GUIDELINES REGARDING THE USE OF INJECTIONS IN LABORATORY ANIMALS

PURPOSE:

The purpose of this policy is to provide standardized methods for injecting common laboratory animal species. This policy provides an overview of needle sizes, routes of administration, and maximum allowable fluid volume that can be administered per route.

GUIDELINES:

The following guidelines outline various circumstances under which injections may be made, and what material is permissible.

LIQUID INJECTABLE MATERIAL

- **Experimental Compounds**: the liquid diluent or vehicle used for injection of cells, infectious agents, or experimental compounds must be sterile to avoid inadvertent introduction of microbial or toxic contaminants into animals, as well as the potential introduction of unwanted variables.
- **Contamination**: if multiple doses are drawn into a syringe for administration to multiple animals, and the syringe is subsequently refilled for dosing additional animals, do not refill the syringe by inserting the used/contaminated needle into the sterile vial. Be sure to use a new, sterile needle when withdrawing solutions form the sterile vial. Do not leave an open needle in the vial as this can lead to contamination of the solution.
- **Veterinary Drugs and Compounding**: anesthetics, analgesics, tranquilizers, injectable saline, and other biologics to be injected into animals must be commercially available pharmaceutical grade compounds. Compounding and use of non-pharmaceutical grade drugs and biologics must be used in consultation with the university veterinarian per IACUC policy.
- **Preparation of Injectable Agents**: if administering large volumes via subcutaneous or intraperitoneal routes, or if administering intravenous solutions, it is recommended that the fluids are warmed to near body temperature. Refrigerated or room temperature solutions may cause hypothermia in small animals following injection and could lead to the death of the animal.
- **Dilution of Injectable Agents**: some drugs are labeled for intravenous use. In small rodents, IV injections may be difficult due to the small size of the animal's veins. Drugs or compounds intended for intravenous use may be irritating or cause tissue damage if administered by other routes. Therefore, such solutions must be diluted in pharmaceutical grade sterile saline prior to use. Adhere to injectable volume recommendations when making dilutions.

INJECTIONS

Methods for injections include: intradermal (ID), subcutaneous (SC), intramuscular (IM), intraperitoneal (IP), intravenous (IP), intraosseous (IO), or retroorbital. Injection of compounds can pose several risks to the animal, including the potential for disease, injury, or infection. The operator also assumes risk of injury when handling injectable agents, so proper training must be given. Risks include injury via struggling or biting animals or accidental self-injection. Do not insert needles to their hubs unless indicated.

MULTIPLE USES OF A NEEDLE

Sterile hypodermic needles are intended for a single use before being discarded. The LU IACUC has chosen to limit the use of a single needle for injection of up to 5 rodents that are housed in a single cage. In order to minimize the potential for spreading infectious particles among rodents, investigators shall not use the same needle to inject rodents in different cages. Needles used for blood withdrawal or intravenous injection may not be used on more than one animal. Needles must be visually inspected for burrs or other defects after each injection and replaced with a new sterile needle if damage is observed.

TABLE I. STANDARD VOLUME AND NEEDLE SIZE RECOMMENDATIONS FOR INJECTABLE SITES IN RODENTS AND RABBITS*

	Intradermal (ID)[1,2]		**Subcutaneous (SC)[3,5]**		**Intramuscular (IM)** [3,5]**		**Intraperitoneal (IP)[3,5,6]**		**Intravenous (IV)[2,5]**		**Intraosseous (IO)*****	
	Needle Length: 1/2-5/8”		Needle Length: 5/8-1”		Needle Length: 1/2- 5/8” GP/Rabbit: 5/8-1”		Needle Length: 1/2-5/8” Rabbit: 3/4-1”		Needle Length: 1/2-5/8” Rabbit: 5/8-1”		Needle Length: 1/2-5/8” Rabbit: 3/4-1”	
Species	**Max Volume (ml) per site**	**Needle Size (gauge)**	**Volume Range (ml)**	**Needle Size (gauge)**	**Max Volume (ml) per site**	**Needle Size (gauge)**	**Volume Range (ml)**	**Needle Size (gauge)**	**Max Volume (ml)**	**Needle Size (gauge)**	**Max Volume (ml)**	**Needle Size (gauge)**
Mouse	0.05	27-30	2-3	25-27	0.05	25-27	2-3	23-27****	0.20	26-30	0.20	25-27[8]
Rat	0.05	25-27	5-10	23-25	0.30	25-27	5-10	23-25	0.50	21-23	0.50	23-26[9]
USDA Regulated												
Gerbil	0.05	27-30	1-3	25-27	0.05	25-27	2-3	23-25	0.10	27-30	0.10	23-25[10]
Hamster	0.05	25-27	3-4	25-27	0.10	25-27	3-4	23-25	0.30	25-27	0.30	23-25[10]
Guinea Pig	0.05	25-27	5-10	23-25	0.30	25-27	10-15	23-25	0.50	25-27	0.50	22-25
Rabbit	0.10	25-27	30-50	21-25	0.50-1.0	23-25	50-100	21-23	1-5	23-25	1-5	20-22[11]

*Retroorbital injections in mice: max volume <0.15ml. Needle size: 27 gauge.[4]
**Irritating substances like ketamine must not be administered IM as they may lead to self-mutilation of the affected limb if muscle or nerve damage occurs.
***Anesthesia is recommended for intraosseous injections. Injections must be given slowly and maximum volumes are those of IV injections.
****Recommended range in Formulary for Laboratory Animals, Third Edition is 25-27 gauge.[2] Jackson Laboratories training modules recommend 23-26 gauge.[7]

REFERENCES

1. AALAS Learning Library. https://www.aalaslearninglibrary.org/demo/course2
2. Donovan, J. and Brown, P. 2006. Parenteral Injections. Current Protocols in Immunology. 73:1.6.1—1.6.10.
3. Hawk CT, Leary SL, Morris TH. 2005. Formulary for Laboratory Animals, 3rd ed. Blackwell Publishing: Ames, Iowa, pp. 157-162.
4. T. Yardeni et al. Retro-orbital injections in mice. *Lab Animal* 2011; 40(5): 155-160.
5. Diehl et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol* 2001; 21: 15-23.
6. http://www.nus.edu.sg/iacuc/files/The%20Laboratory%20Mouse.pdf
7. http://jax.org/contact/index.html. Personal communication with technical services on 1/29/2012 at 2:45pm. Standard operating procedures for Jackson Laboratories recommend 23-26 gauge needles for intraperitoneal injections in adult mice.
8. Uehara H, Kim SJ, Karashima T, et al. Effects of blocking platelet-derived growth factor-receptor signaling in a mouse model of experimental prostate cancer bone metastases. *J Natl Cancer Inst* 2003;95:558-70.
9. Nakamura K, Kurokawa T, Aoyama I, et al. Stimulation of bone formation by intraosseous injection of basic fibroblast growth factor in ovariectomised rats. *Int Orthop* 1998;22:49-54.
10. The Merck Veterinary Manual on-line. 2011. Merck, Sharp, & Dohme Corp, Inc.; Whitehouse Station, NJ.
11. Porzio P, Pharr JW, Allen AL. Excretory urography by intraosseous injection of contrast media in a rabbit model. *Vet Radiol Ultrasound* 2001;42:238-243.