be collected.

vii. Ear Vein (pig)

1. Local anesthesia can be applied to the site 30 minutes before sample collection.
2. The ear should be warmed to dilate the vessel.
3. Blood flow should be stopped by applying pressure for approximately two minutes.
4. Small volumes can be collected.

b. Cannulation (all species)

i. When multiple blood samples are needed over a period of time, a cannulated animal (with the cannula surgically implanted by the animal vendor) can be used. Surgery to implant a cannula can also be done by the lab, as written and approved in the animal use protocol.

ii. Blood volumes taken must follow the guidelines below.

c. Cardiac Puncture (mice, rats, hamsters, guinea pigs, rabbit)

i. This is a terminal procedure and must be performed under deep general anesthesia.

ii. Maximum volume of blood can be collected.

d. Points to remember

i. Before starting the blood sample collection, assure that all necessary supplies are available (needles, lancets, blood collection tubes, anesthetic, fluid replacement, gauze, etc.).

ii. Not more than three attempts should be made to collect blood from any one animal at any one time to prevent pain and distress.

iii. To improve vasodilation, it may be helpful to warm the entire animal or part of the animal. Warming chambers, heat lamps, warm water baths or similar can be used.

iv. Fluid replacement should be considered if taking large volumes.

e. Maximum volumes

i. Approximately 10% of the total circulating blood volume (CBV) can be safely removed every 2 to 4 weeks, 7.5% every 7 days, and 1% every 24 hours.

ii. Volumes greater than what is recommended must be justified in the animal use protocol and appropriate fluid replacement provided.

iii. The chart below shows the species, blood volume (in ml/kg), example body weights and maximum sample volumes for the stated sampling frequencies.

1. To calculate the maximum volume for an animal of a specific weight use the following calculation:

a. Body weight x blood volume for that species = CBV. CBV x percent allowed = maximum volume.

b. Example: 25 g mouse: CBV = 0.025 kg x 75 ml/kg = 1.875 ml. Maximum volume to be taken every 7 days (7.5% of CBV) = 0.075 x 1.875 = 0.14 ml.

Species	Blood Volume (ml/kg)	Body Weight	1% CBV (ml) every 24 hours	7.5% CBV (ml) every 7 days	10% CBV (ml) every 2-4 weeks
Mouse	75 ml/kg	20 g	.015	.11	.15
		30 g	.022	.17	.22
		40 g	.03	.22	.3
Rat	65 ml/kg	125 g	.08	.6	.81
		150 g	.097	.73	.97
		200 g	.13	.97	1.3
		250 g	.16	1.2	1.6
		300 g	.19	1.46	1.95
		350 g	.28	1.7	2.28
Hamster	80 ml/kg	85 g	.068	.51	.68
		100 g	.08	.6	.8
		150 g	.12	.9	1.2
Guinea Pig	70 ml/kg	600 g	.42	3.15	4.2
		800 g	.56	4.2	5.6
Rabbit	55 ml/kg	1 kg	.55	4.125	5.5
		2 kg	1.1	8.25	11
		3 kg	1.65	12.375	16.5
		4 kg	2.2	16.5	22
		5 kg	2.75	20.625	27.5
Pig	70 ml/kg	50 kg	35	262.5	350

CBV = Circulating Blood Volume

D. REFERENCES

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