LAB_056 Glucose Tolerance Test in Mice (Expiry: March 2026)

I. OBJECTIVE

To describe the general procedural expectation for performing safe, effective, and humane glucose tolerance tests (GTT), following glucose loading in mice.

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." (NHMRC, 2013).

Glucose Tolerance Test (GTT) – A test performed to measure the rate of glucose clearance from the blood following administration of a glucose load (i.e. glucose tolerance). GTT detects deviations from normal glucose metabolism, which is useful in studying human diseases such as metabolic syndrome and diabetes mellitus.

Intraperitoneal Glucose Tolerance Test (IPGTT) – glucose is administered via intraperitoneal (IP) injection

Intravenous Glucose Tolerance Test (IVGTT) – glucose is administered via intravenous (IV) injection

Oral Glucose Tolerance Test (OGTT) – glucose is administered via oral gavage (OG)

High Fat Diet (HFD) - A diet which contains 45-60% fat, as compared to standard rodent chow diet, which contains ~10% fat.#

III. COMMENTS / RECOMMENDATIONS

- Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application: route of glucose administration, method of blood collection, and any intended variation to this SOP.
- The route of glucose administration is critically linked to the scientific question being investigated. For example, OGTT has the broadest application as it is most physiological and clinically relevant. IPGTT is commonly applied in concert with an OGTT, 2 -5 days apart, to delineate whether alterations in glucose metabolism is resultant of gastrointestinal or the pancreatic defects. IVGTT is far more invasive and specialised, and as such, requires specific justification.
- If planning to perform cannulation for IVGTT, wherever possible the cannula used for glucose administration should also be used for blood collection (to avoid repeat venepuncture). For more detail, see Procedure Annex: Cannulation of a Blood Vessel.
- GTT data outcomes and reproducibility of these results are significantly influenced by suboptimal and inconsistent procedures. It is critically important for the scientific validity of data obtained that GTT is well planned, and performed in a precise and controlled manner, as outlined in this SOP, and summarised within textbox 1, within the reference information section.
- Matching of animal-groups is an important consideration as age, sex, strain, body weight, reproductive status, and health status, among others animal characteristics, are all known to influence insulin sensitivity and glucose tolerance.
- Stress significantly impacts glucose metabolism. The test environment should be managed as a "low stress" environment, free from uncontrolled external stimuli such as human traffic, unnecessary noise, and intense lighting. It is recommended in the days to weeks prior to commencing experimentation that the mice are trained to tunnel handling or other low-stress handling techniques by the person/s that will be completing

the GTT. This period of acclimatisation will enable more efficient handling, and reduced stress for the mice throughout the procedure (Gouveia *et al.*, 2017; Henderson *et al.*, 2020).

- All other stimuli must be controlled as much as possible. For example, light/dark cycles, animal feed, ventilation, caging systems, and environmental enrichment should all be standardised. Note: glucose tolerance has significant variability dependent on the time of day, and light/dark phase period (Carroll *et al.*, 1973; la Fleur *et al.*, 2001). Study design should account for and aim to control this variability.
- "Diseased" or "sick" mice (i.e. animals with any symptoms, exposure history, or diagnostic results which indicate sub-optimal health) should not be used within GTT procedures, unless that disease state is considered part of the study design (e.g. pharmaceutically induced diabetes mellitus, phenotypic SOD1 mice).
- The dosage of glucose administered must be established relative to the experimental design. This dose should be calibrated relative to the individual mouse's gross body mass, or lean body mass (for obese animals). Generally, the glucose dose should be limited to 2 g/kg.
- Fasting is a standard component of GTT. Fasting is usually done as either a "morning fast" (4-6hrs) or an "overnight fast" (10-12hrs). A duration anywhere between 4 to 12 hrs, during either the dark- or light-phase, is generally considered acceptable. It is essential that animal users review current literature and previous datasets to ensure the selected fasting period is appropriate relative to their animal model and planned scientific outcomes. For example, glucose intolerance between animals fed high-fat diet (HFD) and standard rodent chow has been found to be more evident from a shorter, less invasive fasting periods of 6 hrs, compared to the more traditional 12 hr overnight fasting (Andrikopoulos *et al.*, 2008; Ayala *et al.*, 2010). Additionally, there has been some academic suggestions that fasting during the light phase, as compared to the dark phase, better mimics experimental conditions used for humans, aiding translatability.
- As nocturnal-feeding animals with relatively high metabolic rates, overnight (dark-phase) fasting >12 hrs in rodents may induce physiological changes which are more like starvation, than fasting, in humans. As such, where fasting >12 hrs is intended to occur for the purpose of performing GTT, the reviewing AEC will expect specific justification as to why this variation is required and why it should be considered ethically acceptable.
- Generally, anaesthetic agents should not be used to facilitate GTT as their use can potentially lead to confounding of experimental data. This is particularly relevant when using *LAB_013 Blood Collection – Tail Tip (Amputation) Bleed in Rats and Mice* to collect blood for GTT (i.e. anaesthesia should not be used to perform tail tip amputation when conducting a GTT)

