

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_021 Oral Gavage in Mice and Rats Institutional author: UQ Biological Resources AEC Reviewed & Approved: October 2025 SOP Expiry: October 2028	Version #4.0 Page 1 of 6
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LAB_021 Oral Gavage in Mice and Rats (Expiry: Oct 2028)

I. OBJECTIVE

To describe the standard oral gavage procedure in mice and rats used across UQ research projects, also reflecting the procedure used to train workers across UQ by UQBR.

NB: The use of (*) indicates this statement is dependent on the facility procedures

NB: The use of () indicates this statement is dependent on AEC Approvals**

II. DEFINITIONS

Competent - “the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments.”¹

Oral gavage – Administering into the stomach or small intestine.

III. COMMENTS / RECOMMENDATIONS

- Oral gavage must be performed by appropriately trained personnel who have been deemed to be competent in the procedures.
- Clean technique** should be used in making up solutions, dilution of substances, drawing up the substance and administering to the animal.
- Test the gavage tip after a bite to identify any holes that leak solution, discard the gavage tip if damaged.
- Using separate gavage tips is strongly recommended between solutions and cohorts.
- Positioning of the rodent (i.e. its body alignment) is critical for efficient technique
- Never force the gavage tip down through the oesophagus. The pathway should be relatively smooth and free of resistance. Forcing the needle can perforate the walls of the oesophagus/stomach.
- Always administer the compound slowly and ensure the injection is complete before beginning to withdraw the gavage needle
- Immediately stop the procedure and assess the animal’s condition if mucous membranes turn blue, the animal is struggling vigorously, appears to have difficulty breathing, or fluid is observed coming out the nose
- This procedure is usually performed on conscious animals. IF anaesthesia is required, this must be detailed and approved by the AEC.

Considerations for neonates

- For neonates consider using low volume syringes with attached flexible tubing to improve volume accuracy. *Commercially available oral gavage needles are difficult to source, common practice in the past has been to create oral gavage needles using a 0.3 mL insulin syringe, 29G needle, then attaching semi-flexible micro-tubing (e.g. size 10 polyethylene tubing). This provides a suitable size and steady administration of the substance.*
Tubing should be measured and trimmed to an appropriate size for the age of the neonates to be gavaged, with enough length so that no part of the needle is inserted into the mouth. Check that the tubing trimmed is blunt with no sharp edges that could damage the oesophagus. Tubing is attached with a pair of forceps to avoid needlestick injury.

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		Page 2 of 6

- Handling pups may change their smell, where possible encourage mother to mark pups.
You can also rub your gloved hands in the dirty bedding in the cage before restraining, this will allow the smell to transfer to your gloves.
- A “full” or “empty” stomach can be identified in pups by the presence and size of a milk spot.
Pups may not tolerate large volumes if the stomach is full (see table 1).
- Ensure holding cage has heat source provided until the animal can access the mother
- Any unexpected loss of pups must be considered as an adverse event. These animal numbers are included in animal usage counts.

Table 1. Recommended values for gavage tips, syringe and maximum injection volume, for oral gavage in mice and rats. Gavage tip and syringe selection will vary depending on animal and substance characteristics.

Rodent species	Body Weight	Tip gauge	Tip Length (inches)	Syringe Size	Maximum injection volume (10mL/kg)
Mice	10g	Consider using micro-tubing†		0.3mL (insulin)	100uL
	15g	24G	1 to 1½”	1mL	150uL
	20g	22 to 20G	1 to 2”	1mL	200uL
	25g			1mL	250uL
	30g	20 to 19G	2 to 3”	1mL	300uL
	40G			1mL	400uL
Rats	50g	20 to 19G	1 to 1½”	1mL	0.5mL
	150g	18G	2 to 3”	3mL	1.5mL
	250g	18 to 16G	3 to 4”	3mL	2.5mL
	350g			5mL	3.5mL
	450g			5mL	4.5mL
	550g			10mL	5.5mL

†Micro-tubing, attached to an insulin syringe is described in within considerations for neonates

IV. SAFETY AND COMPLIANCE

- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Possible risks include mouse bite injury, needle stick injury, spills, exposure to infectious agents, repetitive task musculoskeletal injury and psychosocial harm.

