

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 2
THE LABORATORY MOUSE

SUBSTANCE ADMINISTRATION AND BLOOD COLLECTION

Substance administration:

- Subcutaneous 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.

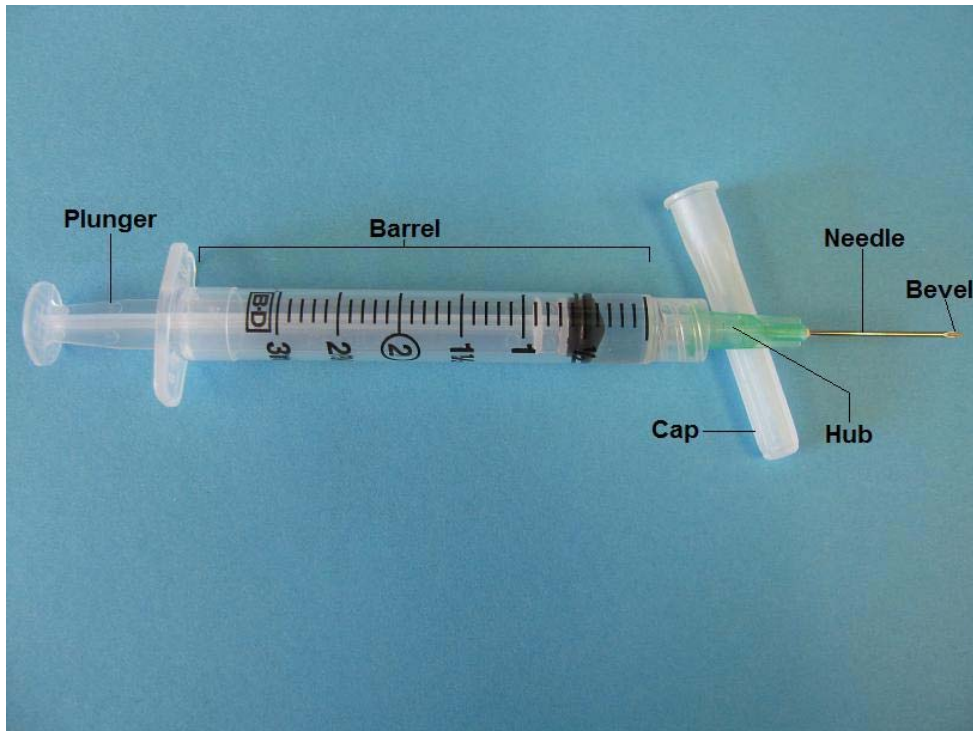
SUBSTANCE ADMINISTRATION

GENERAL INFORMATION

Substance

- Verify that the pH of solutions injected subcutaneously or intramuscularly is between 7.3 and 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.
- Proper restraint is important in order to reduce the risk of tissue damage at the injection site.
- Check proper placement of the needle prior to injection. Withdraw 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)

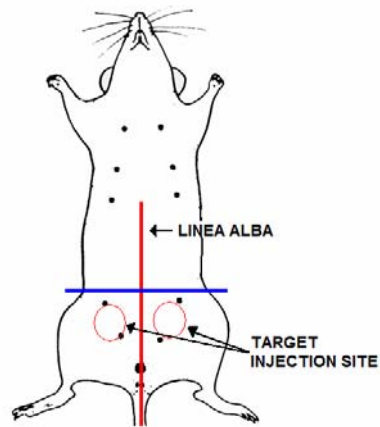
- Recommended injection site: loose skin over the neck and dorsum.
- Recommended volume: 0-10 ml/kg bodyweight
- Needle size: 25G or smaller
- Subcutaneous administration should be limited to 2 to 3 sites per day.
- The mouse can be placed in a restraining device or restrained by hand on the wire-bar lid 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.

