

UBC ANIMAL CARE COMMITTEE

TECH 03a – Intravenous Tail Vein Injections in the Adult Mouse SOP

Last date revised: December 2020

Date approved: February 2021

Version No. 2

PURPOSE:

- To describe the procedure for administering intravenous (IV) injections into the lateral tail veins of adult mice.
- This Standard Operating Procedure (SOP) follows the Canadian Council on Animal Care (CCAC) current guidelines for acceptable injection volumes and sites in rodents.

RESPONSIBILITY:

- Those trained persons listed on an approved Animal Care Committee (ACC) Animal Care Protocol who are responsible for performing intravenous injections.
- All animal users performing intravenous tail vein injections in rodents must have successfully completed the UBC Animal Care Services (or equivalent) Introduction to Working with Rodents in Research (IWRR), Restraint/SQ/IP (RSCIP) injection, Rodent Anesthesia (RA; if applicable) and the Tail Vein (TVINJ) Injection courses.

MATERIALS: *(can be purchased from Animal Care Services)*

- Appropriately sized animal restrainer (if performing conscious tail vein injections)
- Anesthetic machine, induction chamber and supportive care equipment (if applicable)
- Sterile needles (27-30 G, 1/2" or smaller)
- Sterile syringes (0.3-1.0 ml)
- Sterile substance to be injected (in sterile, multi-dose vial)
- 70% isopropyl alcohol
- 2" x 2" gauze
- Cotton Tipped Applicators
- Sharps container
- Weigh Scale
- Safe heat source for heating mice prior to injecting
- Electronic thermometer (to measure temperature of heated environment)



Table 1 - RECOMMENDED NEEDLE SIZE AND MAXIMUM VOLUME OF ADMINISTRATION

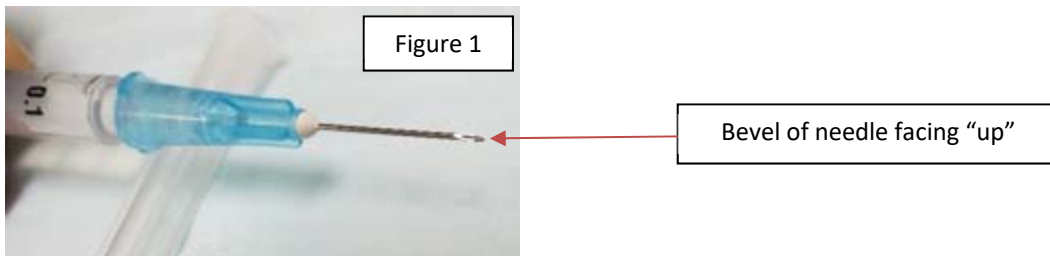
Species	Needle Gauge	Maximum Volume for IV Injection*
Mouse	27-30 G	5ml/kg – bolus injection **
		10ml/kg – slow bolus injection **

*Greater than the recommended volume should not be given unless justified and approved on the Animal Care Protocol and increased monitoring for complications have been implemented.

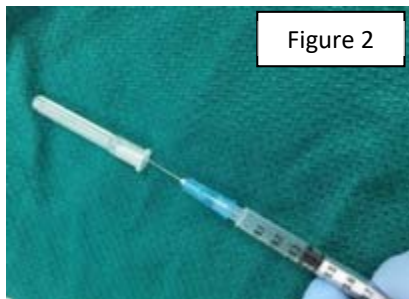
** ≤5 ml/kg injected as a bolus (over 1-2 seconds) is the recommended maximum volume to reduce the incidence of hemodilution, distress, and abnormal pulmonary and cardiac effects on the animal. Larger bolus volumes (up to 10 ml/kg) are tolerated if injected more slowly (typically over 5-6 seconds).

PROCEDURE:

1. Warm up the container of substance to be administered.
 - a. E.g. heating pad, water bath, or holding vial/prepared syringe in hand to warm up
 - b. Do not overheat beyond 37°C
2. Weigh the animal and calculate the volume to be administered (refer to Table 1 for maximum recommended volumes and below for how to calculate volume).
3. Disinfect the top of the container with an alcohol-moistened gauze.
4. Safely uncap the needle. Maintain sterility of the needle.
5. Draw up the amount of pre-warmed solution to be administered into the syringe and needle. Ensure that no air bubbles are present in the syringe or substance to be injected.
 - a. It is helpful to turn the needle so that the bevel points “up”, and turn the syringe so the numbers on the syringe barrel can be read (see Figure 1).



