

LAB_021 Oral Gavage in Mice and Rats

I. OBJECTIVE

To describe the oral gavage technique used in mice and rats within UQBR facilities.

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. COMMENTS / RECOMMENDATIONS

- Positioning of the rodent (i.e. its body alignment) is critical for efficient technique
- Never force the gavage needle into place
- 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, or appears to have difficulty breathing, or fluid is observed coming out the nose
- Coating the gavage needle tip with sucrose immediately prior to use may improve efficiency as well as animal welfare outcomes
- Rodents may be conscious or anaesthetised for the technique (however, any anaesthetics must be detailed and justified to the AEC, and only short acting anaesthetics should be used e.g. methoxyflurane or isoflurane)

In relation to human safety:

- Facility and procedure appropriate PPE use is essential when handling laboratory rodents
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form. This procedure has particular risks of:
 - needle stick and mouse bite injury – take appropriate care
 - splash back into the face or eyes when injecting – wear appropriate PPE
 - musculoskeletal injury when performed regularly – consider suitable ergonomic design wherever possible
- In the event of a spill follow facility emergency spill procedures relative to SDS details.

III. 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 needle (rigid or flexible, bulb tipped needle, tubing), see table 1 for needle gauge and length
- Substance for administration (**)
- Syringe, see table 1
- Clinical waste bin
- Change station or Biosafety Cabinet (*)(**)

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IV. PREPARATION

1. Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work
AEC approved activities are considered literally (as detailed in the application), they are not subject to discretion – check that detail are consistent and that the relevant personnel are approved.
2. Set up equipment items
There should be no contamination of needles or the substance for gavage during this process.
3. Turn on Change station or Biosafety Cabinet *
4. Wipe surfaces with disinfectant *Ensure equipment is operating as required.*

Clean Technique

Use clean technique when performing this procedure, this will minimise contamination from pathogens and subsequently infection in research animals.

Preparation of Injection Substance

- Confirm the concentration and volume with the approved AEC protocol
The NHMRC Guidelines for oral administration depends on the size (volume) of the stomach and the amount of food and water already present in the stomach. To maximise 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 clearly cited and justified 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 oral gavage needle
The oral gavage needle gauge is determined by the viscosity of the substance to be administered and the size of the animal.
To correctly select an oral gavage needle length, estimate distance by measuring from nose to the stomach. This can be achieved by measuring tubing against the body. Restrain the animal with head tilted backwards with the neck is extended and measure from the nose to the last rib of the rodent. 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.
- 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 of the risks to workers involved in the project. This includes risk of performing the procedure as well as the 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

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Do not administer chilled substances.

- Load syringe

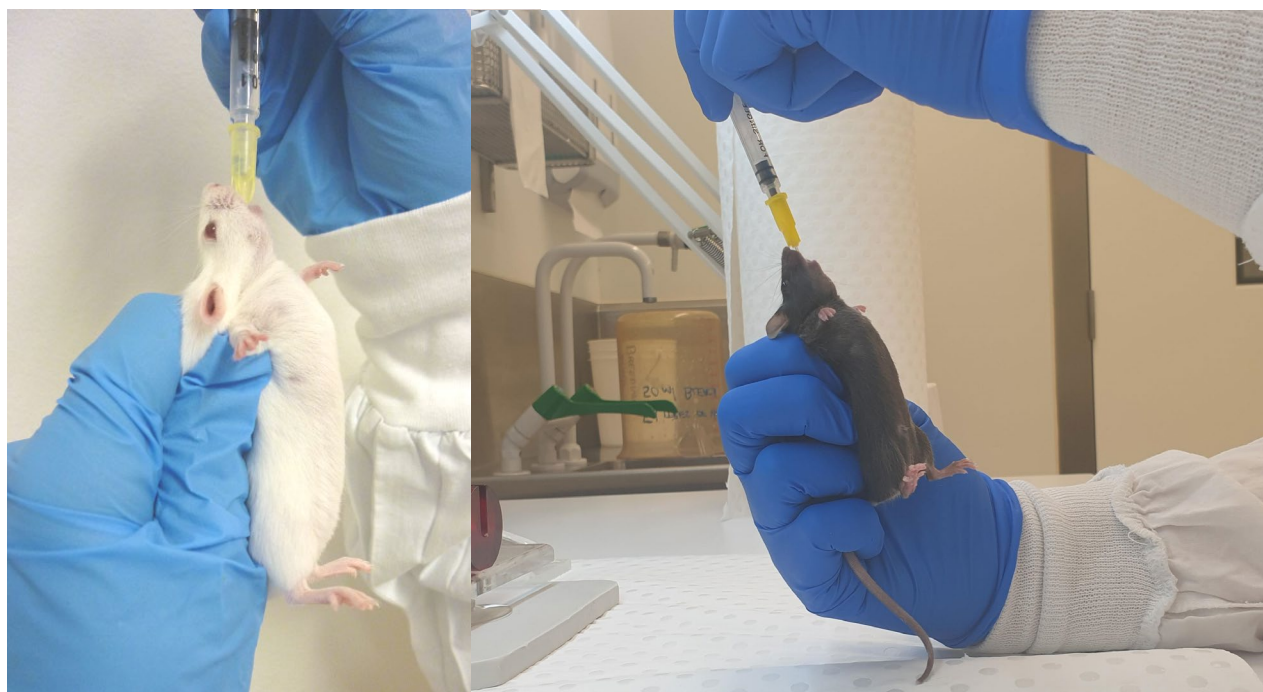
Consider the most appropriate way to load the syringe and gavage tube for accuracy. A measured volume of added solution may need to be drawn up to ensure accuracy of dosing because some solution will remain in the gavage needle. In some circumstances loading from the gavage needle may be possible for palatable solutions. In general avoid the outside of the gavage tube or needle bulb making contact with the solution to decrease exposure to unpalatable solutions. If the substance spills onto the outside of the gavage tube or needle, ensure the needle is cleaned prior to insertion (depending on the substance this may mean simply wiping the substance off with a Kim-Wipe®)

- Attach gavage tube removing dead space and air bubbles.