IV. EQUIPMENT

- Personal Protective Equipment (PPE)
  Requirements vary dependent on facility and the type of work being conducted.
- Permanent Marker
- Disinfectant (e.g. 70% ethanol)
- Electronic weigh scales
- Local anaesthetic ointment (e.g. Emla® cream)
- Heat source, e.g. heat mat, regulated warming box, or warm water bath,
  As per the relevant blood collection SOP, listed under step 5
- Clean restraint tubes (appropriate size and type)
  As per LAB_006 Handling and Restraint in Mice and Neonates
- Venepuncture instrument/tool, e.g. sterile lancet, number 11 scalpel blade, 25 - 30G needle
- Blood collection tubes (if not collecting blood directly onto the glucose test strip)
- Gauze swabs

- 20% Glucose solution (sterile, using an innocuous vehicle e.g. saline or PBS)
- Glucometer and compatible glucose test strips
- Sharp's container and clinical waste bin

V. PREPARATION

- Check AEC approvals, procedural documents, and animal identification, to ensure that the correct animals and procedures (including personnel and location details) have been selected for the scheduled work.
- Ensure appropriate experimental record sheets are organised (see table 1 for an example template, within the reference information section).
- Ensure the glucometer is functioning appropriately and 'ready to go' (e.g. there are sufficient numbers of compatible test strips available, and the device has sufficient battery charge).

VI. PROCEDURE

1. Measure and record the gross body weight of each mouse. Animals may be marked at the base of the tail using the permanent marker to assist with identification.

   Animal handling is performed in accordance with LAB_006 Handling and Restraint in Mice and Neonates.

2. Place the mice in a clean cage with routine access to water, but no access to food. Commence fasting of the mice for 4 to 12 hrs (see comments/ recommendations for guidance on fasting duration).

3. Measure and record gross body weight of each mouse, post fasting.

   At this time topical local anaesthetic ointment (e.g. Emla® cream) may be applied to the venepuncture site and the mice may be warmed, as per the relevant blood collection SOP, listed under step 5

4. Calculate and record the amount of glucose to be administered to each animal, relative to body weight.
   a) OGTT and IPGTT, glucose dose = 1 - 2g / kg

      To achieve 1.5g of glucose/kg body mass, (using a 20% glucose solution)

      Volume of glucose for injection (µL) = 7.5 x body weight (g)

      For example, a 25 g mouse will require an injection volume of 187.5µL

   b) IVGTT, glucose dose = 0.25 - 1g / kg

      To achieve 0.5g of glucose/kg body mass, (using a 20% glucose solution)

      Volume of glucose for injection (µL) = 2.5 x body weight (g)

      For example, a 25 g mouse will require an injection volume of 62.5µL

   Ensure injection volumes calculated do not exceed the maximum limits as prescribed within the relevant SOPs, listed under step 6.

   In relation to 20% glucose, this solution is hypertonic relative to blood and interstitial fluid (20% glucose is ~1000mOsmol/L, whereas serum is <300mOsmol/L). As a "once-off" injection this concentration difference is not expected to result in noticeable adverse reactions, however, the risk of irritation, particularly phlebitis associated with IV injection, is something for which operators should be aware.

5. Restrain the first mouse as required and collect a blood sample. This initial blood sample is used to measure and record the fasting basal glucose level. Blood samples are collected directly onto a glucose test strip for immediate assessment. Alternatively, a blood collection tube may be used.

   Blood collection may be performed via any of the methods listed below:

   - *LAB_013 Blood Collection – Tail Tip (Amputation) Bleed in Rats and Mice (without anaesthesia)*
   - *LAB_019 Blood Collection - Tail Bleed in Rats and Mice*

- *LAB_020 Blood Collection – Facial Bleed (Sub-Mandibular) in Mice*
- *LAB_036 Blood Collection – Saphenous Vein in Mice*
- *LAB_037 Blood Collection – Pedal Vein in Bleed Mice*
- *Blood collection via an indwelling cannula*

Following any blood collection method, ensure the collection site has stopped bleeding. If ongoing bleeding is observed gentle pressure using a gauze swab may be applied. In the case of tail tip amputation, Emla® cream applied directly to the amputated tip can act as a useful occlusive layer/bandage.