6. Slide the needle back into the cap loosely without handling either the cap or the needle (see Figures 2 and 3 below) while you restrain the animal.
 - a. Take care to avoid touching the needle to a non-sterile surface or blunting the needle tip.

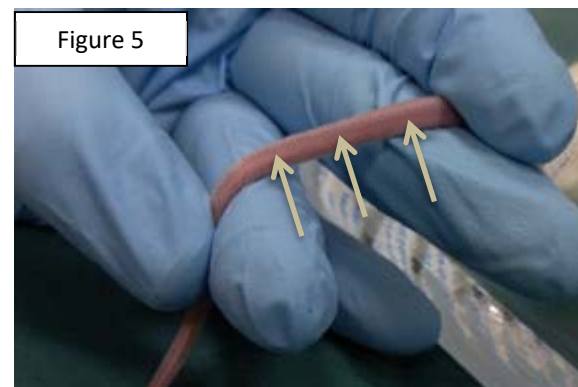
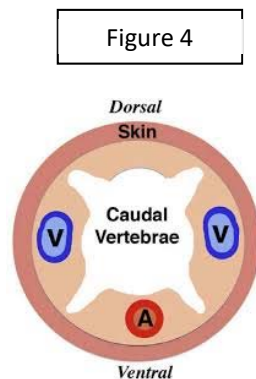


7. Have all equipment and supplies prepared (alcohol, gauze/cotton tipped applicators, needle/syringe loaded with substance to be injected) before placing the animal in a restraint device or anesthetizing.
8. Vasodilate the veins of mice prior to intravenous injections to help with visualization and injection into the vein. This can be performed in 1 of 3 ways: placing the cage on top of a safe heat source, exposing the mice to a safe heat source from above the cage, or directly heating the tail itself. **The animal(s) must not be left unattended.**
 - a. If all mice in a cage are to be injected, place home cage on or under the heat source. If only one mouse in a cage is to be injected, place that mouse in a separate cage on or under the heat source.
 - b. If using a heat source placed above the cage, position it 1 foot (30 cm) above the mice so they won't overheat. Place your hand just over the mice and leave it in place for several seconds to assess if the temperature feels acceptable; if not, adjust the position of the heat source accordingly.
 - c. The heat source should be positioned so only half the cage is on or under the heat so mice can move to the cooler end of the cage if they begin to overheat.

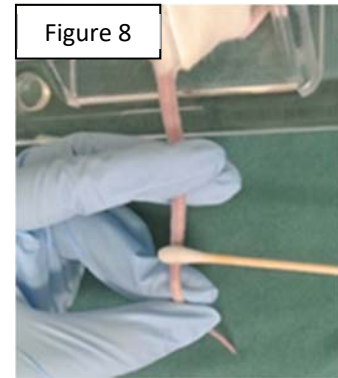
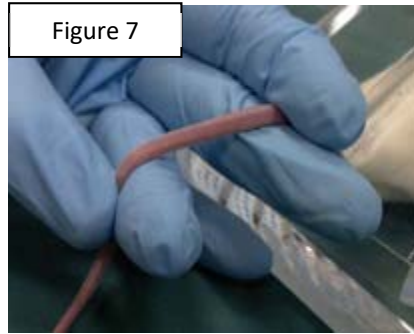
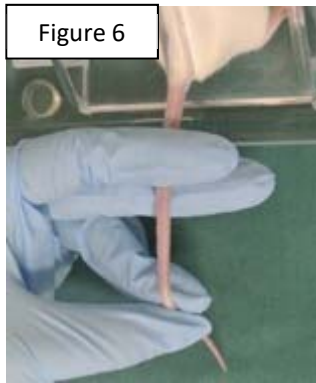
- i. Examples of heat sources, in order of preference, are hot water heating pads, electric heating pads or heat lamps.

Note: Care must be taken if using heat lamps or heating pads since they pose a higher risk of causing hyperthermia (overheating) and burn related injuries (especially the ears) to the animals.

