
1. PURPOSE

This Standard Operating Procedure (SOP) describes the recommended administration sites, volumes, and needle sizes for common routes of substance administration.

2. RESPONSIBILITY

2.1. Principal investigator (PI) and their research staff, veterinary care staff.

3. CONSIDERATIONS

- 3.1. Animals should be acclimated to handling and restraint to facilitate substance administration and reduce stress.
- 3.2. Dose volumes should be reduced to the minimum that is compatible with compound formulation and accuracy of administration.
- 3.3. If the substance is known to be toxic or an irritant, consideration should be given to minimizing the dose and choosing a route with the least potential complications.
- 3.4. The route of administration should be determined by the purpose of the experiment, the species, the possible effects of the dosing technique on the animal, the substance properties, and the frequency of dosing.
- 3.5. Warm the solutions to body temperature or room temperature immediately prior to administration, whenever possible.
- 3.6. The vehicle chosen should be biologically inert, have no effect on the biophysical properties of the compound, and have no toxic effects on the animals at the quantities administered. If a component of the vehicle has biological effects, its total amount should be limited such that these effects are minimized. The viscosity of the vehicle should be suitable for the route of administration.
- 3.7. All substances to be administered to animals must be discarded on or before the manufacturer's expiration date to ensure safety, sterility, and stability. Expired substances must not be administered to animals.
 - 3.7.1. Diluting and mixing drugs or agents may change drug chemical composition and shelf life. It is recommended to discard dilutions and mixtures within 30 days of preparation, unless otherwise specified by the manufacturer or when peer-reviewed evidence is available.

4. ENTERAL ROUTES OF ADMINISTRATION

- 4.1. The enteral routes of administration deliver substances via the gastro-intestinal tract and include oral, gastric, and rectal administration.
- 4.2. Voluntary consumption:
 - 4.2.1. Substances can be mixed in foods or fluids. Mixing with a palatable substance can facilitate administration.
 - 4.2.2. Animals should be separated to ensure adequate consumption of doses.
- 4.3. Gavage:
 - 4.3.1. Requires the use of a feeding tube or feeding needle. The size (gauge and length) and type of tubing should be carefully selected to minimize discomfort during dosing.
 - 4.3.2. If administering the recommended volume, withdrawal of food prior to oral administration is not required. However, restricting the food intake before dosing may be beneficial in preventing discomfort and allowing for better absorption of some substances.
 - 4.3.3. Administration of large volumes can result in reflux and aspiration into the lungs which can lead to serious complications.
 - 4.3.4. Limit oral gavage to once per day.
 - 4.3.5. Refer to the table in [Annex 2](#) for recommended volumes of administration.

4.4. Rectal administration:

4.4.1. Rectal instillation can be performed using a small-gauge, soft, flexible tubing with smooth edges.

5. PARENTERAL ROUTES OF ADMINISTRATION

5.1. The parenteral routes of administration are all routes other than those that are not enteral, generally injections.

5.2. The substance:

- 5.2.1. Verify that the pH of solutions injected subcutaneously or intramuscularly is of 7.3 to 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
- 5.2.2. Verify the solubility of the substance. Precipitation may cause the formation of large particles which, if injected intramuscularly, can be painful. For intravenous injections, solutions, not suspensions, must be administered.
- 5.2.3. Inject separate substances at different sites to avoid cross reaction of chemicals.
- 5.2.4. Avoid injecting highly viscous liquids as they can cause discomfort and require a larger needle size for injection.
- 5.2.5. 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.

5.3. Needles:

- 5.3.1. Do not reuse needles. Use a new, sterile needle for every animal.
- 5.3.2. When withdrawing a solution through a rubber stopper, needles can become dull. Switch to a new, sterile needle for injection.
- 5.3.3. Use the smallest gauge of needle possible that allows accurate injection of the substance.
- 5.3.4. Note that up to 0.05 mL of the solution may be lost in the syringe hub, depending on the syringe and needle used.
- 5.3.5. Recommended needle sizes:
 - 5.3.5.1. Refer to the table in [Annex 1](#).
 - 5.3.5.2. As birds and fish species vary greatly in size, needle sizes should be selected according to specific species.

5.4. Volume of administration:

- 5.4.1. Use the smallest possible volume of administration, administering less than the recommended volumes whenever possible.
- 5.4.2. Recommended volumes for substance administration:
 - 5.4.2.1. Refer to the table in [Annex 2](#).
 - 5.4.2.2. As bird species vary greatly in size, administration volumes should be selected according to specific species.
- 5.4.3. If the volume administered must exceed the recommended volumes listed in section 4.4.2, justification must be provided and will require approval by the FACC.
- 5.4.4. Possible maximal administration volumes:
 - 5.4.4.1. Refer to table in [Annex 3](#).