V. TRAINING CONSIDERATIONS

- All unsupervised animal procedures must be performed by appropriately trained personnel who have been deemed competent in the procedure.
- Training for injections must be undertaken on models or cadaver animals initially.
- Note for UQBR Training purposes, 100uL may be administered once per rodent.

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---	--	--

- For UQBR training purposes animals may kept for monitoring. Adverse effects may take time to develop and can assist with the assessment of competency. These include gastro-oesophageal trauma or perforation, pulmonary aspiration (due to incorrect needle placement or injection technique), as well as physical/psychological stress to the animal (in rodents that have not been habituated to handling).

VI. EQUIPMENT

- PPE. *
Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious material.
- Disinfectant. *
- Gavage tip (flexible medical grade polyethylene micro tubing, or a bulb tipped needle), see table 1 for needle gauge and length.
- Substance for administration. (**)
- Syringe, see table 1.
- Clinical waste bin.
- Change station or Biosafety Cabinet. (*)

VII. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
Variations can occur between approved procedures listed versus what is planned with the animal – check AEC approvals.
- Set up equipment items.
There should be no contamination of equipment or substances during this process.
- Turn on Change station or Biosafety Cabinet. *
- Wipe surfaces with disinfectant.
Ensure equipment is operating as required.

Preparation of Injection Substance

- Confirm the concentration and volume with the approved AEC protocol.
The NHMRC Guidelines for oral administration reflect dosing limitations based on the size (volume) of the stomach and the amount of food and water already present in the stomach. If dosing using the maximum gavage volume, food can be removed a few hrs before the procedure with AEC approval. As a guide, oral gavage volume is 10ml/kg. Any volume larger than this should be stated and approved in the AEC application. Consider temperature, pH, injection of hazardous substances (cytotoxic, radioactive, infectious), and highly viscous liquids to improve success of procedure. These considerations can impact safety and animal welfare, refer to Reference Information below for information about these variables.
- Select the appropriate gauge and length of the gavage tip.
The gavage tip gauge is determined by the size of the animal (see Table 1). Determine the correct gauge by measuring from the lateral commissure of the lips to the xiphoid process of the rib. This can be achieved by measuring tubing against the body (see Figure 1). This is the length required to reach the rodent's stomach. It may be useful to mark this length on the needle (with a non-permanent marker pen) prior to use.

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---	--	--

- It is the responsibility of the researcher to convey all risks associated with compounds and materials to be used. This may include lab specific risk assessments and SDS and other OHS obligations.
If substances to be used are experimental or off label (i.e. no Safety Data Sheet is available), the laboratory is responsible for conveying all risks to workers involved in the project. This includes risk of performing the procedure as well as risks associated with animal husbandry, such as, waste management of cage bedding and cadavers that UQBR staff may be exposed to. Exposure maybe acute or chronic.
- If required, remove the solution from the fridge to reach room temperature.
It is not recommended to administer solutions that are very cold as this could cause discomfort.
- Load syringe and attach gavage tip removing dead space and air bubbles.
Ensure the solution is drawn up without the gavage tip, then push the solution forward to the tip of the gavage for accurate dosing. It can be helpful to overdraw the solution to account for the dead space, then expel the excess. Avoid contact between the outside of the gavage tip and the solution to minimise exposure to unpalatable substances. If contact, occurs, clean the gavage top before inserting e.g. wiping with a Kim-Wipe (C).

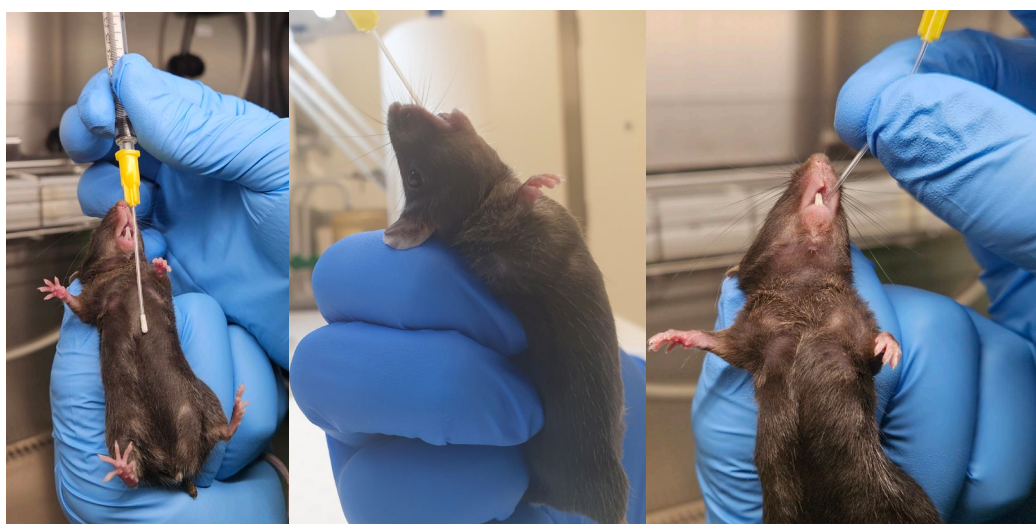


Figure 1. (Left) Measuring for correct tip gauge. **(Middle)** Secure restraint for oral gavage. **(Right)** correct positioning of the gavage tip when inserting

VIII. PROCEDURE

- Securely restrain the rodent, as per **LAB_006 Handling and Restraint in Mice and Neonates** for mice and **LAB_039 Handling and Restraint in Rats and Neonates** for rats. The head must be totally immobilised to position the gavage needle.
This procedure places pressure on the throat. If there is concern that breathing is being restricted, immediately release the rodent or reduce restraint pressure and ensure rhythmic breathing is present.
- With your dominant hand, insert the gavage tip in the diastema (gap between teeth) of the rodent's mouth.
Insert the gavage needle to the side of the front teeth (see Figure 1), taking care to avoid placing tubing directly towards the front of the mouth as the rodent is likely to bite the bulb of the needle. If the gavage tip is bitten, broken or shows signs of cracking, immediately remove and replace it.

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---	--	--

3. Direct the gavage tip toward the oesophagus at the back of the throat, gently press the needle on the back of the mouth and allow the animal to swallow the needle. It is important to use very gentle pressure, as too much force will rupture the oesophagus.
There should be little resistance. If resistance is felt, you are likely entering the trachea or placing pressure on the oesophagus wall. Withdraw the gavage tip and re-insert. The rodent may swallow as the gavage tip is passed into the oesophagus; this is a normal reflex. Sometimes gently twisting the tubing will assist in movement of the gavage tip into the oesophagus. It may be helpful to use the gavage tip to ensure the rodent's head remains in line with the body.
4. Slowly press on the plunger to administer the solution.
The plunger should be slowly depressed as the stomach may distend; this can minimise potential reflux.
5. Once the injection is complete, wait 1 second then carefully withdraw the gavage needle.
Remove the gavage needle from the side of the rodent's front teeth to avoid opportunities to bite on the gavage tip. If the gavage tip is broken or shows signs of cracking, immediately replace the gavage tip.
6. Release the rodent into holding cage and continue to monitor for recovery and health.
Following the procedure, the animal should immediately return to normal behaviour. Observe the animal for the following signs: laboured breathing, mouth breathing intermittent and violent abdominal movement, discharge from the nose or mouth, ruffled fur, hunched posture, lethargy, minimal responsiveness. If any of these symptoms are observed, contact your animal facility manager and/or Veterinary services for advice, referring to LAB_022 Veterinary Care Program.
7. Repeat steps 1-6 for any remaining rodents in the cohort.
8. Place the gavage tip and syringe into clinical waste bin. *
If re-using the gavage tips for the remainder of the treatment period, you can instead remove the gavage tip, flush with saline, and store in a zip lock bag. Only keep gavage tips that are undamaged and functional.
9. Complete record keeping requirements – note procedure, date and initials on cage card, log procedure on relevant AEC animal monitoring paperwork and the relevant research sample collection labelling/records.
Injection procedures should also include the substance and volume injected. Records need to be clear and legible on each record to allow others to read and understand.

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		Page 6 of 6

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Version #	Reviewing AECs	AEC Review Date	Outcome
3.0	Molecular Biosciences AEC, Laboratory Biomedicine AEC, Anatomical Biosciences AEC, Health Sciences	March 2025	Extended to March 2026
4.0	Molecular Biosciences AEC, Laboratory Biomedicine AEC, Anatomical Biosciences AEC, Health Sciences	December 2024-October 2025	Approved

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