6. Immediately following step 5, administer the predetermined glucose load (identified in step 4), via:
   a) LAB_021 Oral Gavage in Mice and Rats for OGTT,
   b) LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates for IPGTT,
   c) or LAB_030 Injections - Intravenous (IV) tail vein, in Mice and Rats for IVGTT.

   If planning to perform cannulation for IVGTT, refer to the Procedure Annex: Cannulation of a Blood Vessel.

7. Record the time of blood collection and glucose administration (from steps 5 and 6, respectively) as the "start time" on the experimental record sheet (and start a timer accordingly).

   Often multiple mice will be undergoing GTT on the one day, at the same time. Careful time management is required to ensure the blood collection points are accurately reflective of the scheduled time points for each mouse. As such, individual animals should be staggered (e.g., using 1–5-minute intervals).

8. Collect serial blood samples at predetermined timepoints post glucose loading. Ensure each timepoint and sample result is recorded on the experimental record sheet.
   a) These timepoints for blood collection generally include: 15, 30, 45, 60, 90, 120 minutes post glucose loading.
   b) The predetermined timepoints may vary between individual models, however, the number of blood collection points should not exceed 10 in total (inclusive of the initial blood collection for establishing the fasting basal glucose level);
   c) Collection volumes at each timepoint should remain <10uL;

      Larger blood volumes may be collected (e.g. 20uL) so long as the following point is observed;

   d) The total volume of blood collected must be the minimum amount required for the project, should not exceed a "moderate bleed", and must not exceed a "major bleed" (see table 2, within the reference information section).

   Blood collection in rodents almost always causes some "wastage" (e.g. oozing or dripping of blood post venepuncture, extravasation of blood into the subcutaneous space). It is critical that this volume of wastage is estimated and accounted for when estimating the total blood loss to the animal.

9. At the end of the experiment return mice to their home cages with food and water and monitor their general behaviour and physical condition. If any signs of pain and distress are observed take immediate action as appropriate – for guidance refer to the animal ethics webpage.

10. Reassess the mice after at least 1 hour, before returning to routine monitoring protocols.

    It is recommended that these animals are then permitted at least a 24hr "respite" period, where they are not used for other procedures.

11. After the GTT procedure, stored blood samples may be assessed further using various assays (*ex vivo*). This testing may require specific blood handling and storage (e.g. collection of blood plasma and storage at ≤ (-)20°C, until analysis).

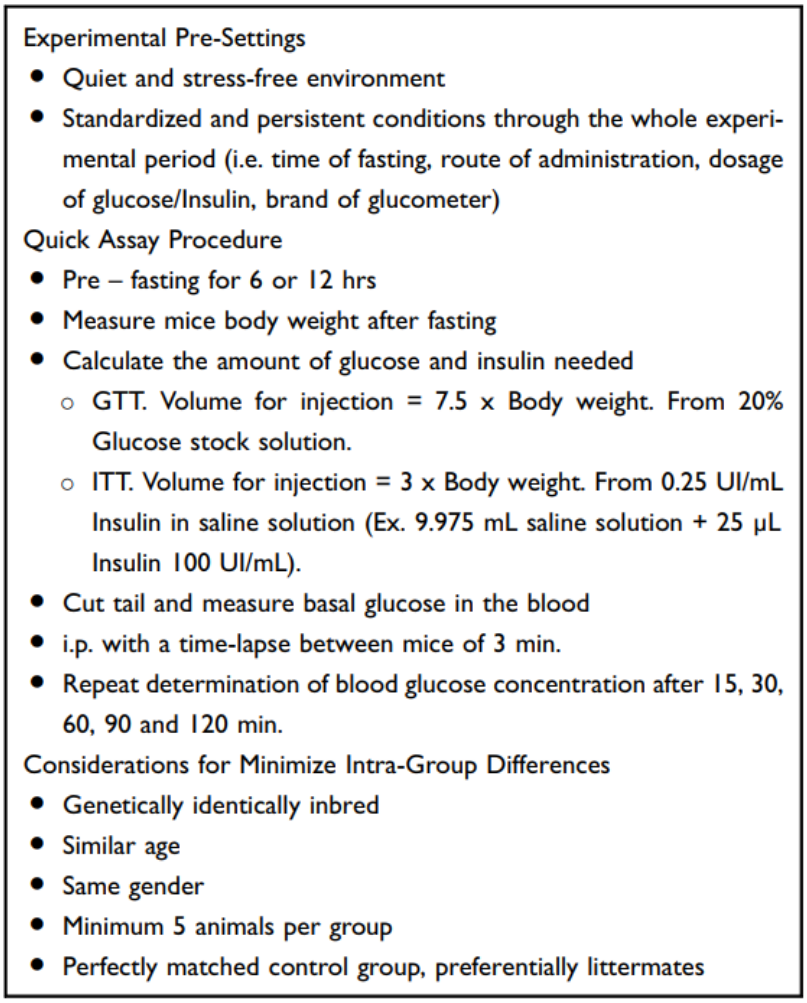
PROCEDURE ANNEX: Cannulation of a Blood Vessel

