

University Animal Care Committee

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 2

THE LABORATORY RAT

SUBSTANCE ADMINISTRATION AND BLOOD COLLECTION

Substance Administration:

- Subcutaneous injection
- Intramuscular injection
- Intraperitoneal injection

Blood collection:

- Saphenous bleed

The UACC would like to acknowledge the invaluable help of the Comparative Medicine Animal Resources Centre technicians in preparing this handout.

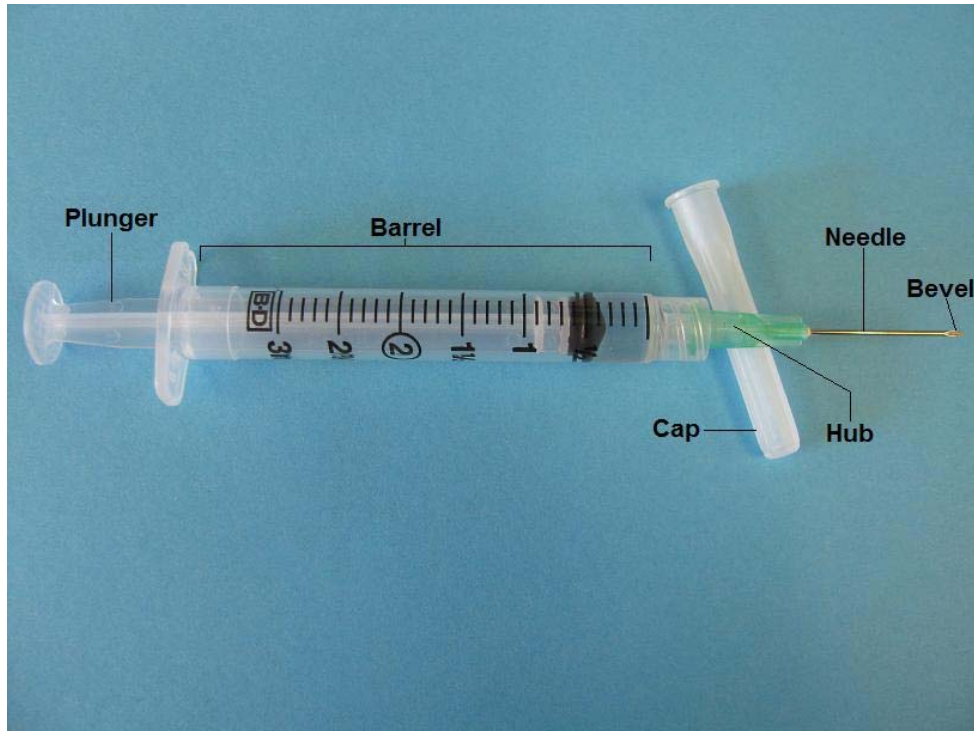
# SUBSTANCE ADMINISTRATION

## General Information

### Substance

- Verify that the pH of solutions injected subcutaneously or intramuscularly have a pH of 7.3 to 7.45 and that solutions are isotonic (same tonicity as blood; 280–310 mosm/L). Non-isotonic solutions must be injected slowly if the intraperitoneal or intravenous routes are used.
- Warm the solutions to body temperature (or at least room temperature) immediately prior to administration, if possible.
- Verify the solubility of the substance. Precipitation may cause the formation of large particles which, if injected intramuscularly, can be painful.
- Inject separate substances at different sites to avoid cross reaction of chemicals.
- Avoid injecting highly viscous liquids as they can cause discomfort and require a larger needle size for injection.
- Substances to be injected must be sterile as contamination can lead to infection or irritation of the injection site. Sterilize solutions by autoclaving or microfiltration and use aseptic technique for injection.

### Syringe anatomy



## Injections

- Do not inject into inflamed or damaged tissue.
- Check proper placement of the needle prior to injection. Withdraw on the syringe plunger; if blood enters the needle hub, the needle has entered a blood vessel. Withdraw the needle slightly and redirect it.
- No resistance should be encountered during injection. Do not apply overt pressure on the syringe plunger. The injected substance should flow freely to prevent any unnecessary pain and tissue damage.
- Give injections at a constant flow rate.
- If bleeding occurs after injection, apply pressure with gauze until bleeding stops.

