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## Standard Procedures for Administration of Substances

Substances should be pharmaceutical grade, stable, as close to pH 7 as possible, and warmed to body temperature before administration.

Investigators who plan to dose greater volumes than specified must consult the veterinary staff and provide justification in the IACUC protocol.

If the route or species you are considering is NOT on this list or if you have questions about your dosing material, please consult with veterinary staff.

Common routes of administration are intravenous (IV), intraperitoneal (IP), intradermal (ID), subcutaneous (SC), intramuscular (IM), and oral gavage.

1. **Intravenous (IV) injection.** For lateral tail vein injection in rodents, secure unanesthetized animal in a restrainer. Warm up the tail using heat lamp or handwarmers for 2-3mins to dilate the vein. Locate the lateral tail vein by swabbing the area with 70% ethanol which helps make the vein visible. Start at the middle or distal part of the tail. Puncture the skin with the needle at a slight angle of about 16-20 degrees with bevel facing up.

Marginal ear vein injection in rabbits is done under anaesthesia. The fur on top of the ear will be clipped to aide in visualization. Alcohol will be used to disinfect the site. The vessels will be occluded by gently holding off at the base of the ear. Insert about ¼ part of the needle not too deep as the vein is very superficial. Secure the syringe and needle by holding the base of the needle by your thumb. Administer a bit of the material into the vein, and if the blood flushes all through out, then you are on proper placement. Once proper placement was obtained, administer the material in a steady, fluid motion.

Ensure that pressure is applied over the injection site simultaneously with the withdrawal of the needle when injection is completed. This pressure should be maintained for a short time to allow the puncture site to form a clot (hemostasis), which prevents a hematoma (bleeding in the surrounding tissues) from forming and keeps the injected material from leaking out of the vein.

2. **Intraperitoneal (IP) injection** is administered into lower abdominal quadrants in rodents. Scruff (for mice) or place your hand around the thorax just behind the elbow (for rats) and hold in a manner that the head is tilted downward to allow abdominal structures to fall out of the way. Disinfect the injection site with 70% ethanol. The needle is inserted, bevel up, at a 30-degree angle into the lower right quadrant of the abdomen. This places the injection away from the abdominal midline so as not to inject into the urinary bladder. Always aspirate the syringe prior to an IP injection to confirm proper needle placement. If any aspirate is seen, the needle is withdrawn and discarded, and the procedure is started again. Apply gentle pressure thereafter.

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3. **Intradermal (ID) injection.** Anaesthesia is commonly used to immobilize the animal for intradermal injections because it is difficult to accurately place the compound in a conscious animal. The skin is shaved and disinfected with 70% ethanol, and the needle is inserted at a shallow angle (10–20 degrees) between the layers of the skin. The needle is advanced only until its bevel is inserted within the skin. Multiple injections are necessary for a larger quantity. Proper placement of an intradermal injection will result in a bleb. Apply gentle pressure thereafter.
  
4. **Subcutaneous injection (SC or SQ).** This is placed between the skin and the underlying muscle. This can be done either on anesthetized or unanesthetized animal. Disinfect the site of injection with 70% ethanol. The skin (neck region or flank region-positioned on the lateral recumbency) is tented to create a large subcutaneous space into which the injection is administered. Orient the hypodermic needle bevel up and parallel to the body at the base of the tent; then insert it through the skin into the subcutaneous space. It is also important to direct the needle away from the fingers creating the tent to prevent needlestick injury and inadvertently injecting yourself with the compound. A bulge, called a bleb, can be observed following the subcutaneous injection of fluid.
  
5. **Intramuscular (IM) injection** is done under anaesthesia. In smaller animals, this is administered into the gluteal or quadriceps muscles. Avoid the sciatic nerve which runs along the caudal aspect of the femur. Grasp and stabilize the muscle mass with one hand and perform the injection with the other hand. Prior to injecting the material, disinfect the site with 70% ethanol, the plunger on the syringe is gently pulled back to ensure that no blood is aspirated into the syringe, which verifies that the needle tip is not inside a vessel. If blood is aspirated, remove the needle and apply pressure to the area to stop haemorrhage, then use another site for the IM injection. Be careful not to inject too much material into a muscle; too large a volume would damage the tissue.
  
6. **Oral gavage** Gavage needles come in various sizes. Select a gavage needle with a metal ball at the end. The animal is restrained so that the head and esophagus are in a straight line. The needle will be measured for proper length from mouth to the last rib. Gently pass the sterile needle along the roof of the mouth down into the esophagus. Movement should be smooth. If any resistance is felt, immediately stop, remove the needle, and start the procedure over. Once the needle tip is in the stomach of the animal, slowly administer the gavage solution into the stomach. Keep the needle stationary until all material is expelled from the syringe; this prevents aspiration of the material into the lungs. Once the administration is complete, care must be taken into consideration when removing the needle to prevent damage to the esophagus. The animal will be monitored for signs of labored breathing, sudden lethargy or poor mucous membrane color. If any of these signs are present, the animal will be euthanized immediately.

Recommended volumes and gavage needle/ needle sizes

	PO	IV	IV	IP	*SC	ID	**IM
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Species	(ml/kg)	(bolus) (ml/kg)	(ml/kg/hr)	(ml/kg)	(ml/kg)	(ml/inj)	(ml/site)
Mice	10; 24-20 ga	5; 25-27 ga	2-4; 23-25 ga	20; 25-27 ga	*10; 23-25 ga	0.05-0.1; <25 ga	**0.05; <23 ga
Rat	10; 20-16 ga	5; 25-27 ga	2-4; 22-25 ga	10; 25 ga	*5; 23-25 ga	0.05-0.1; <25 ga	**0.1 ga; <21 ga
Rabbit	10; 6-8 French feeding tube	2; 25-27 ga	2-4; 22-25 ga	5; 21-25 ga	*1; 23-25 ga	0.05-0.1; <25 ga	**0.25; <21 ga

\* no more than 2-3 subcutaneous sites per day

\*\* no more than 2 intramuscular sites used per day

#### References:

1. A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes. *JOURNAL OF APPLIED TOXICOLOGY. J. Appl. Toxicol.* 21, 15-23 (2001)
2. <https://oacu.oir.nih.gov/sites/default/files/uploads/training-resources/rodentinjection.pdf>
3. AALAS Learning Library. 2012 LAT Training Manual. Common Technical Procedures. Lesson 3. Injections
4. Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. *Journal of the American Association for Laboratory Animal Science.* Vol 50, No 5 September 2011 Pages 600-613