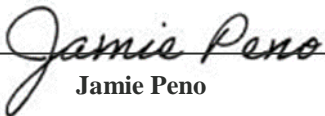
 <div> <div>DIVISION OF RESEARCH &amp; INNOVATION</div> <div>Research Integrity &amp; Compliance</div> </div>		<b>Institutional Animal Care and Use Committee</b>  <b>Standard Operating Procedures</b>	
<p align="center"><b>Title: Blood Collection in Research Animals</b></p>			
<b>Effective Date:</b>	December 22, 2020	<b>Document Number:</b>	IACUC-SOP-02-22.00
<b>Approval/Date:</b>  <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">               _____              Jamie Peno              Director, Research Integrity and Compliance           </div> <div style="text-align: center;">             12/22/2020              _____              Date           </div> </div>			
<b>REVISION HISTORY</b>			
<b>Date</b>	<b>Section</b>	<b>Author</b>	

## PURPOSE

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. Principal Investigators should use these guidelines to choose the most appropriate technique for blood sampling in a humane and efficient manner. Deviations from these guidelines should be specifically approved by the UNT IACUC and Attending Veterinarian in an approved Animal Use Protocol with provided justification.

## SCOPE

It is the responsibility of the Principal Investigator to choose the best method of blood collection for the volume needed and the welfare of the animal, based on these guidelines.

It is the responsibility of the PI to 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).

It is the responsibility of the PI to ensure personnel performing blood collection in research animals have appropriate and documented training in blood collection techniques in the species being used and to ensure that they are approved to do so in a UNT approved AUP.

It is the responsibility of the IACUC to assure that investigators and staff are following this SOP.

## DEFINITIONS AND ABBREVIATIONS

UNT- University of North Texas, Denton

IACUC- Institutional Animal Care and Use Committee  
SOP/SOP's- Standard Operating Procedure(s)  
AUP- Animal Use Protocol  
PI- Principal Investigator  
AV- Attending Veterinarian  
CBV- Circulating Blood Volume

## PROCEDURES

- I. Blood samples from research animals may be needed for analysis of:
  - A. Biochemical, metabolic, toxicological, or immunological parameters.
  - B. Examination or culture of micro-organisms
  - C. Production of antibodiesParenteral Administration of Substances
- II. Methods of Blood Collection
  - A. Venipuncture
    1. 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.
      - a) Superficial Temporal Vein, or Mandibular Vein (adult mice only)
        - i. This is a humane, efficient, and economical way to bleed laboratory mice.
        - ii. A lancet (Goldenrod Animal Lancet found at [http://www.medipoint.com/html/animal\\_lancets.html](http://www.medipoint.com/html/animal_lancets.html) or a needle can be used.
        - iii. No anesthesia is required.
        - iv. Multiple blood draws are possible using this method (from the same mouse multiple times in a day or daily, depending on the volume taken).
        - v. Large volumes can be taken.
      - b) Retro-Orbital Sinus/Plexus (mice, rats, hamsters)
        - i. The animal must be anesthetized with general anesthesia.
        - ii. This procedure must be performed by skilled personnel.
        - iii. A minimum of 10 days should be allowed for tissue repair before repeat sampling of the same orbit.
        - iv. Care must be taken to ensure adequate hemostasis following the procedure.
        - v. 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.
        - vi. This method is considered to be painful. Topical ophthalmic anesthetic may provide pain relief after the procedure.
        - vii. Justification to use this method must be included in the protocol.
        - viii. Large volumes can be collected.
      - c) Saphenous Vein (mice, rats, hamsters, guinea pigs)
        - i. Anesthesia is not required.
        - ii. 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.
        - iii. Moderate to large volumes can be collected.
      - d) Tail Vein (mice, rats)
        - i. Anesthesia is not required.
        - ii. The animal should be effectively restrained.
        - iii. Can be done by cannulating the blood vessel (with a needle) or by superficially nicking the vessel.
        - iv. With tail nicking, the clot/scab can be gently removed for repeated small

- samples (example: for glucose measurements).
  - v. Warming the tail with a heat lamp or warm water bath will aid with blood collection.
  - vi. 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.
  - vii. A small volume can be collected in mice and a moderate volume can be collected in rats.
- e) Marginal Ear Vein (rabbit)
  - i. The rabbit should be placed in a restrainer.
  - ii. The marginal ear vein can be cut to collect blood in a tube or a needle can be used.
  - iii. The ear can be warmed by hand before blood collection.
  - iv. Local anesthetic cream can be applied 30 minutes before sampling.
  - v. Blood flow should be stopped before placing the animal back into its home cage.
  - vi. Small to moderate amounts of blood can be collected.

**B. Cannulation (all species)**

1. When multiple blood samples are needed over a period of time, a cannulated animal (with the cannula surgically implanted) can be used.
  - a) Surgery to implant a cannula can be done by the lab, as written and approved in the animal use protocol or can be performed by the vendor prior to animal shipment.
2. Blood volumes taken must follow the guidelines below:
  - a) 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.
3. Points to remember:
  - a) Before starting the blood sample collection, assure that all necessary supplies are available (needles, lancets, blood collection tubes, anesthetic, fluid replacement, gauze, etc.).
  - b) Not more than three attempts should be made to collect blood from anyone animal at any one time to prevent complications and/or pain and distress.
  - c) To improve vasodilation, it may be helpful to warm the entire animal or part of the animal.
    - i. Warming chambers, heat lamps, warm water baths or similar can be used.
  - d) Fluid replacement should be considered if taking large volumes.

**III. Maximum volumes**

- A.** 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.
1. CBV should be calculated using recent animal weights.
  2. Body weight x blood volume for that species = CBV.
  3. CBV x percent allowed = maximum volume.
  4. Example: 25 g mouse:  $CBV = 0.025 \text{ kg} \times 75 \text{ ml/kg} = 1.875 \text{ ml}$ .  
 Maximum volume to be taken every 7 days (7.5% of CBV) =  $0.075 \times 1.875 = 0.14 \text{ ml}$ .
- B.** Volumes greater than what is recommended must be justified and approved in the Animal Use Protocol and appropriate fluid replacement provided.
- C.** The chart below shows the species, blood volume (in ml/kg), example body weights and maximum sample volumes for the stated sampling frequencies:

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

#### I. CBV = Circulating Blood Volume

## REFERENCES

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## APPENDICES

### IACUC Standard Operating Procedures