## Needles

- Use the smallest gauge of needle possible that allows accurate injection of the substance.
- Always use sharp needles

### 1 Subcutaneous injection (SC)

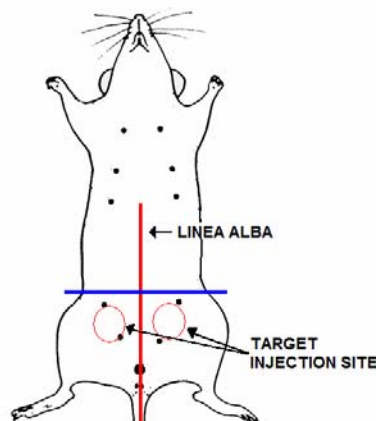
- Recommended injection site: loose skin over the neck and dorsum.
- Recommended volume: 0-5 ml/kg
- Needle size: 25G or smaller
- Subcutaneous administration should be limited to 2 to 3 sites per day.
- The rat can be placed in a restraining device (e.g towel or plastic conical restraining bag) or hand-held on the wire grid of the cage.
- Procedure:
  - Grasp a fold of skin over the neck or dorsum of the mouse with the tips of your middle finger and thumb.
  - Use your index finger to create a flat surface in the tented skin to create a space of injection often called the "tent".
  - Insert the needle, bevel up, in the lower part of the "tent" to avoid injuries to your fingers and direct it parallel to the mouse's body to make sure that you do not inadvertently puncture the body of the mouse.
  - Check proper placement of the needle prior to injection by withdrawing the syringe plunger and inject.
  - Return the animal in its home cage.

## 2. Intramuscular injection (IM)

- Recommended injection site: quadriceps or posterior thigh muscles.
- Recommended volume: 0-0.1 ml/per site
- Needle size: 25G or smaller
- Avoid trauma to the sciatic nerve and blood vessels by directing the needle away from the line of the bone.
- Intramuscular administration should be limited to 2 sites at one time.
- During an experiment, sites of injection should be alternate.
- Procedure
  - Using a towel or a conical plastic restraining bag
    - Wrap the rat in towel or insert it in the conical plastic restraining bag.
    - Hold the rat in the inner part of your non-dominant hand.
    - Take the external paw of the rat out of the restraining device and grab a fold of skin in front of the hip area between your thumb and index to extend the leg. (e.g. If you hold the rat with your left hand, you will extend his right hind paw).
    - Insert the needle perpendicular to the plane of the muscle in order to avoid the sciatic nerve which runs along the bone.
    - Check proper placement of the needle prior to injection by withdrawing on the syringe plunger and inject.
    - Return the animal to its home cage

## 3. Intraperitoneal injection (IM)

- Recommended injection site: Both side of the lower abdomen (On females, injection can be done between the two last nipples).
- During an experiment, sites of injection should be alternate.
- Recommended volume: 0-10 ml/kg
- Needle size: 23G to 25
- Procedure



- Using a conical plastic restraining bag
- Place the rat in the conical restraining bag.
- Hold the rat head down at a 30-45 degree angle.  
This allows the abdominal contents to move away from the injection site.
- Fold a part of the bag to have access to the lower abdomen.
- Place your needle parallel to the linea alba.
- Insert the needle with an angle of 30 to 45 degree in one of the two lower quadrants of the abdomen.
- Check proper placement of the needle prior to injection by withdrawing the syringe plunger and inject.
- Return the animal to its home cage.
- Using a towel
  - Wrap the rat in a towel. Make sure you can have access to the lower abdomen.
  - With your non-dominant hand, hold the ventral part of the rat against your body with your hand and a small portion of your forearm. The rat will have his head down and will be in a diagonal position.
  - With your thumb flip his paw to visualize the lower abdomen.  
(eg. If you hold the rat with your left hand, you will flip left hind paw of the rat)
  - Place your needle parallel to the linea alba.
  - Insert the needle in one of the two lower quadrants of the abdomen.
  - Check proper placement of the needle prior to injection by withdrawing on the syringe plunger and inject.
  - Return the animal to its home cage.

Note: A brown to greenish substance aspirated into the hub of the needle may indicate that the intestine was punctured. The contents of the syringe are then contaminated and must be discarded.

A yellow substance aspirated into the hub of the needle may indicate that the bladder was punctured. The contents of the syringe are then contaminated and must be discarded.