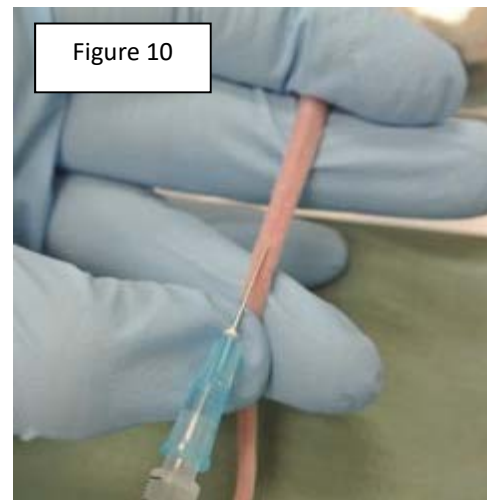
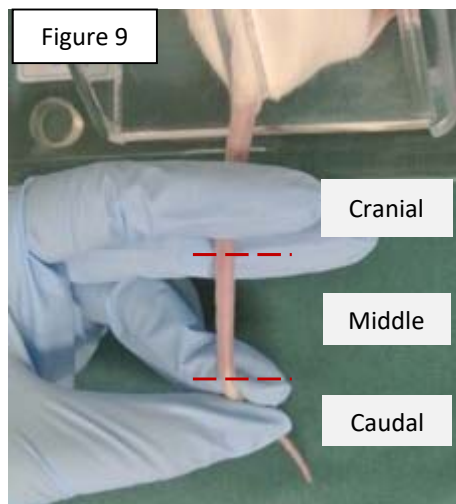
- d. Increase the environmental temperature in the cage to approximately 28-30°C for up to 30 minutes. To prevent mouse overheating, use an electronic thermometer to ensure that the ambient temperature does not exceed 30°C.
 - e. For heating the tail of the mouse directly, restrain the mouse (i.e. place in a restrainer if conscious or anesthetize) and:
 - i. Submerge the tail in warm water for 1-2 minutes
 - ii. Wrap tail in an exam glove filled with warm water for 1-2 minutes
 - iii. Place tail on heating pad and cover with hand
 - iv. The warm water or surface of the heating pad should be between 30 and 35°C (measured with a thermometer; if no thermometer available, ensure it is comfortable to hold against the skin of the inside of the wrist for 1 minute).
 - v. Ensure the animal does not overheat
 - vi. Dry the tail before attempting the tail vein injection.
9. Place the animal in a restraint device, OR, if anesthetizing, place one mouse at a time in a warmed induction chamber. Once anesthetized, move it to a nose cone and maintain on isoflurane for the procedure.
- NOTE: If anesthetizing the animal, perform the IV injection as quickly as possible after removal from the induction chamber, since vasoconstriction from anesthesia will make it difficult to access the vein.*
10. Grasp the warmed tail with your non-dominant hand and locate one of the two lateral tail veins.
- a. You may have to gently rotate the tail slightly (see Figures 4 (blue V) & 5 (grey arrows) for location).



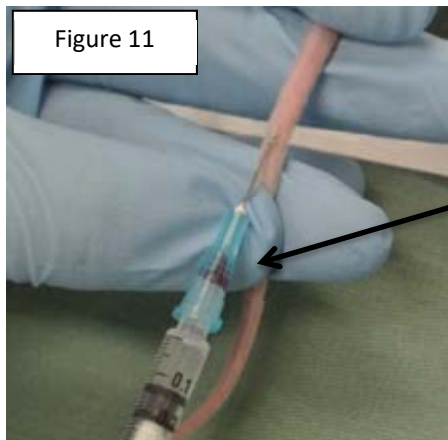
11. Occlude the tail vein (optional) and apply gentle traction to the tail.
 - a. Gently squeeze the tail close to its base with your first finger on top and your middle finger underneath to hold the tail in place and occlude the vein (see Figures 5 & 6).
 - b. With your ring finger under the tail, use your thumb to gently roll the tip of the tail over your ring finger (see Figures 6 and 7).
 - c. Apply gentle traction between the cranial and caudal ends of the tail to keep the tail taut (not flexible) or the needle will be difficult to insert under the skin. (see Figure 7).