- If cannulation is proposed to administer the glucose load, the following information is expected to be provided with justification within the animal ethics application: why cannulation is necessary (why is the OGTT or IPGTT not sufficient to investigate your scientific question), where the cannula is intended to be placed and why that location (e.g. tail vein vs jugular vein vs carotid artery), if the cannula will be used for blood collection in addition to glucose administration, any other relevant procedural details (e.g. how the cannula will be placed, how it will be maintained, any variations this presents to the standard GTT procedure described above, associated anaesthesia and analgesia protocols).
- Wherever possible, a cannula used for glucose administration should also be used for the subsequent blood collection (to avoid repeat venepuncture). The difficultly associated with maintaining a tail vein cannula in mice (due to the blood vessel size and movement of the animal) is an important consideration – as such, the jugular vein and carotid artery often remain the only viable options for cannulation in mice.
- Jugular vein and carotid artery cannula placement are surgical procedures,
    - which are more invasive than tail vein cannulation, so should only be performed where necessary,
    - must follow the principles described within LAB_001 Aseptic Technique for Laboratory Animal Surgery or LAB_002 Clean Technique for Laboratory Animal Surgery,
    - require general anaesthesia (as per LAB_060 Rodent Anaesthesia - Isoflurane),
    - and require analgesia (as guided by Guideline – Rodent Analgesia (Procedure Specific)).

VII. REFERENCE INFORMATION

TEXTBOX 1 | Summary of General Experimental Considerations, relative to GTT (image source: Benedé-Ubieto *et al.*, 2020). *In the context of this SOP, please consider the following comments:*

- "same gender", should be considered to read as "sex matched"
- minimum animal numbers per group, should be established based on power analysis
- *Insulin Tolerance Test (ITT), is not considered part of this SOP. For information relative to this procedure please refer to LAB_056 Insulin Tolerance Test in Mice.*



Experimental Pre-Settings

- Quiet and stress-free environment
- Standardized and persistent conditions through the whole experimental period (i.e. time of fasting, route of administration, dosage of glucose/Insulin, brand of glucometer)

Quick Assay Procedure

- Pre – fasting for 6 or 12 hrs
- Measure mice body weight after fasting
- Calculate the amount of glucose and insulin needed
  - GTT. Volume for injection = 7.5 x Body weight. From 20% Glucose stock solution.
  - ITT. Volume for injection = 3 x Body weight. From 0.25 UI/mL Insulin in saline solution (Ex. 9.975 mL saline solution + 25 µL Insulin 100 UI/mL).
- Cut tail and measure basal glucose in the blood
- i.p. with a time-lapse between mice of 3 min.
- Repeat determination of blood glucose concentration after 15, 30, 60, 90 and 120 min.

Considerations for Minimize Intra-Group Differences

- Genetically identically inbred
- Similar age
- Same gender
- Minimum 5 animals per group
- Perfectly matched control group, preferentially littermates

TABLE 1 | Example experimental record sheet for conducting GTT.

Mouse ID#	BW (g)	Glucose (µL)	Time of glucose injection	Glucose levels (mg/dL)						
				0min	15min	30min	45min	60min	90min	120min

TABLE 2| Recommended blood collection volumes based on a mouse's live body weight (NHMRC 2008).
The total amount of blood loss from any blood collection procedure must take into account the sample volume collected as well as any circumstantial bleeding (e.g. prolonged bleeding post venepuncture).

Mouse Weight	TOTAL BLOOD VOLUME (TBV) *[equates to 5-7% of body weight]*	Minor bleed *(<7.5% of TBV)*	Moderate bleed *(7.5-10% of TBV)*	Major Bleed *(10-15% of TBV)*
Recovery period required between bleeds, relative to volume collected:		1 week recovery	2 weeks recovery	3 weeks recovery
18g	1.2mL	<90uL	90-120uL	120-180uL
22g	1.5mL	<115uL	115-150uL	150-225uL
26g	1.8mL	<140uL	140-180uL	180-270uL

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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
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Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approved Until
2	LBM	01/12/2022	01/12/2025

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