## BLOOD COLLECTION

### 1. Sapheneous bleed

- Survival procedure.
- Observe animals prior to sample collection for weakness, illness, dehydration, obesity, or anemia. If any of these signs are observed, contact the veterinary staff of your facility.
- Do not puncture a site presenting inflammation or a hematoma.
- Limit the number of punctures to four punctures per day with no more than two punctures per site.
- Replace isotonic fluids if >10% of total blood volume is required. It is recommended to replace collected blood volume by 3–4 times with isotonic fluids (i.e. fluids with same tonicity as blood, such as 0.9% saline, 5% dextrose or Lactated Ringer's solution).
- It is possible to warm the animal prior to the procedure to create a vasodilation using a red heat lamp, for example.
- Using an anesthetic agent will not be helpful as it decreases the peripheral blood pressure.
- Procedure
  - Weigh the animal.
  - Use the tables below to calculate the maximum amount of blood to be collected.
  - Hold the restrainer with your non-dominant hand in the inner part of your fingers.
  - Take the external paw of the rat out of the restraining device and grab a fold of skin in front of the hip area between your thumb and index to extend the leg.  
(e.g. If you hold the rat with your left hand, you will extend his right hind paw).
  - Clip the hair with an electric shaver or a scalpel blade or pluck the fur on the exterior side of the leg.
  - Apply petroleum jelly or other water-insoluble lubricant on the shaved area to prevent migration of blood into the surrounding hair.
  - Puncture the vein at a 90 degree angle using a 23G needle.
  - As drops of blood appear collect them directly into micro-hematocrit tubes or collection tubes.
  - To increase the blood flow during blood collection, gently flex the paw
  - Following blood collection, release the fold of skin between your thumb and index, then apply a gentle pressure on the leg with a piece of gauze until the bleeding stops.
  - Place the animal back in its home cage.

- Monitoring
  - If too much blood is withdrawn too rapidly or too frequently without replacement (approximately 2% of the animal's body weight at one time), the animal may go into hypovolemic shock.
  - Monitor the animal during and after blood sampling for signs of shock.
  - Contact the veterinary care staff if any signs of hypovolemic shock are observed. Signs of shock include the following:
    - Fast and thready pulse
    - Pale dry mucous membranes
    - Cold skin and extremities
    - Restlessness
    - Hyperventilation
    - Sub-normal body temperature

PERCENT OF BLOOD VOLUME COLLECTED IN A SINGLE SAMPLING	RECOVERY PERIOD (weeks)
7.5%	1
10%	2
15%	4

Single sampling means that you take the whole quantity of blood required during one blood collection.

PERCENT OF BLOOD VOLUME COLLECTED OVER A 24-HOUR PERIOD (MULTIPLE SAMPLES)	RECOVERY PERIOD (weeks)
7.5%	1
10 - 15%	2
20%	4

Multiple samples over a 24 hours period means that you do more than one blood collection during a 24 hour period.

SPECIES	CIRCULATING BLOOD VOLUME (ml/kg BW)	7.5% (ml/kg BW)	10% (ml/kg BW)	15% (ml/kg BW)	20% (ml/kg BW)
Rat	64	4.8	6.4	9.6	12.8

Sample calculation:

You need to collect 10% of the blood volume of a 200 g rat in a single sample.

The blood volume recommended to be collected in a single sampling of 10% is 6.4 ml/KG of body Weight (BW).

200g = 0.2Kg

0.2kg x 6.4 ml → 1.28 ml

*Therefore you can safely collect 1.28ml of blood from a 200g rat every 2 weeks.*

Blood volumes – Rat

Body weight (g)	Total circulating blood volume (mL)	Acceptable volume for collection (mL)			
		7.5%	10%	15%	20%
150	9.6	0.72	0.96	1.44	1.92
160	10.2	0.77	1.02	1.54	2.05
170	10.9	0.82	1.09	1.63	2.18
180	11.5	0.86	1.15	1.73	2.30
190	12.2	0.91	1.22	1.82	2.43
200	12.8	0.96	1.28	1.92	2.56
210	13.4	1.01	1.34	2.02	2.69
220	14.1	1.06	1.41	2.11	2.82
230	14.7	1.10	1.47	2.21	2.94
240	15.4	1.15	1.54	2.30	3.07
250	16.0	1.20	1.60	2.40	3.20
260	16.6	1.25	1.66	2.50	3.33
270	17.3	1.30	1.73	2.59	3.46
280	17.9	1.34	1.79	2.69	3.58
290	18.6	1.39	1.86	2.78	3.71
300	19.2	1.44	1.92	2.88	3.84
310	19.8	1.49	1.98	2.98	3.97
320	20.5	1.54	2.05	3.07	4.10
330	21.1	1.58	2.11	3.17	4.22
340	21.8	1.63	2.18	3.26	4.35
350	22.4	1.68	2.24	3.36	4.48
360	23.0	1.73	2.30	3.46	4.61
370	23.7	1.78	2.37	3.55	4.74
380	24.3	1.82	2.43	3.65	4.86
390	25.0	1.87	2.50	3.74	4.99
400	25.6	1.92	2.56	3.84	5.12
410	26.2	1.97	2.62	3.94	5.25
420	26.9	2.02	2.69	4.03	5.38
430	27.5	2.06	2.75	4.13	5.50
440	28.2	2.11	2.82	4.22	5.63
450	28.8	2.16	2.88	4.32	5.76
460	29.4	2.21	2.94	4.42	5.89
470	30.1	2.26	3.01	4.51	6.02
480	30.7	2.30	3.07	4.61	6.14
490	31.4	2.35	3.14	4.70	6.27
500	32.0	2.40	3.20	4.80	6.40