12. Swab the tail with alcohol to increase visibility of the vein and clean the skin (see Figure 8).
 - a. Use a cotton-tipped applicator dampened with 70% isopropyl alcohol to wipe over a lateral vein between your two sets of fingers.
 - b. Do not soak the tail in alcohol as that may cause vasoconstriction.
13. Insert the needle into the distal portion of the middle 1/3 of the tail with the dominant hand (see Figure 9). See Appendix 1 for different examples of holding the syringe.
 - a. Slide the needle a few millimeters through the skin and into the tail vein with the bevel facing up (see Figure 10).
 - b. Ensure the needle is completely parallel to the vein and just slightly under the skin (not too deep into the tail).
 - c. Be prepared for the mouse to react and move its tail when it feels the needle (if it is conscious).
 - d. A successful insertion should feel as if the needle 'slides' easily into the vein.
 - e. If a second attempt at accessing the vein is needed, move further up the tail (toward the mouse's body).

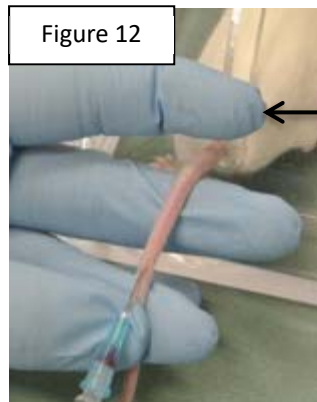


14. Apply gentle negative pressure to the syringe (i.e. pull plunger back) to look for blood (**optional**).
 - a. Do not allow the needle to move further in or out when pulling back on the plunger or injecting.
 - b. Wait 1-2 seconds for blood to enter the hub of the needle indicating the needle is in the vein (see Figure 11 – black arrow).

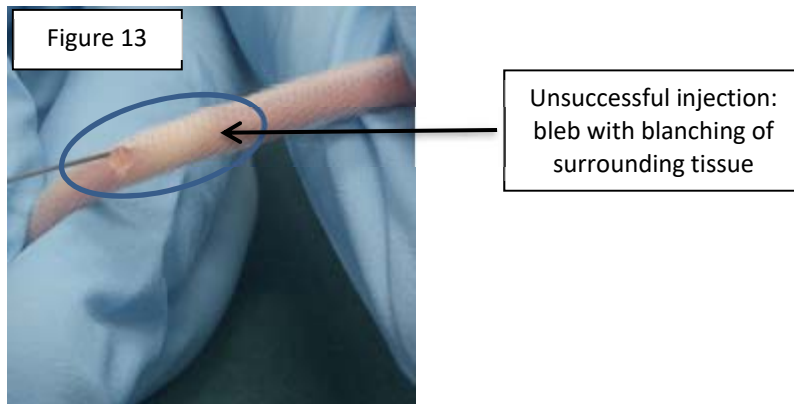


Note: *If using insulin syringes, the plunger doesn't move as freely as it does in regular syringes. If the needle is not in the vessel, and the plunger is pulled back to look for blood, air can enter the syringe. If this happens, you must remove the needle from the tail and remove the air before performing the injection.*

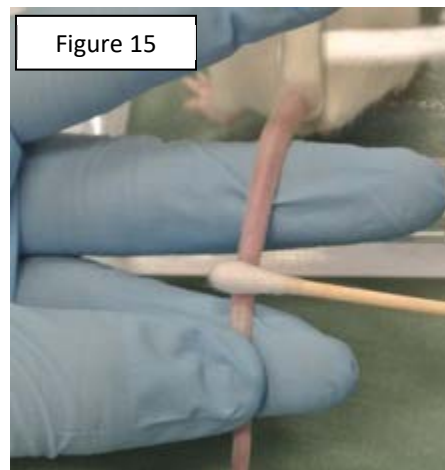
15. Gently release the vein where occluded and inject the substance. The speed of injection is dependent on the volume being injected.
 - a. Slowly lift the index finger holding the top/base of the tail (see Figure 12 – black arrow).
 - Move the finger slowly so that the mouse does not react and move its tail (if conscious).
 - b. Ensure you still have a good grip on the distal end of the tail with thumb and ring finger.



16. Slowly press the plunger of the syringe to inject the substance into the vein.
 - a. Start by injecting a very small volume to test whether you are in the vein.
 - b. If the needle is in the vein, there will be no resistance while injecting and the vein will blanch (become clear as the blood is no longer seen while injecting).
 - c. If not in the vein, you will feel some resistance when injecting and the fluid will cause blanching around the vein or form a subcutaneous 'bleb' (swelling; see Figure 13).
 - d. If not in the vein, first attempt to redirect the needle slightly without coming out of the tail. Move the needle a millimeter further into the tail without going too deep under the skin.
 - e. If unsuccessful, remove needle from the tail and re-attempt injection at a location more cranial to (up) the tail towards the animal's body, or into the other lateral tail vein.
 - f. **No more than two attempts** should be made on each tail vein.



