Intravenous Glucose Tolerance Test

Version: 1
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(note that the following list should be linked to the appropriate location.)

Summary:

The intravenous glucose tolerance test is used to assess insulin sensitivity, glucose disposal and islet function *in vivo* by measuring glucose and insulin responses following intravenous glucose administration.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Ketamine/Xylaxine		
Isoflurane		
Clippers		
Indwelling Catheter		
Iris Scissors		
Forceps		
Sterile Gauze		
Sterile Saline		
Non absorbable suture		
Wound Clips		
Tissue Glue		
Cotton tipped applicators		
Analgesia (described in parent protocol)		
Microhematocrit tube		
Ophthalmic anesthetic		
Sterile Lancets		
Handheld Glucometer		
Glucometer test strips		
50% dextrose solution		
27G or 25G needle/syringe		
Betadine		
Alcohol (70% ethanol)		

Anesthesia machine (if inhalant anesthesia described in protocol)		
Microscissors		
Lock solution		
Stop watch/timer		

Protocol:

WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.

Intravenous Glucose Tolerance Test (IVGTT):

1. Fast mice prior to IVGTT evaluation. The fasting duration may vary depending on the investigators preference but may not exceed 18 hours.
2. Prior to intravenous injection, obtain a baseline blood sample using one of the 3 described methods below (in-dwelling catheter, retro-orbital (RO) bleed, peripheral bleed).
3. Administer a 0.25-1g/kg intravenous glucose bolus via the tail vein. Timed sampling will begin at the time of glucose administration.
4. Obtain a blood sample at 0 (baseline), 1, 5, 10, 20, 30, and 50 minute time points following tail vein glucose injection. Specific time points can be modified as per the investigators needs.

IMPORTANT: All blood sample collection will adhere to the UC Davis IACUC policy for maximum blood volume in rodents.

Blood Sampling Methods:

1. Awake Indwelling Catheter Method:
    a. Anesthetize the mouse with the anesthetic approved by the given parent protocol (either inhalant or injectable).
    b. Place mouse in dorsal recumbency.
    c. Once in a surgical plane of anesthesia, clip the hair covering the designated surgical site and perform sterile scrub.
    d. Create a 1-1.5cm incision in the jugular furrow region and gently dissect away the subcutaneous tissue to expose the vessel of interest (either Jugular or Carotid, as defined in the parent protocol).
    e. Using non-absorbable suture, occlude the cranial and caudal end of the vessel to occlude blood flow.
    f. Create a small incision in the vessel to insert the catheter.
    g. Stabilize the catheter in the vessel with non-absorbable suture.
    h. Test the patency of the catheter by injecting and flushing lock solution.
    i. Tunnel the distal end of the catheter though the subcutaneous tissue and using a stab incision, exteriorize the end between the scapulae on the dorsum.
    j. Secure the catheter at the exit point with suture.
    k. Close the incision at the jugular furrow using tissue glue, absorbable suture or wound clips.

IMPORTANT: All animals will be provided with analgesics peri-operatively and post-operatively as approved in the parent protocol. All mice will be allowed to fully recover from the surgery prior to the IVGTT being performed.

2. Retro-orbital (RO) Blood Sampling:
    a. Lightly anesthetize mouse with inhalant anesthesia just prior to blood collection unless prolonged anesthesia is required; if prolonged anesthesia is needed, injectable anesthesia can also be used.
    b. Apply ophthalmic anesthetic to the eye that will be used for sampling.
    c. Using a micro-hematocrit tube, penetrate the intraorbital capillary plexus to obtain approximately 100uL of blood.

IMPORTANT: In the case that mice or data will be negatively impacted by anesthesia an exemption can be made to allow RO blood collection in awake mice. Only highly trained personnel with experience performing RO bleeds will be permitted to perform RO blood collection in awake mice. This option is only reserved for investigators with strong scientific justification that anesthesia will negatively impact their study.

3. Peripheral Sampling:
    a. Gently restrain mouse.
    b. Using a sterile lancet puncture the peripheral blood vessel of interest (tail, saphenous or submandibular).
    c. Blood can be measured directly from the lanced site with a handheld glucometer or a hematocrit tube can be used to collect the blood droplet for future analysis.