5.5. Injections:

- 5.5.1. Do not inject into inflamed or damaged tissue.
- 5.5.2. Limit the number of puncture attempts to 3. After 3 unsuccessful attempts, request the assistance of another trained individual.
- 5.5.3. 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.
- 5.5.4. Give injections at a constant flow rate.
- 5.5.5. If bleeding occurs after injection, apply pressure with gauze until bleeding stops.

- 5.6. Intramuscular injections:
 - 5.6.1. Due to their small muscle mass, intramuscular injections should be avoided in smaller species as it is technically difficult and painful for the animals.
 - 5.6.2. Check proper placement of the needle prior to injection by withdrawing the syringe plunger; if blood enters the needle hub, the needle has entered a blood vessel. Withdraw the needle slightly and redirect it.
 - 5.6.3. Intramuscular administration should be limited to 2 sites per day.
 - 5.6.4. Injection sites should be rotated.
- 5.7. Intraperitoneal injections:
 - 5.7.1. This technique is not recommended in late-term gestating animals.
 - 5.7.2. Become familiar with the internal anatomy of the species used to avoid penetration of internal organs during injection.
 - 5.7.3. Check proper placement of the needle prior to injection by withdrawing the syringe plunger; if blood, urine, or digesta enters the needle hub, withdraw the needle and discard solution.
 - 5.7.4. Limit intraperitoneal injections to twice per day.
 - 5.7.5. Injection sites should be rotated.
 - 5.7.6. Osmotic minipumps can be surgically implanted intraperitoneally when repeated dosing is required.
 - 5.7.7. In birds, the intracoelomic route is used.
- 5.8. Subcutaneous injections:
 - 5.8.1. Subcutaneous administration should be limited to 3 sites per day.
 - 5.8.2. Check proper placement of the needle prior to injection by withdrawing the syringe plunger; if blood enters the needle hub, the needle has entered a blood vessel. Withdraw the needle slightly and redirect it.
 - 5.8.3. Osmotic minipumps can be surgically implanted subcutaneously when repeated dosing is required.
- 5.9. Intravenous injections:
 - 5.9.1. Limit the number of punctures to 5 per site, per day.
 - 5.9.2. Rotate injection sites, if possible.
 - 5.9.3. For continuous infusion, cannulas or catheters may be fixed or surgically implanted.
- 5.10. Intranasal instillation:
 - 5.10.1. Brief anesthesia may be administered (e.g., isoflurane anesthesia).

6. REFERENCES

- 6.1. Diehl, K.-H. et al., "A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes", J. Appl. Toxicol., 21, 15–23 (2001)
- 6.2. Guide to the Care and Use of Experimental Animals, Vol. 1 (2nd ed), Canadian Council on Animal Care, Canada, 1993.
- 6.3. Gehling, A. M., Kuszpit, K., Bailey, E. J., Allen-Worthington, K. H., Fetterer, D. P., Rico, P. J., Bocan, T. M., & Hofer, C. C. (2018). Evaluation of Volume of Intramuscular Injection into the Caudal Thigh Muscles of Female and Male BALB/c Mice (*Mus musculus*). *Journal of the American Association for Laboratory Animal Science* : JAALAS, 57(1), 35–43.
- 6.4. CCAC guidelines: Experimental procedures (Part A – Administration of substances and biological sampling), Draft for Public Review – September 2021.
- 6.5. Refining procedures for the administration of substances. (2001). *Laboratory Animals*, 35(1), 1–41. <https://doi.org/10.1258/0023677011911345>
- 6.6. Turner P.V., Brabb T., Pekow C. and Vasbinder M.A. (2011a) Administration of substances to laboratory animals. Part A: Routes of administration and factors to consider. *Journal of the American Association of Laboratory Animal Science* 50(5):600-613.

SOP REVISION HISTORY

DATE	NEW VERSION
2017.01.17	4.4 Intramuscular injections, volume/site given in ml/kg
2017.08.31	4.1.3. Verify the solubility of the substance. Precipitation may cause the formation of large particles which, if injected intramuscularly, can be painful. For intravenous injections, solutions, not suspensions, must be administered.
2021.12.08	1. PURPOSE This Standard Operating Procedure (SOP) describes the recommended volumes, injection administration sites, volumes, and needle sizes for common routes of substance administration.
2021.12.08	3. MATERIALS 3.1. Needles 3.2. Syringes 3.3. Administration volumes chart 3.4. Substance to be administered
2021.12.08	3.1. Animals should be acclimated to handling and restraint to facilitate substance administration and reduce stress. 3.2. Dose volumes should be the minimum that is compatible with compound formulation and accuracy of administration. 3.3. If the substance is known to be toxic or an irritant, consideration should be given to minimizing the dose and choosing a route with the least potential complications. 3.4. The route of administration should be determined by the purpose of the experiment, the species, the possible effects of the dosing technique on the animal, the substance properties, and the frequency of dosing. 3.6. The vehicle chosen should be biologically inert, have no effect on the biophysical properties of the compound, and have no toxic effects on the animals at the quantities administered. If a component of the vehicle has biological effects, its total amount should be limited such that these effects are minimized. The viscosity of the vehicle should be suitable for the route of administration.
2021.12.08	4. ENTERAL ROUTES OF ADMINISTRATION 4.1. The enteral routes of administration deliver substances via the gastro-intestinal tract and include oral, gastric, and rectal administration. 4.2. Voluntary consumption: 4.2.1. Substances can be mixed in foods or fluids. Mixing with a palatable substance can facilitate administration. 4.2.2. Animals should be separated to ensure adequate consumption of doses. 4.3. Gavage: 4.3.1. Requires the use of a feeding tube or feeding needle. The size (gauge and length) and type of tubing should be carefully selected to minimize discomfort during dosing. 4.3.2. If administering the recommended volume, withdrawal of food prior to oral administration is not required. However, restricting the food intake before dosing may be beneficial in preventing discomfort and allowing for better absorption of some substances. 4.3.3. Administration of large volumes can result in reflux and aspiration into the lungs which can lead to serious complications. 4.4. Rectal administration: 4.4.1. Rectal instillation can be performed using a small-gauge, soft, flexible tubing with smooth edges.
2021.12.08	5. PARENTERAL ROUTES OF ADMINISTRATION 5.1. The parenteral routes of administration are all routes other than that are not enteral, generally injections.
2021.12.08	5.3. Needles: 5.3.1. Always use sharp Do not reuse needles. Use a new, sterile needle for every animal. 5.3.2. Use the smallest gauge of needle possible that allows accurate injection of the substance. 5.3.3. When withdrawing a solution through a rubber stopper, needles can become dull. Switch to a new, sterile needle for injection. 5.3.4. Note that up to 0.05 ml of the solution may be lost in the syringe hub. 5.3.5. Recommended Maximum acceptable needle sizes (G):
2021.12.08	5.4.3. 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. Unless it is an IV injection, withdraw the needle slightly and redirect it.
2021.12.08	5.4. Volumes for injection:

2021.12.08	<p>5.5. Intradermal injections:</p> <p>5.5.1. General anesthesia may be required to immobilize animals.</p> <p>5.5.2. Clip fur and clean the skin with 70% alcohol or an antiseptic solution prior to injection.</p> <p>5.5.3. Intradermal injection should be limited to 6 sites per day.</p>
2021.12.08	<p>5.6. Intramuscular injections:</p> <p>5.6.1. Due to their small muscle mass, intramuscular injections should be avoided in smaller species as it is technically difficult and painful for the animals.</p> <p>5.6.2. Check proper placement of the needle prior to injection by withdrawing the syringe plunger; if blood enters the needle hub, the needle has entered a blood vessel. Withdraw the needle slightly and redirect it.</p> <p>5.6.3. Intramuscular administration should be limited to 2 sites per day.</p> <p>5.6.4. Injection sites should be rotated.</p>
2021.12.08	<p>5.7. Intraperitoneal injections:</p> <p>5.7.1. This technique is not recommended in pregnant animals or birds.</p> <p>5.7.2. Check proper placement of the needle prior to injection by withdrawing the syringe plunger; if blood, urine, or digesta enters the needle hub, withdraw the needle and discard solution.</p> <p>5.7.3. Limit intraperitoneal injections to twice once per day.</p> <p>5.7.4. Injection sites should be rotated.</p> <p>5.7.4. Osmotic minipumps can be surgically implanted intraperitoneally when repeated dosing is required.</p>
2021.12.08	5.8.1. Subcutaneous administration should be limited to 2 to 3 sites per day.
2022.07.07	4.3.5. Refer to the table in Annex 2 for recommended volumes of administration.
2022.07.07	5.3.4. Note that up to 0.05 mL of the solution may be lost in the syringe hub, depending on the syringe and needle used .
2022.07.07	<p>5.3.5. Maximum acceptable Recommended needle sizes (G):</p> <p>5.3.5.1. Refer to the table in Annex 1.</p> <p>5.3.5.2. As birds and fish species vary greatly in size, needle sizes should be selected according to specific species.</p>
2022.07.07	<p>5.4. Volumes for injection of administration:</p> <p>5.4.1. Use the smallest possible volume for injection of administration, administering less than the recommended volumes whenever possible.</p> <p>5.4.2. Recommended volumes for substance administration:</p> <p>5.4.2.1. Refer to the table in Annex 2.</p> <p>5.4.2.2. As bird species vary greatly in size, administration volumes should be selected according to specific species.</p> <p>5.4.3. If the volume administered must exceed the recommended volumes listed in section 4.4.2, justification must be provided and will require approval by the FACC.</p> <p>5.4.4. Possible maximal administration volumes:</p> <p>5.4.4.1. Refer to table in Annex 3.</p>
2022.07.07	6.3.2. Become familiar with the internal anatomy of the species used to avoid penetration of internal organs during injection.
2022.07.07	6.5.3. For continuous infusion, indwelling cannulas or catheters may be fixed or surgically implanted.
2022.07.07	<p>6.5. Intravenous injections:</p> <p>6.5.4. Recommended injection sites:</p>
2022.07.07	<p>7.3. Gehling, A. M., Kuszpit, K., Bailey, E. J., Allen-Worthington, K. H., Fetterer, D. P., Rico, P. J., Bocan, T. M., & Hofer, C. C. (2018). Evaluation of Volume of Intramuscular Injection into the Caudal Thigh Muscles of Female and Male BALB/c Mice (Mus musculus). Journal of the American Association for Laboratory Animal Science : JAALAS, 57(1), 35–43.</p> <p>7.4. CCAC guidelines: Experimental procedures (Part A – Administration of substances and biological sampling), Draft for Public Review – September 2021.</p> <p>7.5. Refining procedures for the administration of substances. (2001). Laboratory Animals, 35(1), 1–41. https://doi.org/10.1258/0023677011911345</p> <p>7.6. Turner P.V., Brabb T., Pekow C. and Vasbinder M.A. (2011a) Administration of substances to laboratory animals. Part A: Routes of administration and factors to consider. Journal of the American Association of Laboratory Animal Science 50(5):600-613.</p>
2022.07.07	<p>3.7. All substances to be administered to animals must be discarded on or before the manufacturer's expiration date to ensure safety, sterility, and stability. Expired substances must not be administered to animals.</p> <p>3.7.1. Diluting and mixing drugs or agents may change drug chemical composition and shelf life. It is recommended to discard dilutions and mixtures within 30 days of preparation, unless otherwise specified by the manufacturer or when peer-reviewed evidence is available.</p>

Recommended needle sizes

SPECIES	SUBCUTANEOUS	INTRAPERITONEAL	INTRAVENOUS	INTRAMUSCULAR	INTRADERMAL
Mouse	25-27	27-30	26-30	29-31	27-30
Rat	25-27	23-27	25-27	25-30	27-30
Rabbit	21-25	21-23	23-25	23-25	25-30
Cat	21-25	21-23	21-25	21-25	25-30
Dog	21-25	21-23	21-25	21-25	25-30
Ferret	23-25	21-23	21-25	21-25	25-30
Marmoset	23-27	23-27	25-27	25-27	25-30
Macaque	21-25	21-23	21-25	21-25	25-30
Frog (Xenopus)	25	n/a	25	25	n/a
Swine	19	19	19	19	25-30
Cow	19	19	19	19	25-30
Sheep	19	19	19-21	21	25-30

