Standard Operating Procedure	Title: Simplified Intra-Peritoneal Glucose Tolerance Test (I.P.G.T.T)	
	Doc. Number: ESLIM_004_001 Rev No. 1	Date Issued: 01/06/04

1.0 Purpose:

1.1 The glucose tolerance test measures the clearance of an intraperitoneally injected glucose load from the body. Animals are fasted for approximately 16 hours, a solution of glucose is administered by intraperitoneal (IP) injection and blood glucose is measured at different time points during the following 2 hours.

2.0 Scope:

2.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure.

2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the Clinical Chemistry, Haematology and Metabolism department leader.

2.3 Any deviances from this protocol must be reported to the Clinical Chemistry, Haematology and Metabolism department leader.

3.0 Safety Requirements:

3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

3.2 Dispose of blades, and needles in sharps waste.

4.0 Associated Documents:

4.1 Glucose meter operating manual

5.0 Notes:

5.1 The validity of results obtained from metabolic and hormonal studies is largely dependent on methods of animal husbandry. It is important that individuals following this procedure are experienced and aware of the animal's welfare, and are familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.

5.2 The majority of mouse metabolic and hormonal studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

5.3 It is recommended that all metabolic experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day.

5.4 The following conditions of animal housing and handling have been defined in order to avoid additional influences on blood parameters:

5.4.1 Mice are housed up to 5 animals per cage.

5.4.2 Each test group contains up to 10 mice per line and sex.

5.4.3 All of the mice are given the same diet (standard diet of the respective animal facility).

5.4.4 Mice must be aged between 12 and 16 weeks.

6.0 Quality Control:

7.0 Equipment:

7.1 Mouse restraining device

7.2 Glucose meter

7.3 Test sticks

7.4 Scalpel blade

7.5 Scales

7.6 Timer

8.0 Supplies:

8.1 Glucose solution 20% (0.9 NaCl)

8.2 Gauge needle (25G 5/8)

8.3 Syringe 1ml

8.4 Microvette tube

8.5 Tissue

8.6 Clean cages and food

8.7 Lignocaine topical anaesthetic cream (optional)

9.0 Procedure:

9.1 Fast mice for 16-18 hours (overnight). Ensure that they have access to drinking water all the time.

9.2 On the following day, at 8a.m., place mice individually in clean cages with water only (no food). Identify cages with a mouse number.

9.3 Prepare an experiment record sheet, syringe and sticks for glucose measurement:

9.3.1 Record the weight of each mouse

9.3.2 Calculate and record the volume of glucose required (2g of glucose/kg) for IP injection as follows: volume of IP glucose injection (µl) = 10 x body weight (g).

9.3.3 Calibrate the glucose meter according to the manufacturer's instructions.

9.4 Optional depending on local regulations: Wearing gloves apply a small amount of Lignocaine topical anaesthetic cream to the tail of the mouse. Spread the cream over the tail evenly and gently massage it in, ensuring that the proposed incision site is fully covered. Massage for approximately for 10 seconds to enhance the effect of the anaesthetic cream. Wipe off excess cream with a piece of tissue.

9.5 Return the mouse to its cage and allow at least 15 minutes for the effect of the anaesthetic to take place.

9.6 Restrain the mouse in the restraining device with the tail exposed. Score the tip of the tail using a fresh or sterilised scalpel blade

9.7 A small drop of blood (<5µl) is placed on the test strip of the blood glucose meter. This is the baseline glucose level (t = 0) and is recorded in the experiment record sheet.

9.8 Remove the mouse from the restraining device.

9.9 Inject the mouse intraperitoneally with the appropriate amount of glucose solution, as previously determined (point 9.3.2) and note the time-point of injection on the record sheet.

9.10 The blood glucose levels are measured at 15, 30, 60 and 120 minutes (t = 15, t = 30, t = 60 and t = 120) after glucose, by placing a small drop of blood on a new test strip and recording the measurements. Start the bleeding again by removing the clot from the first incision, massage the tail if blood flow is inadequate. Results are recorded in the record sheet.

9.11 Ensure that further blood loss from the incision is minimal by applying pressure to the incision for 1-2 minutes after each measurement. At the end of the experiment place mice in a clean cage and make sure that a plentiful supply of water and food is made available to the animals.

10.0 Supporting information:

11.0 History Review:

No glucose meter manufacture specified – method more generalised.
Anaesthetic cream optional depending on local regulations (compulsory in UK).

12.0 Emergency Procedures: