ST VINCENT'S HOSPITAL MELBOURNE
A FACILITY OF ST VINCENT'S HEALTH AUSTRALIA

St Vincent's Hospital
(Melbourne) Limited
ABN 22 052 110 755

41 Victoria Parade Fitzroy VIC 3065
PO Box 2900 Fitzroy VIC 3065

Telephone 03 9288 2211
Facsimile 03 9288 3399
www.svhm.org.au

Induction of diabetes in mice

SCOPE

Injection of streptozotocin (STZ) is a common method of inducing diabetes in healthy mice. The instructions of preparation of STZ must be meticously followed. The success rate is estimated to be 50%.

AUTHORISATION TO UNDERTAKE PROCEDURE

Authorisation must be given by AEC for persons to undertake this procedure.
The investigator must be named in an AEC approved protocol and if training is required this must be provided by a person experienced in this technique.

SPECIAL REQUIREMENTS/SAFETY

- Procedures with the potential for producing Streptozotocin (STZ) aerosols should be conducted within an approved chemical fume hood whenever possible.
- Needles used for STZ injection are disposed of in approved sharps containers immediately following use.
- Needles used for STZ injection should never be bent, sheared, or recapped.
- Bench paper utilized during preparation of STZ stock should be lined with an impervious backing to limit potential for contamination of work surfaces in the event of the occurrence of minor spills.
- Areas where STZ is prepared and/or administered should be cleaned and decontaminated immediately following each task. Bench tops, BSC interiors, equipment, and laboratory surfaces with potential for STZ contamination should be routinely cleaned with bleach water (20% bleach), sulfamic acid solution, or other suitable deactivating agent:.
- The available scientific literature indicates that STZ and its metabolites are primarily (up to 20%) excreted in urine and to a much lesser extent in faeces. The drug undergoes rapid renal clearance within 48 hours after an acute administration. (Karunanyake et al., 1974). The metabolism and potential risks associated with STZ use require that all potentially contaminated carcasses, bedding, and other materials be disposed of as regulated medical waste through incineration.

MATERIALS/EQUIPMENT

- Personal Protective Equipment
- Scales (for weighing mice)
- STZ, scales, tubes, ice, KOH, ethanol, sodium citrate buffer (Preparation of STZ)
- Vortex
- Filter-sterilize (0.22 μm)
- 26G needle
- 1ml syringe
- AccuChek glucose strips
- AccuChek blood glucose monitor
- Humulin R 100 U/ml insulin

PROCEDURE

Preparation of Na Citrate buffer
(As per Animal Models of Diabetic Complications Consortium (AMDCC))

- Dissolve 1.47 g of Na Citrate in 50 ml ddH_2O
- pH and adjust buffer to pH 4.5 .
- Buffer should be made fresh with every group of injections

Preparation of STZ
(Immediately prior to use; active half-life of STZ is 15min)

- Weigh and record mouse weights (see appendices).
- Calculate the volume of STZ required for administration using the following formula:
- Volume to be administered =

$$\frac{\text{Dose to be administered (mg/kg)}}{\text{Weight of STZ (mg)}} \times \text{weight of mouse (g)}$$

- Wearing appropriate personal protective equipment (i.e. gloves, mask and lab coat), weigh out appropriate amount of STZ into a 5 ml tube for no more than 10 mice per tube. This is to ensure that active STZ is administered to all mice within 15 minutes of reconstitution.
- Place the tubes on ice.
- Once an appropriate number of tubes have been prepared, spray the work surface and equipment with KOH to inactivate STZ. Wash twice with water and spray area with ethanol, and wipe.
- Proceed to BRC.
- Transfer the desired mice to the BRC experimental room
- Dissolve STZ in the correct volume of Na Citrate* as determined by the above equation.
- Vortex until STZ is completely in solution.
- Filter-sterilize (0.22 μm)
- Restrain the animal and inject the appropriate volume (e.g. 0.5 ml/25 g mouse) i/p (as per SOP 52) using a 1 ml insulin syringe.
- Release the animal back into the cage and continue administering STZ to the remainder of the mice.

A. Injection of Single High Dose Streptozotocin (SHDS)

Method 1: Injection of STZ using Na Citrate buffer

STZ dose:

Mouse strain	Concentration (IV injection)	Concentration (IP injection)
BALB/c	225 mg/kg	275 mg/kg
CBA/J	225 mg/kg	275 mg/kg
C57BL/6	225 mg/kg	250 mg/kg
nude	150 mg/kg	175 mg/kg
SCID Beige	150 mg/kg	175 mg/kg
C57BL/6 Rag	150 mg/kg	175 mg/kg

NB: the preferred method of diabetes is via intraperitoneal (i/p) injections. Intravenous injection via the penile or tail vein can also be used but is considerably more stressful to the animal.

