LAB_026 Intranasal Delivery in Mice and Rats (Expiry: May 2028)

I. OBJECTIVE

To describe the standard intranasal delivery 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. CONDITIONS FOR USING THIS SOP

- When citing this SOP in your ethics application, you must also describe your chosen restraint or anaesthetic technique (or quote the relevant SOP you will be following).
- You must state the volume you will be administering in your ethics application. Maximum volumes are outlined in the table below.
- Frequency should be as low as possible. provide justification and measures to reduce complications to the AEC in your application.

III. 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]
Intranasal – lying within or administered by way of the nasal structures

IV. COMMENTS / RECOMMENDATIONS

- Intranasal delivery must be performed by appropriately trained personnel who have been deemed to be competent in the procedures.
- **Aseptic technique** should be used in making up solutions, dilution of substances, drawing up the substance and administering to the animal. This includes using a new pipette tip for each animal.

Neonate comments

- 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.
- Ideally select pups that have recently fed by identifying a prevalent milk spot. There is a possibility pups may not feed soon after procedures.
- 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 maximum volumes and relative targets for intranasal delivery of substances rodents.

Values	Small volumes	Large volumes
Maximum Volume	5uL-10uL per nostril (10-20uL total)	10-25uL per nostril (20-50uL total) Volumes above 50uL require specific justification and AEC approval (**). Rats may tolerate slightly larger volumes than mice.
Target site of delivery (generalised)	Upper respiratory tract Central nervous system	Lower respiratory tract
Anaesthesia?	Not required	Requires anaesthesia as they are more likely to expectorate the substance and experience inappropriate levels of distress.

V. 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.

VI. TRAINING CONSIDERATIONS

- All unsupervised animal procedures must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training must be undertaken on models or cadaver animals initially.
- Note for UQBR Training purposes, 5uL may be administered per nostril.
- For UQBR training purposes animals may be kept for monitoring. Adverse effects may take time to develop and can assist with the assessment of competency. These include unresolved respiratory distress and suffocation.

VII. EQUIPMENT

- PPE. *
- Disinfectants. *
- P20 Pipette.
- Micro-pipette tips.
- Sharps container.
- Substance for administration. **
- Change station/Bio-safety cabinet. *
- Vaporous anaesthetic and associated delivery apparatus.
  (e.g. isoflurane vaporiser induction chamber)
- Oxygen supply.

VIII. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
  Deviations can occur between approved procedures listed versus what is planned with the animal – check that these match and that the relevant personnel are approved.
- 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.

IX. PROCEDURE

Conscious Animals (Small Volumes)

1. Correctly identify and restrain the animal, 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.
   It is imperative that the animal cannot freely move its head. If it can move its head the correct dose may not be administered, or you may injure the rodent during the procedure.
2. Draw up the solution with the pipette.
3. Slowly push the plunger to form a small droplet at the end of the pipette tip.
4. Place the droplet near the nostril allowing the rodent to inhale the solution.
5. Repeat above steps for remaining volume, alternating nostrils for each drop inhaled.
6. Discard the pipette tip into the sharp's container. *
7. Place the rodent into holding cage and continuously monitor the animal for 5 minutes. If any abnormal behaviour is observed (e.g. increased respiratory sounds) establish a plan for ongoing management (e.g. on-going oxygen supplementation and monitoring or immediate euthanasia) and consult a UQBR veterinarian, as required.
8. Complete all record keeping requirements e.g. updating the cage cards and animal monitoring records.

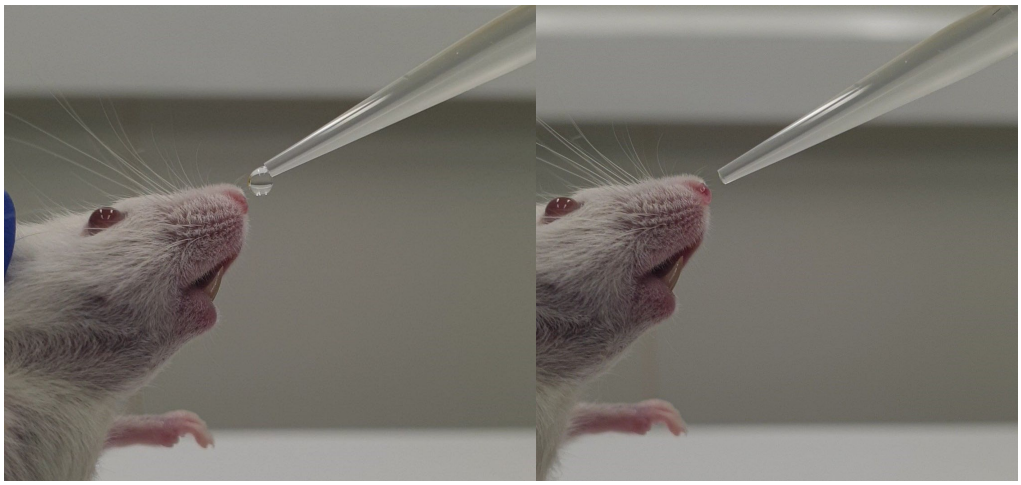

Figure 1. Droplet on the end of the pipette tip being inhaled by a mouse.