Mouse restraint for gavage

Refer to LAB_006 Handling and Restraint of Mice and neonates

- When performing oral gavage in mice ensure the rodent is restrained so the head is tilted back to extend the neck is but remain in line with the body in an upright position.



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Figure 1. Restraint of a mouse and weaner mouse during oral gavage

Rat restraint for Gavage

Refer to LAB_039 Handling and Restraint in Rats and Mice

- The recommended restraint method is the 1-person technique for visual inspection and held upright.

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. It is recommended that the tubing is attached with a pair of forceps.
- 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 is able to access the mother

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- Note any unexpected loss of pups must be considered as an adverse event. Note these animal numbers are included in animal usage counts.

V. PROCEDURE

Oral Gavage Procedure

1. Securely restrain the rodent, as explained above

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
2. With your dominant hand insert the gavage needle into the side of the rodent's mouth

Insert the gavage needle to the side of the front teeth, take 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 tube is bitten, broken or shows signs of cracking, immediately remove and replace it, preparing the oral gavage needle again.
3. Advance the bulb tip of the gavage tube down the oesophagus to the required depth.

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 tube and re-insert. The rodent may swallow as the tube is passed into the oesophagus, this is a normal reflex. Sometimes gently twisting the tubing will assist in movement of the needle into the oesophagus. It may be helpful to use the gavage needle 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 pressed slowly to slowly distend the stomach and minimise potential for reflux
5. Only once the injection is complete, 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 tube. If the gavage tube is broken or shows signs of cracking, immediately replace the tube.
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 movement once placed back in the cage. , if you see the animal behaving abnormally once in their home cage such as laboured breathing, gasping, hunched, or limited movement, take action to manage their condition, including veterinary advice and referring to SOP LAB_022 Veterinary Care Program.
7. Place the gavage needle and syringe into clinical waste bin *

Generally the gavage needle is disposed following use for a cohort of animals. Seek advice from the research group to confirm if the same gavage needle can be used for a cohort in a given day.
8. 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.
9. Repeat these steps for the next animal or if finished, pack and clean up equipment and space.

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VI. REFERENCE INFORMATION

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

Rodent species	Body Weight	Needle gauge	Needle Length (inches)	Syringe Size	Maximum injection volume (10mL/kg)
Mice	10g	Consider using micro-tubing†		0.3mL (insulin)	100uL
	15g	24G	1 to 1½"	<0.5mL	150uL
	20g	22 to 20G	1 to 2"	<0.5mL	200uL
	25g			<0.5mL	250uL
	30g	20 to 19G	2 to 3"	<0.5mL	300uL
	40G			<0.5mL	400uL
Rats	50g	20 to 19G	1 to 1½"	0.5 to 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. PREPARATION

UQBR Training Consideration

As part of UQBR training sessions (using this technique) animals may be maintained for a number of days post procedure to monitor for potential development of adverse outcomes. 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).

Injection Considerations **

Temperature – Consider if the substance has been stored in the fridge, if possible allow it to reach room temperature before injecting into the animal due to comfort and possible impact on body temperature.

Experimental Substances – A need for increased monitoring is generally required for experimental substances

Cells – When injecting cells, a larger gauge needle may need to be used. In a mouse a 25g needle will safely inject most cells. Depending on the research there may be a need to handle the needle and syringe in a specific manner for successful cell delivery.

Non-biological pH – There are mechanisms to improve pH of a substance for injection. For example, increasing the dilution, change of delivery vehicle, or anaesthetising the animal. This can decrease the risk of internal tissue necrosis and improve procedure outcomes.

If the substance is not a neutral pH of ~7, it may be acidic or alkaline, replace the needle that was used to drawn up the solution before injection to decrease any pain on entry to the animal.

Radioactive Substances – Additional approvals and safety precautions are required and will be included in the risk assessment. It is common to require safety goggles, additional gloves and shielding. You may also be required to work under a licensed person.

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Infectious – Additional approvals and safety precautions are required and will be included in the risk assessment. Additional training may be required to ensure containment of infectious agents and waste management to protect other research projects and human health.

Cytotoxic – Additional approvals and safety precautions are required and will be included in the risk assessment. Additional training may be required to ensure containment of cytotoxic agents and waste management to protect other research projects and human health.

Non-TGA approved and off label substance use – If substances are experimental there may not be an SDS available. Ensure the risk assessment for the use and management of the substance includes excretion of the substance from the animal, chronic versus acute exposure, waste management of bedding/cage handling.

Injecting Schedule 7, 8 or 9's – The use and possession of these scheduled drugs requires special QLD Health Approval. Please ensure you have QLD Health 'Researcher Approval to 'possess', 'use' and 'dispose' of these drugs during project planning. Seek further advice about this from UQBR or your local area Drugs Officer.

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