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Version 3

UC Davis - Intravenous Glucose Tolerance Test V.3

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Protocol status: Working

We use this protocol and it's working

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Abstract

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.

Materials

Reagents and Materials:

- Ketamine/Xylazine
- 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

Troubleshooting

1 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.

2 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 through 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.