

LABORATORY ANIMAL BIOMETHODOLOGY WORKSHOP

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

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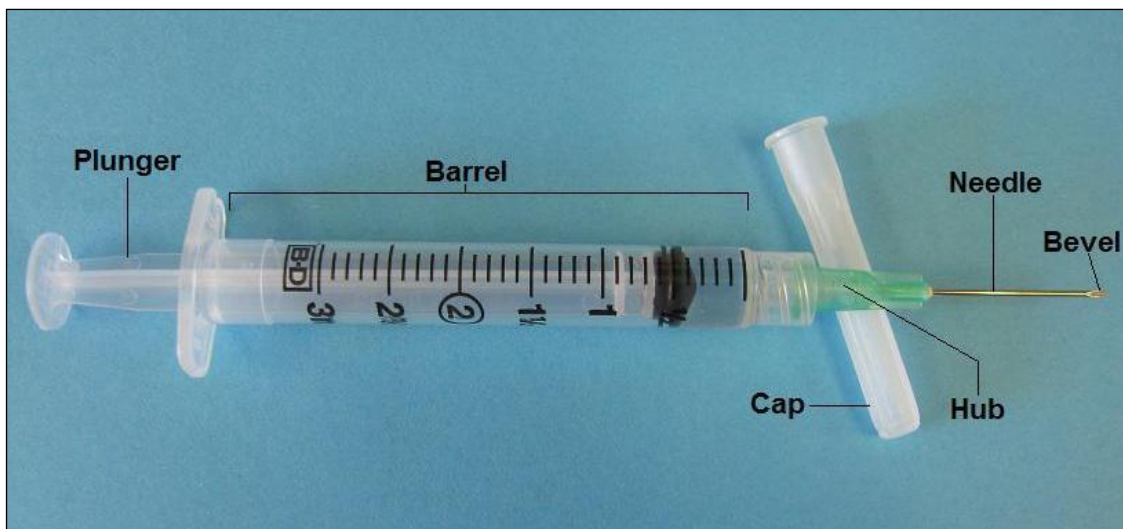
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1. GENERAL INFORMATION

1.1. 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.

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: 0-5 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 rat with the tip 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 rat’s body to make sure that you do not inadvertently puncture the body of the rat.
- Check proper placement of the needle prior to injection by withdrawing the syringe plunger and inject.
- Return the animal to its home cage.



2.2. Intraperitoneal injection (IP)

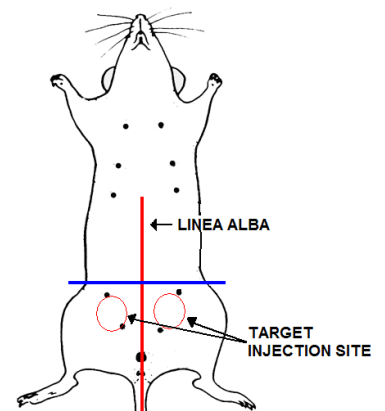
- This technique is not recommended for gestating animals.
- Recommended injection site: Either side of the lower abdomen (on females, injection can be done between the two last nipples).
- During an experiment, injection sites should be alternated.
- Recommended volume: 0-20 ml/kg bodyweight
- Needle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

Procedure

- Place your needle parallel to the linea alba.
- Insert the needle at 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 pulling back on the syringe plunger and inject.

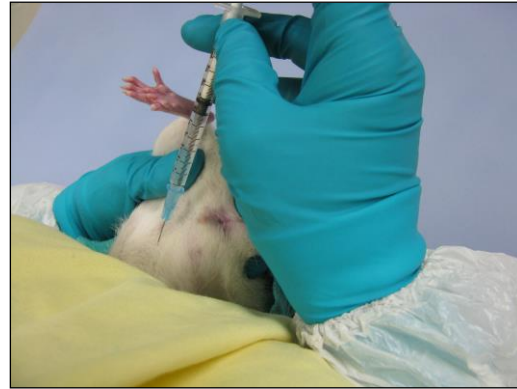
Note: A brown to greenish substance aspirated into the hub of the needle may indicate that the intestine was punctured. A yellow substance aspirated into the hub of the needle may indicate that the bladder was punctured. In both cases, the solution is contaminated. The needle and solution should be replaced.

- Return the animal to its home cage.



Restraint using a conical plastic restraining bag:

- Place the rat in the conical restraining bag.
- Hold the rat head down, in dorsal recumbency, at a 30-45 degree angle. This allows the abdominal contents to move away from the injection site.
- Fold over a part of the bag to have access to the lower abdomen.
- Proceed with the injection as described above.



Restraint using a towel:

- Wrap the rat in a towel, head first, making sure that you have access to the lower abdomen.
- With your non-dominant hand, hold the rat with its abdomen against your body using your hand, wrist and a small portion of your forearm to restrain the animal. The rat will have his head pointing down and back, diagonally against the side of your abdomen.
- Using your thumb, flip the hind paw to visualize the lower abdomen (i.e., if you hold the rat with your left hand, you will flip left hind paw of the rat).
- Proceed with the injection as described above.

Two-person procedure:

- Use the 'V' grip to restrain the rat, avoid pressure on the trachea. Gently wrap your fingers around the thoracic cavity. Support the rear feet and tail.
- Gently stretch the rat's body holding the head lower than the body and restrain the rat's hind limbs.
- Proceed with the injection as described above.



2.3. Intramuscular injection (IM):

- Recommended injection site: quadriceps or posterior thigh muscle.
- 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 alternated.

Procedure:

- Use a towel or a conical plastic bag to restrain the rat.
- Hold the rat in your non-dominant hand, paws resting on the palm of your hand.
- Take the paw that faces away from you out of the restraining device. Using your thumb and index fingers grasp the fold of skin between the hip area and body to extend the leg (i.e., 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 directing the needle away from the line of the bone in order to avoid the sciatic nerve.
- 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. 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 to medium
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 2 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)
Rat	64	4.8	6.4	9.6	12.8

Sample calculation:

You need to collect 10% of the blood volume for a 300g 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.

$$300\text{g} = 0.3\text{kg}$$

$$0.3\text{kg} \times 6.4 \text{ ml/kg} = 1.92 \text{ ml}$$

Therefore you can safely collect 1.9 ml of blood from a 300g rat every 2 weeks.

3.3. Rat blood volumes

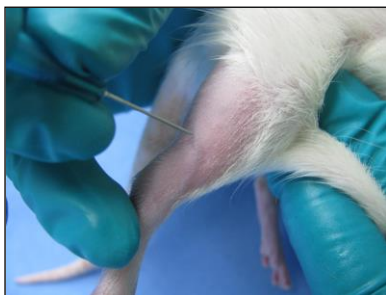
Body weight (g)	Total circulating blood volume (mL)	Acceptable volume for collection (mL)			
		7.5%	10%	15%	20%
200	12.8	0.96	1.28	1.92	2.56
210	13.4	1.01	1.34	2.02	2.69
220	14.1	1.06	1.41	2.11	2.82
230	14.7	1.10	1.47	2.21	2.94
240	15.4	1.15	1.54	2.30	3.07
250	16.0	1.20	1.60	2.40	3.20
260	16.6	1.25	1.66	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

3.4. Saphenous vein 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. wrap the rat in a towel or insert it in a conical plastic restraining bag.).
- Hold the rat in your non-dominant hand, paws resting on the palm of your hand.
- Take the paw that faces away from you out of the restraining device. Using your thumb and index fingers grasp the fold of skin between the hip area and body to extend the leg (i.e., if you hold the rat with your left hand, you will extend his right hind paw).
- 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.
- 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.
- 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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