Recommended Needle Sizes, Trocar Implant Needle Use, Injection Sites, and Volumes for Injection

Species	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous	Intragastric	Intratumoral	Intradermal
Rat [250g]	Scruff, back, up to 1.25-2.50 ml, 21 G or smaller [up to 5-10 µl/g]	Quadriceps/posterior thigh, up to 300 µl, 23 G or smaller [up to 100 µl per site]	up to 2.5-5 ml, 23 G or smaller [up to 10-20 µl/g]	Lateral tail vein, up to 2- 5 ml, 25 G or smaller [up to 10-20 µl/g]	up to 5 ml, ball end or disposable gavage needle [up to 20 uL/gm]	up to 100 µl, dependent on tumor size, 23 G or smaller	up to 0.05ml (50ul), 27G or smaller
Mouse [25g]	Scruff, up to 250-1000 µl, 21 G or smaller [up to 10-40 µl/g]	Quadriceps/posterior thigh, up to 50 µl/site [2 site max], 25 G or smaller [up to 50 µl per site]	up to 0.5-2 ml, 23 G or smaller [up to 20-80 µl/g]	Lateral tail vein, up to 250-625 µl, 25 G or smaller [up to 10-25 µl/g]	up to 500 uL, ball end or disposable gavage needle* [up to 20 uL/gm]	up to 100 µl, dependent on tumor size, 25 G or smaller	up to 0.05ml (50ul), 27G or smaller

Adapted from *A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes (Diehl et al, Journal of Applied Toxicology, 21, 15-23, 2001).*

**Needle size for intragastric injection: 20 G or smaller (Adults >20g); 22G or smaller (wealings/young adults 10-20g); Consult LAM for any mice less than 10g body weight.*

GENERAL ADMINISTRATION NOTES

- Recommendations are based on the adult body weight of a rat [250 g] and the adult body weight of a mouse [25 g]. The dose volumes listed above are the maximum volumes an animal can received based upon these average weights.
- These volumes should be reduced if the injected material is likely to irritate tissues OR if using an oil- based vehicle for gavaging.
- Changing needles also serves to keep the injection needle from becoming dulled if passed through a stoppered vial.
- As a safety precaution, the vial of material should be placed in an upright position on a hard, flat surface when inserting a needle into the vial. Once the needle is in place, the vial can then be picked up.
- For IV injections, it is recommended to use the lateral tail vein [2 available], while the mouse is enclosed within a commercial or custom made restrainer. Dilation of the vein is accomplished by warming of the tail by immersion in warm water or use of a heat lamp. Five minutes of supplemental heat may be necessary for optimal dilation. Monitor carefully to prevent the animal from being burned or overheated by the heat source. The vein is best entered in the proximal 1/3 of the tail, and a successful injection is obvious to the user [based on lack of resistance as the plunger is depressed]. If repeat injections are performed, it is recommended that one start further down the tail and to work upwards with subsequent injections. In most cases a maximum injection volume of 0.5 ml can be safely given to an adult mouse [based on experience and veterinary observations]. Most users try to limit the injection to 0.1 ml/10g [a standard veterinary recommendation]. For cell injections, excessive cell densities may lead to embolism in lung capillaries and death of the recipient. The tendency to clump is cell line dependent. Mastering of the injection technique takes practice and training sessions may be scheduled by contacting the facility manager or the LAM veterinary staff.
- For hydrodynamic gene therapy, the volumes range from 1.6 to 2.5 ml, which is given over 3-5 seconds or 6-8 seconds depending on the volume. The standard needle size used for Balb/c mice is 2 5 gauge and for mice with smaller tail veins [i.e., C57BL/6] a 27-gauge needle is used. Please note that it can take up to 15 seconds to administer the agent if a 27-gauge needle is used. Based on veterinary observations, the appearance of the mice post-injection is strain dependent. The Balb/c mice are not as affected by the injections as the B6 mice. The usual clinical manifestation post- injection ranges from mild immobility with the lower volume to extended periods of immobility with the higher volume. Most often, the animals are alert but have a reduced activity level [stunned appearance]. Severe dyspnea has been reported by others and if observed for more than five minutes the animals must be humanely euthanized.
- For routine hydrodynamic-based transfection, anesthesia is not recommended prior to the injection due to observed gasping and delayed recovery as compared with unanesthetized animals
- For **Intradermal** injections: This method is generally not recommended and scientific justification must be provided. Using a fine needle (27G or smaller) and Tuberculin or Hamilton syringe is recommended. The mouse should be anesthetized, fur clipped (if necessary), and area wiped with 70% ethanol. The skin is held tautly with the thumb and index finger as the needle is inserted, bevel up and at a shallow angle, just under the superficial layer of epidermis. Resistance should be felt as the needle is advanced and the compound is injected. A hard bleb will be seen upon

successful intradermal injection, even with small quantities. If multiple sites are injected, adequate spacing is necessary to prevent coalescing of lesions.

- For intragastric (gavage) injections: Always measure the gavage needle for the correct length, by placing it along the outside of the animal so that the ball tip is at the last rib and the other end of the needle is by the animal's nose. It is recommended to mark the tube at the nose so that the tube is not passed further than this point, risking perforation of the stomach. NEVER force insertion of a gavage needle. Using a disposable, flexible gavage needle has less potential for complications and is recommended over using a rigid, metal gavage needle.

GUIDELINES FOR NEEDLE USE

- Reuse of needles on multiple animals can lead to dulling of the needle, increasing the discomfort associated with injections, and can lead to disease transmission and/or contamination of vials of material to be injected. Therefore, the ACUC requires justification be provided if the reuse of needles exceeds the following criteria:
    - A needle must be replaced if there is evidence it is becoming dull, typically after five but after no more than ten injections. The gauge and route of administration will affect the dullness (e.g., needle is difficult to insert through skin).
    - A needle, once used on an animal, may not be reintroduced into the vial of material being injected unless the vial is to be discarded immediately after dosing (avoids the possibility of significant bacterial contamination at subsequent use).
- Trocar implant needles [10 gauge or smaller] should be replaced or professionally sharpened when it is difficult to push the needle through the skin or subcuti. To decrease the rate of dulling, a nick incision can be made with another instrument [scalpel, surgical scissors] prior to trocar needle use.

Sharps with safety features should be used when practical. Examples of these features include self-retracting or self-sheathing hypodermic needles and an excellent discussion of available safety features may be found online at:
https://search.usa.gov/search?affiliate=usdoloshapublicwebsite&query=safe+sharps.

Reference to works such as 'An evaluation of sharp safety hypodermic needle devices. Nursing Standard. 25, 35, 39-44. 2010 ' will aid in selecting a hypodermic needle that is both safe and easy to use.