Complete the quiz after reading this handout.

You must score 8/10 to successfully complete the training.

Score will be available immediately after completion.

Don’t forget to review any incorrect answers once the quiz is graded.

Module 2 Quiz
1. GENERAL INFORMATION

Administering substances to animals can have a significant impact on their welfare. If carried out incorrectly, not only can animal welfare be compromised, but the scientific goals of the study can be affected. If the administration is for a therapeutic purpose, then incorrect administration can lead to a failure of the treatment.

The selection of a particular route of administration must balance a number of factors – for example the volume and physicochemical properties of the substance, the required speed of onset, and other factors such as the degree of tissue irritation that could be caused. If a study requires repeated injection of substances the use of Osmotic minipumps may represent a significant refinement.

All animals benefit from being first accustomed to being handled, and this can be done during the period of acclimatization needed before they are used on a research procedure. If injections are to be repeated on a regular basis, then familiarization with restraint assumes even greater importance, and training of the animal to co-operate with the procedure may also be helpful.

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, may alter the concentration of the solution.
- 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. Syringes

- Syringes are available in different volumes. The 1cc and 3cc syringes are most commonly used in rats.
- Slip tip syringes: These syringe barrels allow the needle to slip over the tip and compress for easy installation/removal.
- Luer lock syringes: these syringe barrels have a threaded tip that allows for Luer lock needles to be screwed on securely. These can be useful when injecting viscous substances.
- When administering very small volumes (below 50uL), insulin syringes with attached needles can be used.
1.3. Needles

- Use the smallest gauge of needle possible that allows accurate injection of the substance.
- The recommended needle lengths for use in rats are $\frac{1}{8}$", $\frac{1}{2}$", and $\frac{3}{8}$".
- It is recommended to use a new needle for each animal. This ensures that the needles are sharp to reduce discomfort and sterile to reduce the risk of any injection-site infection.
- Needles should not be bent, broken, or recapped.

1.4. 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.
- Use the smallest volume possible that allows adequate administration of the substance.
- No bubbles should be present in the syringes when preparing substances for administration.
- 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)

- Subcutaneous administration of material often causes minimal pain or discomfort, provided the material is non-irritant, has a near-neutral pH, and is not excessively hypertonic or hypotonic.
- Recommended injection site: loose skin over the neck and dorsum.
- Maximum recommended volume: 10ml/kg bodyweight
- 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 restrained by hand.

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 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 involving repeated injections, injection sites should be alternated.
- Maximum recommended volume: 20ml/kg bodyweight
- Needle size: 25G to 27G

Restraint using a conical plastic restraining bag:

- Place the rat in the conical restraining bag. The perforation at the tip of the bag allows for ventilation.
- 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 the large opening of the bag to access to the lower abdomen.
- Proceed with the injection as described below.

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 forearm to restrain the animal. The rat will have his head pointed down, resting against 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 below.

Two-person procedure:

- One person uses the ‘V’ grip to restrain the rat, avoid pressure on the trachea. Gently wrap 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.
- The second person proceeds with the injection as described below.
Procedure:

- Restrain the animal using one of the techniques described above.
- Hold the animal, 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 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 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.

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 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 (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. Inject.
- Return the animal to its home cage
3. BLOOD COLLECTION

3.1. General information

- The common survival blood collection sites are:

<table>
<thead>
<tr>
<th>SITE</th>
<th>GENERAL ANESTHESIA REQUIRED</th>
<th>REPEAT SAMPLING (DAILY)</th>
<th>OBTAINABLE VOLUME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saphenous vein</td>
<td>No</td>
<td>Yes</td>
<td>Medium to large</td>
</tr>
<tr>
<td>Tail vein or artery</td>
<td>No</td>
<td>Yes</td>
<td>Small</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>Yes</td>
<td>Yes</td>
<td>Large</td>
</tr>
</tbody>
</table>

- 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

<table>
<thead>
<tr>
<th>PERCENT OF BLOOD VOLUME COLLECTED IN A SINGLE SAMPLING</th>
<th>RECOVERY PERIOD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5%</td>
<td>1</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
</tr>
<tr>
<td>15%</td>
<td>4</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>PERCENT OF BLOOD VOLUME COLLECTED OVER A 24-HOUR PERIOD - MULTIPLE SAMPLES</th>
<th>RECOVERY PERIOD (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5%</td>
<td>1</td>
</tr>
<tr>
<td>10 - 15%</td>
<td>2</td>
</tr>
<tr>
<td>20%</td>
<td>4</td>
</tr>
</tbody>
</table>

Multiple samples over a 24-hour period means that you perform more than one blood collection during a 24-hour period.
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CIRCULATING BLOOD VOLUME (ml/kg BW)</th>
<th>7.5% (ml/kg BW)</th>
<th>10% (ml/kg BW)</th>
<th>15% (ml/kg BW)</th>
<th>20% (ml/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>64</td>
<td>4.8</td>
<td>6.4</td>
<td>9.6</td>
<td>12.8</td>
</tr>
</tbody>
</table>

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.

\[
30\text{g} = 0.03\text{kg} \\
0.03\text{kg} \times 6.4 \text{ml/kg} = 1.92 \text{ml}
\]

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

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Total circulating blood volume (mL)</th>
<th>Acceptable volume for collection (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7.5%</td>
</tr>
<tr>
<td>150</td>
<td>9.6</td>
<td>0.72</td>
</tr>
<tr>
<td>160</td>
<td>10.2</td>
<td>0.77</td>
</tr>
<tr>
<td>170</td>
<td>10.9</td>
<td>0.82</td>
</tr>
<tr>
<td>180</td>
<td>11.5</td>
<td>0.86</td>
</tr>
<tr>
<td>190</td>
<td>12.2</td>
<td>0.91</td>
</tr>
<tr>
<td>200</td>
<td>12.8</td>
<td>0.96</td>
</tr>
<tr>
<td>210</td>
<td>13.4</td>
<td>1.01</td>
</tr>
<tr>
<td>220</td>
<td>14.1</td>
<td>1.06</td>
</tr>
<tr>
<td>230</td>
<td>14.7</td>
<td>1.10</td>
</tr>
<tr>
<td>240</td>
<td>15.4</td>
<td>1.15</td>
</tr>
<tr>
<td>250</td>
<td>16.0</td>
<td>1.20</td>
</tr>
<tr>
<td>260</td>
<td>16.6</td>
<td>1.25</td>
</tr>
<tr>
<td>270</td>
<td>17.3</td>
<td>1.30</td>
</tr>
<tr>
<td>280</td>
<td>17.9</td>
<td>1.34</td>
</tr>
<tr>
<td>290</td>
<td>18.6</td>
<td>1.39</td>
</tr>
<tr>
<td>300</td>
<td>19.2</td>
<td>1.44</td>
</tr>
<tr>
<td>310</td>
<td>19.8</td>
<td>1.49</td>
</tr>
<tr>
<td>320</td>
<td>20.5</td>
<td>1.54</td>
</tr>
<tr>
<td>330</td>
<td>21.1</td>
<td>1.58</td>
</tr>
<tr>
<td>340</td>
<td>21.8</td>
<td>1.63</td>
</tr>
<tr>
<td>350</td>
<td>22.4</td>
<td>1.68</td>
</tr>
<tr>
<td>360</td>
<td>23.0</td>
<td>1.73</td>
</tr>
<tr>
<td>370</td>
<td>23.7</td>
<td>1.78</td>
</tr>
<tr>
<td>380</td>
<td>24.3</td>
<td>1.82</td>
</tr>
<tr>
<td>390</td>
<td>25.0</td>
<td>1.87</td>
</tr>
<tr>
<td>400</td>
<td>25.6</td>
<td>1.92</td>
</tr>
<tr>
<td>410</td>
<td>26.2</td>
<td>1.97</td>
</tr>
<tr>
<td>420</td>
<td>26.9</td>
<td>2.02</td>
</tr>
<tr>
<td>430</td>
<td>27.5</td>
<td>2.06</td>
</tr>
<tr>
<td>440</td>
<td>28.2</td>
<td>2.11</td>
</tr>
<tr>
<td>450</td>
<td>28.8</td>
<td>2.16</td>
</tr>
<tr>
<td>460</td>
<td>29.4</td>
<td>2.21</td>
</tr>
<tr>
<td>470</td>
<td>30.1</td>
<td>2.26</td>
</tr>
<tr>
<td>480</td>
<td>30.7</td>
<td>2.30</td>
</tr>
<tr>
<td>490</td>
<td>31.4</td>
<td>2.35</td>
</tr>
<tr>
<td>500</td>
<td>32.0</td>
<td>2.40</td>
</tr>
</tbody>
</table>
3.4. Saphenous vein procedure

- Weigh the animal.
- Use the table to calculate the maximum amount of blood to be collected according to the recovery period.
- Place the animal in the appropriate restraint device, towel or 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. The paw should remain pointing down at all times.
- Following blood collection, release the fold of skin and apply gentle pressure on the puncture site using a gauze until the bleeding stops. At this point, the paw is held up.
- 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:
  - Fast and thready pulse
  - Pale dry mucous membranes
  - Cold skin and extremities
  - Restlessness
  - Hyperventilation
  - Sub-normal body temperature

4. REFERENCES

4.1. McGill Standard Operating Procedure (SOP) 404 Substance Administration

4.2. McGill Standard Operating Procedure (SOP) 403 Guidelines for Blood Collection Volumes and Frequency

The UACC would like to acknowledge the invaluable help of the Comparative Medicine and Animal Resources Centre technicians in preparing this handout.
Rat Module 2 Quiz

You must score 8/10 to successfully complete the training.

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Start the Quiz