- 17.** After injecting, leave needle in tail for 2-3 seconds to allow injected substance to clear the vein, then remove the needle and immediately apply pressure to the puncture site to prevent unnecessary blood loss.
- As the needle is removed, begin immediate hemostasis by gently bending the tail over the middle finger, where the needle was inserted, in order to collapse the vein and stop the blood flowing (see Figure 14).
 - Apply pressure with a dry piece of gauze or cotton tipped applicator until the bleeding has stopped (approximately 10 seconds). Once pressure is applied over the injection site, the bend in the tail can be relaxed (see Figure 15).



- 18.** Discard syringe and needle directly into a sharps container.
- DO NOT RECAP once it has been in an animal.
- 19.** Once bleeding has stopped, either remove animal from the restrainer or recover animal from anesthesia.
- Clean the restraint device between each animal to remove blood, urine, feces and stress pheromones
- 20.** If using inhalant anesthesia, turn off the vaporizer and place animal in a clean recovery cage with no bedding but the bottom covered in paper towel (no longer heated) and observe for 5-10 minutes for complications (see below).

a. Provide oxygen and heat support, until the animal starts to wake up and move around. Ensure the animal is fully conscious before placing it back in its home cage.

b. Return the animal to its home cage once fully recovered.

*** Do not leave the mouse unattended until it is fully conscious and able to care for itself.**

21. Observe animal for 5-10 minutes for complications (see below).

a. Monitor the animal for 5-10 minutes to ensure hemostasis (bleeding has stopped) and that the animal is acting normally.

b. If bleeding does begin again, remove the mouse from the cage and apply gentle pressure to the bleeding site until it stops. Remove all blood from the cage and clean the blood off the mouse.

22. Return the animal to its home cage once hemostasis is achieved.

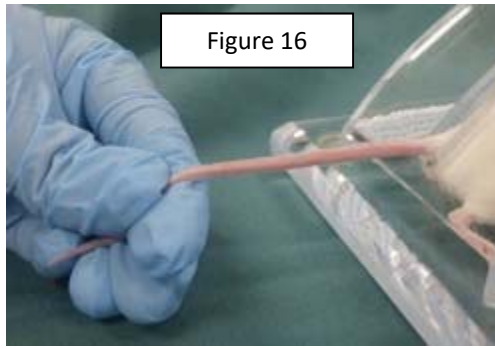
23. Note procedure (drug, dose, route, volume and any complications) on cage card/monitoring records.

CALCULATING VOLUME (IN ML) TO BE ADMINISTERED:

- Convert animal's weight from grams to kilograms
 - Divide the weight in grams by 1000
 - E.g. 30g mouse \div 1000 = 0.030kg
- Calculate the volume to give in ml
 - volume (mL) = dose (ml/kg) x weight of animal (kg)
 - E.g. For a 30g mouse getting 5 ml/kg
Volume (ml) = (5 ml/kg x 0.030 kg) = **0.15 ml**

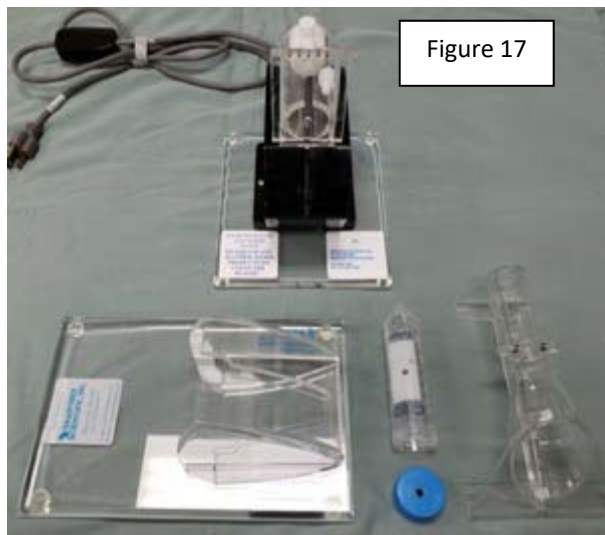
TIPS:

- Reasons why you may not see blood in the needle hub:
 - The needle is not in the vein (perivascular): Without taking the needle out of the tail, make slight adjustments (1-2 mm at a time) from side to side or back and forth - pull the needle back slightly, redirect the needle and slowly advance the needle forward again. If not successful, you will have to remove the needle and re-attempt in a new location.
 - The animal's tail is not warm enough/vein is not dilated enough: Warm tail and try again.
 - Pulling back too much on plunger may collapse vein against the side of the needle, so even if you are in the vein, you will not see blood in the hub: Only gently pull back a small amount or do not pull back the plunger at all if confident the needle is in the vein.
- If sufficiently vasodilated, some people do not occlude the vein and instead apply gentle traction to the tail to hold it straight and taut while injecting into the vein (see Figure 16 below).
** It is important to be aware of how tight the tail is being grasped. Too much pressure/pinching, can result in bruising and necrosis of the tip of the tail.*



- When injecting cells intravenously, it is not recommended to pull back on the plunger prior to injection as it may result in clumping of the cells in the syringe.
 - Inject slowly once you feel the needle is in the correct location (moving freely in the vein)
 - Look for clearing of the tail vein (blanching) as you inject
 - Ensure no swelling (“bleb”) forms under the skin which indicates the injection is going perivascular; if seen, stop the injection, remove needle from vein and re-insert the needle at a location closer to the base of the tail

There are multiple types of restrainers available (see Figure 17). Contact your facility manager or facility Veterinarian for sources.



IMPORTANT NOTES:

- Use the manufacturer’s recommended route of injection since some drugs may have adverse side effects or cause discomfort if injected via a non-recommended route.
- Compounds must be sterile, formulated for in vivo use and physiologically compatible unless otherwise approved in the Animal Care Protocol since systemic infection, clinical illness and/or irritation at the site of injection may occur.
- A new sterile syringe and needle must be used for each animal.
- Ensure no air is in the syringe or needle as it can cause a fatal air embolus if injected.
- The volume to be injected should be the smallest volume possible and not exceed the current recommended volume guidelines (see Table 1 above).
- Follow the recommended needle size (see Table 1 above). Using a larger than recommended needle size must be approved on the Animal Care Protocol (i.e. if injecting viscous liquids).

- Warm substances to body temperature, if this does not damage the compound, since injection of cold substances can cause discomfort and influences the absorption of the drug.
- If injecting a hazardous substance (biohazard, radiation hazard or chemical hazard), include precautions in the Animal Care Protocol for human safety (e.g. anesthesia).

COMPLICATIONS:

- **Perivascular (outside the vein) injection of substances or injections of irritating substances:**
 - **Cause:** Reaction to the injection of an irritating substance outside the vein (as the needle may have gone too deep, alongside, or through the vessel).
 - **Clinical signs:**
 - Pain, redness
 - Infection
 - Sloughing or necrosis of the tissues surrounding the injection site
 - Response:** Contact your Facility Veterinarian for treatment options, which may include analgesics, antibiotics and/or topical treatment of any wounds. Severe wounds or necrosis will require euthanasia.
- **Vasculitis (inflammation or irritation of the vessels)**
 - **Cause:** Reaction of the vessel walls to an irritating substance even though the injection was successful.
 - **Clinical signs:**
 - Redness and swelling along the length of the vessel
 - Decrease of loss of circulation to the tip of the tail (tail tip goes red, blue or black)
 - Difficulty in additional injections using that tail vein
 - Animal demonstrates pain during the injection (struggling during injection and not just when needle enters skin, vocalizing)
 - Difficult to inject even when in blood vessel (bleb forms or back pressure when injecting).
 - **Response:** Stop injecting the substance. Contact your facility Veterinarian for treatment options if tail is damaged or painful, and to discuss options for the irritating substance (further dilution, adjustment of pH, etc.).
- **Hyperthermia (Overheating):**
 - **Cause:** The animal is exposed to temperatures that are too hot and cannot get away. E.g. hot spots on a heating pad, heating pad temperature is set too high, or heat lamp placed too close to the cage.
 - **Clinical signs:**
 - Bright red extremities
 - Rapid respiration or open mouth breathing
 - Licking of tail and feet/increased salivation
 - Attempting to escape the heat (jumping off the cage bottom or climbing on top of cage mates)
 - Decreased activity or lying flat on the cage bottom as far from the heat source as possible.
 - Response:** Immediately remove animal from the heat source or restraint device and place in a cool cage (not on heat) to cool down. If animal is not moving normally, administer oxygen and wet the tail and feet with cool water. Once the animal is acting normally (normal respiratory effort/rate and extremities are pink rather than bright red), you can attempt your procedure again but take care not to overheat the animal (move heat source further away or

turn down temperature of heat source; ensure part of the cage is not on the heat source to allow animal to escape the heat if desired).