Method 2: Injection of STZ using PBS

STZ dose: 0.25 mg/g body weight (a single injection)

Preparation
(Immediately prior to use; active half-life of STZ is 15min):

- Wearing appropriate safety equipment (i.e. gloves, mask and lab coat), draw 4ml of PBS into a 5ml syringe and needle (needle size not important) remove lid of 50mg bottle of STZ (S0130-50MG, Sigma-Aldrich), pierce the top safety seal with the needle and slowly inject the 4 ml of PBS.
- Gently stir the bottle for 15 to 20 seconds to dissolve the STZ.
- Carefully draw the 4ml of fluid back into the syringe and then withdraw the needle and syringe.
- Discard the bottle and needle appropriately.
- Attach a 0.22µm sterile filter to the syringe and push the fluid through the filter into a sterile tube.
- Inject appropriate volume, 0.5 ml/25 g mouse, i/p (as per SOP 52) using 1 ml insulin syringes.

Method 2 is the preferred method for inducing diabetes in SCID and NOD/SCID mice as the majority of the mice become diabetic.

B. Injection of Multiple low dose Streptozotocin (MLDS) in C57BL/6 mice

Note: preparation of STZ is the same as described above in Method 1 with the exception of STZ dose (see below).

Mice are injected with 5 daily doses of 50mg STZ/kg body weight IP as per SOP 52.

MONITORING REQUIREMENTS

Monitoring of blood glucose

48 hours post-STZ injection:

- Monitor blood glucose levels (BGLs) via tail vein prick, using AccuChek glucose strips and glucometer.
- Record BGLs and
- Examine cages for onset of polyuria (excessive urine production). In the event of polyuria, replace cage bedding as required.
- Measure non-fasting blood glucose levels

- **SHDS model:** daily (morning) and maintain below 20 mM by subcutaneous injection (as per SOP 53) of insulin (Humulin R 100 U/ml) until mice are required for experimentation.

  The insulin dose is determined as follows:

BGL	Insulin
<12	None
12-20	10 units
20-25	15 units
>25	20 units

- 7 days post-STZ injection, weights and BGLs are recorded.

- Non-diabetic mice are culled by cervical dislocation on basis of:
  - BGL < 20 mM
  - No evidence of polyuria, i.e. dry bedding.

- Diabetic mice (BGLs >20 mM) exhibiting excessive weight loss (body weight <18 g) are also culled (as per SOP 26) because they are unlikely to survive transplantation.

- Diabetic mice (BGLs >20 mM observed for 3 consecutive days) with body weight >18 g are transplanted with islets.

- **MLDS model:** At least twice weekly (morning). Mice treated in this model do not require administration of insulin.

EXPECTED RISKS

Complications associated with diabetic state in SHDS model.

In rare cases, STZ-treated mice maintained by insulin injection become hypoglycaemic (BGL <3 mM) and lapse into a diabetic coma, becoming motionless and cold.

Under these conditions the following recommendations are made:

- Cease insulin administration
- Warm mouse on heat pad, cage separately and monitor food and water intake

- Confirm hypoglycaemia by remeasuring BGL
- If BGL is <3 mM and mouse remains cold and lethargic, administer 10% glucose or PBS solution by i/p injection
- If normoglycaemia cannot be achieved by the second reading post-treatment, cull mouse by cervical dislocation.

ASSOCIATED PROCEDURE/S

The data should be recorded on the appropriate data sheets (see appendices):

1. Mouse weights.
2. Streptozotocin doses.
3. Blood glucose results, insulin and glucose doses.
4. Polyuria assessment.

SOP 26: *Euthanasia of Mice and Rats*
SOP 50: *Intra-venous injection [tail vein] in mice*
SOP 52: *Intra-peritoneal injection in the mouse*
SOP 53: *Subcutaneous injection in mice*

REFERENCES

Australian code for the care and use of animals for scientific purposes (8th Edition 2013)

'The synthesis of [14C] Streptozotocin and its distribution and excretion in the rat' Karunanayake EH, Hearse DJ, and Mellow G Biochem J. 142 (1974) 673-683

Diabetic Complication Consortium: www.diacomp.org

Author: Evelyn Salvaris and Jo Chia	
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