Anaesthetised Animals (Large Volumes)

Wherever possible, supplementary oxygen should be provided from anaesthetic induction, through the procedure, until the animal has recovered from anaesthesia.

1. Correctly identify the animal and induce anaesthesia as per the UQ AEC Approved SOP or ethics protocol.
2. Once anaesthetised, check the animal is at an appropriate depth of anaesthesia, then collect the animal from the anaesthetic delivery apparatus and position them so that you can gently restrain the head.
   When appropriately anaesthetised the rodent has lost its righting and withdrawal reflexes.
3. Draw up the solution with the pipette.
4. Slowly push the plunger to form a small droplet at the end of the pipette tip.
5. Place the droplet near the nostril allowing the rodent to inhale the solution.
6. Repeat above steps for remaining volume, alternating nostrils for each drop inhaled.
   Steps 2-6 must be done with consideration that the animal could start to regain consciousness. For this reason, these steps must be done without being wasteful of time. If the rodent is becoming "too light" in the anaesthetic plane (i.e. regaining consciousness), gently place it back into the anaesthetic delivery apparatus and recommence the intranasal dose delivery from step 2.
7. Discard the pipette into the sharp's container. *
8. Place the rodent into holding cage and continuously monitor the animal until it can perform deliberate conscious movements (e.g. able to walk, eat, drink and toilet normally). If any abnormal behaviour is observed (e.g. increased respiratory sounds) establish a plan for ongoing management (e.g. on-going oxygen supplementation and monitoring or immediate euthanasia) and consult a UQBR veterinarian, as required.
9. Complete all record keeping requirements e.g. updating the cage cards and animal monitoring records.

Monitoring of Animal Condition

- If there are any concerns relative to the animal's ability to breathe, immediately discontinue the procedure, release restraint, and provide supplementary oxygen: refer to **LAB_022 UQBR Veterinary Care Protocol**.
- If the animal's ability to breathe has been affected by the intranasal delivery, the volume was likely incorrectly calculated (i.e. >60uL) or it was administered too rapidly.
- In the event that severe respiratory distress is observed, immediately perform euthanasia of the animal.
  Severe respiratory distress is characterised by irregular, laboured breathing that causes significantly reduced activity in the animal and which is unresponsive to intervention (e.g. releasing the animal from restraint and providing oxygen supplementation does not resolve the symptoms).

X. REFERENCES

1. Erdő, F., Bors, L. A., Farkas, D., Bajza, Á., & Gizurarson, S. (2018). Evaluation of intranasal delivery route of drug administration for brain targeting. Brain Research Bulletin, 143, 155-170. doi:https://doi.org/10.1016/j.brainresbull.2018.10.009
2. Hanson, L. R., Fine, J. M., Svitak, A. L., & Faltesek, K. A. (2013). Intranasal Administration of CNS Therapeutics to Awake Mice. JoVE(74), e4440. doi:doi:10.3791/4440
3. Hanson, L. R., Fine, J. M., Svitak, A. L., & Faltesek, K. A. (2013). Intranasal administration of CNS therapeutics to awake mice. Journal of visualized experiments : JoVE(74), 4440. doi:10.3791/4440
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5. Turner, P. V., Brabb, T., Pekow, C., & Vasbinder, M. A. (2011). Administration of substances to laboratory animals: routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science : JAALAS, 50(5), 600-613.
6. University of Boston. (2014). Administration Of Drugs and Experimental Compounds in Mice and Rats. Research Ethics - Procedures. Retrieved from http://www.bu.edu/researchsupport/compliance/animal-care/working-with-animals/procedures/administration-of-drugs-and-experimental-compounds-in-mice-and-rats/
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Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Outcome
4.0	Molecular Biosciences AEC and Health Sciences AEC	February 2025	Approved
4.0	Anatomical Biosciences AEC and Laboratory Biomedicine AEC	May 2025	Admin changes required
4.1	Admin changes made to SOP as per May 2025 outcomes	N/A	SOP finalised

Conditions:
- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).