2. Intramuscular injection (IM)

- This method is not commonly used in small rodents.
- Recommended injection site: quadriceps or posterior thigh muscles.
- Recommended volume: 0-0.05 ml/per site
- Needle size: 27G or smaller
- Avoid trauma to nerves 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 alternated.
- Procedure:
 - Using a restrainer device
 - Insert the mouse in the restrainer.
 - Hold the restrainer in the palm of your non-dominant hand.
 - Take the paw that is farthest from you out of the tube and grasp a fold of skin between your thumb and index fingers while extending the leg.
 - Insert the needle perpendicular to the plane of the muscle and direct it away from the line of the bone to avoid trauma to nerves and blood vessels.
 - Check proper placement of the needle prior to injection by withdrawing the syringe plunger and inject.
 - Cross-leg technique
 - Restrain the mouse using the basic manual restraint technique without holding the tail.
 - If you are using your left hand to restrain the mouse, grasp the left ankle between your ring finger and middle finger. If restraining with the right hand, grasp the right ankle.
 - Insert the needle perpendicular to the plane of the muscle and direct it away from the line of the bone to avoid trauma to nerves and blood vessels.
 - Check proper placement of the needle prior to injection by withdrawing the syringe plunger and inject.

3. Intraperitoneal injection (IP)

- Recommended injection site: Either side of the lower abdomen (on females, injection can be done between the two last nipples).
- During an experiment, sites of injection should be alternated.
- Recommended volume: 0-20 ml/kg bodyweight
- Needle size: 25G to 27G
- Procedure:



- Restraint the mouse using the basic manual restraint technique.
- Hold the mouse, head-down, at a 30-45 degree angle. This allows the abdominal contents to move away from the injection site.
- 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 the syringe plunger and inject.

Note: A brown to greenish substance aspirated into the hub of the needle may indicate that the intestine was punctured.

A yellow substance aspirated into the hub of the needle may indicate that the bladder was punctured.

BLOOD COLLECTION

1. Saphenous 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 care 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 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.
 - Place the animal in the appropriate restrainer. (e.g. a 50 ml conical tube with end cut off)
 - Hold the restrainer with your non-dominant hand in the inner part of your fingers.
 - Take the paw that is farthest from you out of the tube and grasp a fold of skin between your thumb and index fingers while extending the leg.
 - 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. The paw should remain downward at all time.
 - Following blood collection, release the fold of skin between your thumb and index, then apply a gentle pressure on the leg until the bleeding stops. At this point, the paw should be held upward.
 - 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-hour period means that you perform 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)
Mouse	72	5.4	7.2	10.8	14.4

Sample calculation:

You need to collect 10% of the blood volume for a 30g mouse in a single sample.

The blood volume recommended to be collected in a single sampling of 10% is 7.2 ml/kg of body weight (BW).

30g = 0.03kg

0.03kg x 7.2 ml/kg = 0.22 ml

Therefore you can safely collect 0.22ml of blood from a 30g mouse every 2 weeks

Blood volumes - Mouse

Body weight (g)	Total circulating blood volume (mL)	Acceptable volume for collection (mL)			
		7.5%	10%	15%	20%
10	0.72	0.05	0.07	0.11	0.14
11	0.79	0.06	0.08	0.12	0.16
12	0.86	0.06	0.09	0.13	0.17
13	0.94	0.07	0.09	0.14	0.19
14	1.01	0.08	0.10	0.15	0.20
15	1.08	0.08	0.11	0.16	0.22
16	1.15	0.09	0.12	0.17	0.23
17	1.22	0.09	0.12	0.18	0.24
18	1.30	0.10	0.13	0.19	0.26
19	1.37	0.10	0.14	0.21	0.27
20	1.44	0.11	0.14	0.22	0.29
21	1.51	0.11	0.15	0.23	0.30
22	1.58	0.12	0.16	0.24	0.32
23	1.66	0.12	0.17	0.25	0.33
24	1.73	0.13	0.17	0.26	0.35
25	1.80	0.14	0.18	0.27	0.36
26	1.87	0.14	0.19	0.28	0.37
27	1.94	0.15	0.19	0.29	0.39
28	2.02	0.15	0.20	0.30	0.40
29	2.09	0.16	0.21	0.31	0.42
30	2.16	0.16	0.22	0.32	0.43
31	2.23	0.17	0.22	0.33	0.45
32	2.30	0.17	0.23	0.35	0.46
33	2.38	0.18	0.24	0.36	0.48
34	2.45	0.18	0.24	0.37	0.49
35	2.52	0.19	0.25	0.38	0.50
36	2.59	0.19	0.26	0.39	0.52
37	2.66	0.20	0.27	0.40	0.53
38	2.74	0.21	0.27	0.41	0.55
39	2.81	0.21	0.28	0.42	0.56
40	2.88	0.22	0.29	0.43	0.58