- **Burns:**

- **Cause:** The heat source has damaged the tissues (temperature was too high or too close to animal). Typically heat lamps are associated with burns to the ears. Heating pads can cause burns to tissues in direct contact with the pad.

- **Clinical signs:**

- Pain, scratching or licking at affected area
- Dark red/black areas on the affected tissue
- Ears will develop shriveled, dry, crusted edges
- Can lead to necrosis or sloughing of the injured tissue

Response: Typically, the animal must be euthanized but minor burns may respond to treatment with facility Veterinarian consultation. Provide analgesia until a treatment plan or euthanasia is determined.

- **Bruising of tail or around insertion site of the needle**

- **Cause:** Tail was held too tightly or pinched; repeated attempts at injection
- **Clinical signs:** Purple discolouration of skin; extensive bruising can lead to necrosis or sloughing of the tail.

Response: Provide analgesia. Monitor the tail closely until it has healed. If any redness or black appearance is seen, contact your facility Veterinarian to determine what treatment is needed.

- **Bleeding:**

- **Cause:** The venipuncture site was not “held off” long enough to allow a stable clot to form.
- **Clinical signs:** After initial hemostasis, the needle puncture site may start to bleed again.

Response: Re-apply pressure to the site, with a dry gauze or cotton tipped applicator, until bleeding has stopped (apply pressure for at least 30 seconds before checking). Hemostasis may take up to a few minutes, depending on the animal’s temperature and what gauge needle was used. If more than 3-4 drops of blood were lost, administer 20 ml/kg of SQ Lactated Ringer’s or 0.9% Normal Saline and monitor the colour of the extremities and behaviour. If animal’s extremities are pale or the animal appears weak or lethargic, contact your facility Veterinarian.

- **Injection of air bubbles or particulate matter:**

- **Clinical signs:** Very small air bubbles, emulsions, or suspended cells usually have no negative clinical effect on the animal but small air bubbles will result in inaccurate dosing. Larger air bubbles, particulate matter or clumped cells may become lodged in blood vessels and if the location of this blockage is a vital organ, such as the brain, heart or lungs, it may impact organ function. The animal may show necrosis of extremities, altered breathing patterns or rate, weakness or paralysis, or death.

Response: If abnormal clinical signs are seen after intravenous injection, administer oxygen and contact your facility Veterinarian to determine what treatment is needed. If animal is having trouble breathing or is in distress, the animal should be euthanized.

- **Injection of non-sterile substances:**

- **Cause:** The compound to be injection was not sterile or the needle/syringe was contaminated. Note: all substances for injection must be sterile and formulated for use in vivo unless otherwise approved on the animal care protocol
- **Clinical signs:** Signs may not be seen for hours to days after the injection
 - Chewing at injection site or along the tail vein causing trauma
 - Wound or lesion may appear at site of injection or along tail vein

- Loss of circulation to distal portion of the tail (tissue will become necrotic and become black and dry)
- Animal may develop signs of a systemic infection (weight loss, hunching, piloerection, lethargy, swelling of extremities (feet or legs), weakness or death).
- **Response:** Contact your facility Veterinarian to determine whether any treatment is available. If the tail becomes necrotic, immediate humane euthanasia is to be performed.

REFERENCES:

- A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes; Diehl, K et al. 2001
<http://onlinelibrary.wiley.com/doi/10.1002/jat.727/abstract>
- Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider; Turner, Pekow, Vasbinder, Brabb, 2011
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3189662/>
- Canadian Council on Animal Care (CCAC) guidelines: mice
https://www.ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Mice.pdf
- UBC Guidelines and Standard Operating Procedures (<https://animalcare.ubc.ca/animal-care-committee/sops-policies-and-guidelines>)
 - UBC ACC Guidelines and SOP for the Maintenance of Fluid Homeostasis in Animals
 - UBC ACC SOP: Adult Rodent Anesthesia

APPENDIX 1:

Examples on how to hold the syringe steady while pulling and pushing plunger of syringe during injection:

