

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

MODULE 2 – Substance Administration and Blood Collection in the Laboratory Mouse

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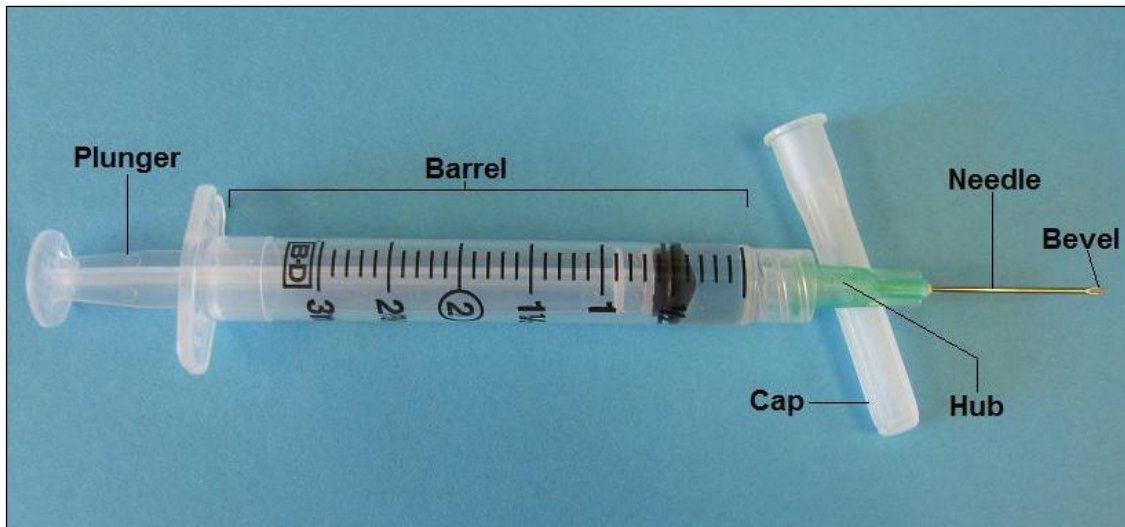
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1. GENERAL INFORMATION

1.1. Substance

- Verify that the pH of solutions injected subcutaneously 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, whenever 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.

1.2. Syringe anatomy



1.3. Proper handling of the syringe



1.4. Needles

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

1.5. 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.

2. SUBSTANCE ADMINISTRATION

2.1. Subcutaneous injection (SC)

- Recommended injection site: loose skin over the neck and dorsum.
- Recommended volume: 0-10 ml/kg bodyweight
- Needle size: 26G 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:

- Using the tips of your middle finger and thumb, grasp a fold of skin over the neck or dorsum of the mouse.
- 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 pulling back on the syringe plunger and inject.

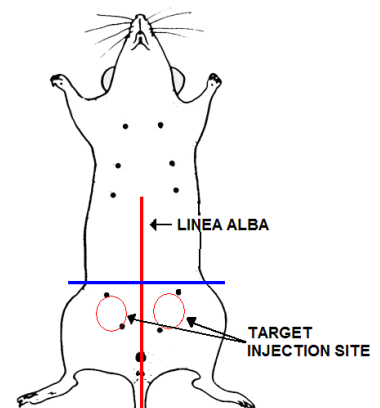


2.2. Intraperitoneal injection (IP)

- This technique is not recommended for gestating animals.
- Recommended injection site: Either side of the lower abdomen (on females, injection can be done between the two last nipples).
- During an experiment, injection sites should be alternated.
- Recommended volume: 0-20 ml/kg bodyweight
- Needle size: 25G to 27G

Procedure:

- Restrain 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. In both cases, the solution is contaminated. The needle and solution should be replaced.

- Return the animal to its home cage.



3. BLOOD COLLECTION

3.1. General information

- The common survival blood collection sites are:

SITE	GENERAL ANESTHESIA REQUIRED	REPEAT SAMPLING (DAILY)	OBTAINABLE VOLUME
Saphenous vein	No	Yes	Medium to large
Tail vein or artery	No	Yes	Small
Submandibular puncture	No	Yes	Medium to large
Tail tip	No	Yes	1-2 drops
Jugular vein	Yes	Yes	Large

- 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 4 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 agents will not be helpful as it decreases the peripheral blood pressure.

3.2. Maximum volumes and recovery periods

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.

$$30\text{g} = 0.03\text{kg}$$

$$0.03\text{kg} \times 7.2 \text{ ml/kg} = 0.22 \text{ ml}$$

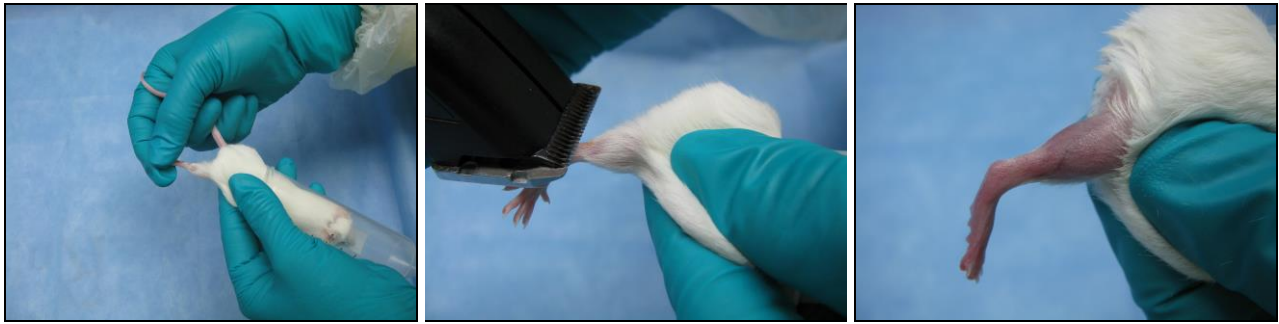
Therefore you can safely collect 0.22ml of blood from a 30g mouse every 2 weeks

3.3. Mouse blood volumes

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

3.4. Saphenous vein procedure

- Weigh the animal.
- Use the tables below to calculate the maximum amount of blood to be collected.
- Use the tables below to calculate the maximum amount of blood to be collected according to the recovery period.
- Place the animal in the appropriate restrainer (e.g., a 50 mL conical tube with end cut off).
- Hold the restrainer in the palm of your non-dominant hand. The head of the mouse should point towards you.
- Take the paw that is facing away from you out of the tube and using your thumb and index finger grasp the fold of skin between the hip area and body to extend the leg.
- Clip the hair on the exterior side of the leg using an electric shaver or a scalpel blade; alternatively you may pluck the fur.



- 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 to the leg using a 23G needle.
- As drops of blood appear collect them directly into collection tubes.
- To increase the blood flow during blood collection, gently flex the paw. The paw should remain pointing down at all times.
- Following blood collection, release the fold of skin and apply a gentle pressure on the puncture site using a piece of gauze until the bleeding stops. At this point, the paw should be held upward.
- Place the animal back in its home cage.



3.5. 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

4. REFERENCES

4.1. CMARC website

www.mcgill.ca/cmARC

4.2. CMARC emails

Veterinary Care	aht.arc@mcgill.ca
Technical Services, Equipment rental (Anesthetic machines)	rts.arc@mcgill.ca
Imports, Transfers and Quarantine	import.cmARC@mcgill.ca
Imaging Services	imaging.cmARC@mcgill.ca
Irradiator Services	irradiator.cmARC@mcgill.ca
Workshop and Training	workshop.cmARC@mcgill.ca
Polyclonal Antibody Production	antibodyproduction.cmARC@mcgill.ca
Materials and drug sales	drss@mcgill.ca
Comparative Pathology	comparative.pathology@mcgill.ca

4.3. McGill Standard Operating Procedures (SOP)

<http://www.mcgill.ca/research/researchers/compliance/animal/sop>

4.4. Animal compliance online theory course

- In order to be approved on the animal use protocol, participant must complete the online theory course.
- Basic level: For participants performing techniques shown in Module 1 only.
- Advanced level: For participants performing techniques shown in Modules 2 and above.
- Link to theory course: <http://animalcare.mcgill.ca/>
- Email: animalcare@mcgill.ca

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