Recommended administration volumes

SPECIES	GAVAGE	SUBCUTANEOUS	INTRAPERITONEAL	INTRAVENOUS BOLUS	INTRAVENOUS INFUSION	INTRAMUSCULAR	INTRADERMAL	INTRANASAL
Mouse	5 mL/kg	5 mL/kg/site	10 mL/kg	< 2 - 4 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/site	0.05 mL/site	35 µL
Rat	5 mL/kg	5 mL/kg/site	10 mL/kg	5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/site	0.05 mL/site	35 µL
Hamster	5 mL/kg	5 mL/kg/site	10 mL/kg	5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/site	0.05 mL/site	35 µL
Gerbil	5 mL/kg	5 mL/kg/site	10 mL/kg	5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/site	0.05 mL/site	35 µL
Guinea pig	5 mL/kg	5 mL/kg/site	10 mL/kg	5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/site	0.05 mL/site	35 µL
Rabbit	5 mL/kg	2.5 mL/kg/site	3 - 5 mL/kg	1 - 5 mL/kg	5 mL/kg	0.05 mL/kg/site	0.05 mL/site	200 µL
Cat	5 mL/kg	2 mL/kg	-	< 5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/kg/site	0.05 mL/site	200 µL
Dog	5 - 8 mL/kg	1 mL/kg	1 mL/kg	< 5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/kg/site	0.05 mL/site	200 µL
Ferret	5 mL/kg	1 mL/kg	1 mL/kg	< 5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/kg/site	0.05 mL/site	200 µL
Marmoset	10 mL/kg	2 mL/kg	5 mL/kg	2.5 - 5 mL/kg	< 2 - 4 mL/kg/hour	0.1 mL/site	0.05 - 0.1 mL/site	50 µL
Macaque	5 - 8 mL/kg	1 mL/kg/site	3 mL/kg	1 - 5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/kg/site	0.05 - 0.1 mL/site	200 µL
Fish	5 mL/kg	1 mL/kg	10 mL/kg	5 mL/kg	< 2 - 4 mL/kg/hour	0.05 mL/kg/site	0.05 mL/site	-
Swine	10 mL/kg	1 mL/kg	1 mL/kg	1 - 5 mL/kg	< 2 - 4 mL/kg/hour	0.25 mL/kg	0.2 mL/site	-
Cow	10 mL/kg	1 mL/kg	1 mL/kg	1 - 5 mL/kg	< 2 - 4 mL/kg/hour	0.25 mL/kg	0.2 mL/site	-
Sheep	10 mL/kg	1 mL/kg	1 mL/kg	1 - 5 mL/kg	< 2 - 4 mL/kg/hour	0.25 mL/kg	0.2 mL/site	-

Possible maximum volumes of administration

SPECIES	GAVAGE	SUBCUTANEOUS	INTRAPERITONEAL	INTRAVENOUS SLOW INFUSION	INTRAMUSCULAR	INTRADERMAL	INTRANASAL
Mouse	20 mL/kg	20 mL/kg into 2-3 sites	20 mL/kg	25 mL/kg	0.1 mL/site	0.1 mL/site	50 µL
Rat	20 mL/kg	10 mL/kg Into 2-3 sites	10 mL/kg	20 mL/kg	0.2 mL/site	0.1 mL/site	50 µL
Hamster	20 mL/kg	10 mL/kg Into 2-3 sites	10 mL/kg	20 mL/kg	0.2 mL/site	0.1 mL/site	50 µL
Gerbil	20 mL/kg	10 mL/kg Into 2-3 sites	10 mL/kg	20 mL/kg	0.2 mL/site	0.1 mL/site	50 µL
Guinea pig	20 mL/kg	10 mL/kg Into 2-3 sites	10 mL/kg	20 mL/kg	0.2 mL/site	0.1 mL/site	50 µL
Rabbit	20 mL/kg	10 mL/kg Into 2-3 sites	10 mL/kg	10 mL/kg	0.5 mL/kg/site	0.1 mL/site	500 µL
Cat	15 mL/kg	5 mL/kg	10 mL/kg	10 mL/kg	0.5 mL/kg/site	0.1 mL/site	500 µL
Dog	15 mL/kg	2 mL/kg Into 2-3 sites	20 mL/kg	10 mL/kg	0.5 mL/kg/site	0.1 mL/site	500 µL
Ferret	15 mL/kg	2 mL/kg Into 2-3 sites	20 mL/kg	10 mL/kg	0.5 mL/kg/site	0.1 mL/site	500 µL
Marmoset	15 mL/kg	5 mL/kg	20 mL/kg	10 mL/kg	0.25 mL/site	0.1 mL/site	100 µL
Macaque	15 mL/kg	2 mL/kg Into 2-3 sites	10 mL/kg	10 mL/kg	-	0.5 mL/site	500 µL
Fish	-	-	-	-	-	0.1 mL/site	-
Swine	10 mL/kg	1 mL/kg	1 mL/kg			0.2 mL/site	-
Cow	10 mL/kg	1 mL/kg	1 mL/kg			0.2 mL/site	-
Sheep	10 mL/kg	1 mL/kg	1 mL/kg			0.2 mL/site	-