Standard Operating Procedure	Title: Bone densitometry	
	Doc. Number: ESLIM_005_001 Rev No.	Date Issued: 01/06/04

1.0 Purpose:

1.1 Measure bone mineral content and density as well as body composition in mice using the pDEXA analyser.

2.0 Scope:

2.1 Individuals who are trained and competent in using DEXA and in handling laboratory animals 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 Bone and Cartilage Research Project Leader.

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3.0 Safety Requirements:

3.1 General laboratory procedures should be followed, which include prohibition of eating, chewing gum, drinking, and 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 Specific X-Ray safety procedures should be followed. Refer to legislation of the resident country for details on X-ray unit applications. While the X-ray source is on the following must be obeyed:

3.2.1 Do not strike the X-ray path.

3.2.2 Do not put metal into the X-ray path (e.g. metal ear clips, transmitters).

4.0 Associated Documents:

5.0 Notes:

5.1 Dual-energy X-ray Absorptiometry (DEXA or DXA) is a method of quantifying bone mineral content and density. DXA uses an X-ray generator of high stability to produce photons over a broad spectrum of energy levels. Its photon output is filtered to produce the two distinct peaks necessary to distinguish bone from soft tissue.

5.2 The technique used for separating photon output into two distinct energy levels is known as 'K-edge' filtration. By placing a filter element in the beam path, energy levels reacting with the filter material are sharply attenuated. The filter effect gradually lessens at higher energy levels, and so a second peak is introduced. The tin filter material used in this system produces energy peaks at 28keV and 48keV. Two solid-state detectors and proprietary energy discrimination are used to determine high and low energy counts.

5.3 The count data is transformed by software into bone and non-bone components, thus generating the bone density values. Information is generated about body weight, body length, fat and bone mass, bone mass density, and lean mass of each mouse.

6.0 Quality Control:

6.1 Calibration of the system is done in daily intervals using the QC and the QA phantoms delivered by the manufacturer. The results from the calibration runs are recorded by the system.

7.0 Equipment:

7.1 pDEXA Sabre X-ray Bone Densitometer (Norland Medical Systems, Inc., Basingstoke, Hampshire, UK)

7.2 Ruler

8.0 Supplies:

8.1 Syringe 1ml

8.2 Needle (25 G, 0.5 mm)

8.3 Anaesthetic solution

8.4 Disinfectant

9.0 Procedure:

9.1 Calculate and record the volume of anaesthetic solution required for intraperitoneal (IP) injection.

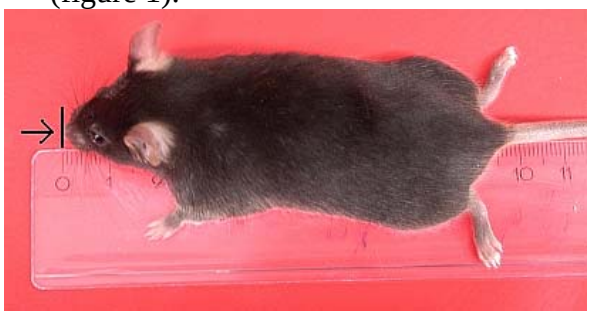
9.2 Administer the anaesthetic solution by IP injection.

9.3 Monitor the animal carefully until unconsciousness ensues to the point of loss of the pedal reflex (toe-pinch).

9.4 Weigh the mouse and record the value.

9.5 Measure the length of the mouse as follows and record the value (accuracy +- 0.5cm)

9.5.1 Place the unconscious mouse on a disinfected ruler so that its nose is at zero (figure 1).

Figure 1: 

9.5.2 To measure the entire length of the head press gently against the ruler (figure 2) and gently pull the tail to ensure that the spine returns to its full length (figure 3).

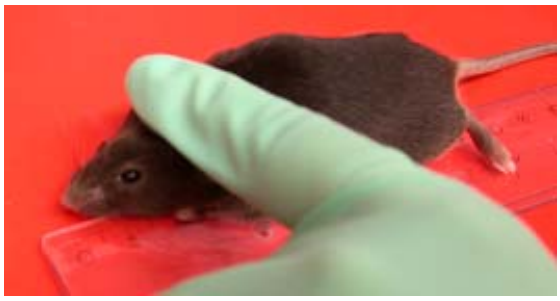
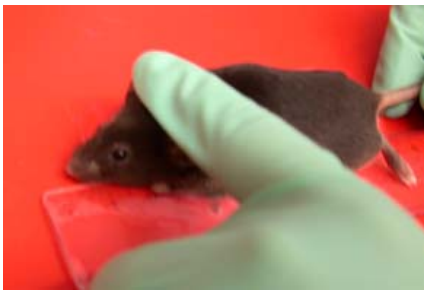
Figure 2: 

Figure 3: 

9.5.3 Measure the length starting from the nose (0cm) to the beginning of the tail (figure 4). Record the measurement – the accuracy is within 0.5cam. For example in figure 4 the length of the mouse is 9.5cm.

Figure 4: 

9.5.4 Disinfect the ruler and contact area after the measurement has been taken.

9.6 Place the unconscious mouse into the pDEXA analyser.

9.7 Perform a scout-scan at a scanning speed of 40 mm/s and a resolution of 1.0 x 1.0 mm/s (HAV: 0.020).

9.8 Optimise the area of interest and perform a measure-scan at a scanning speed of 20mm/s and a resolution of 0.5 x 1.0 mm/s (HAV: 0.020).

9.9 Note that the exposure dose per mouse is 300µSv.

9.10 For the analysis of the data, regions of interest must be defined. The standard analysis comprises of a whole body analysis, and a whole body analysis excluding the head area.

10.0 Supporting information:

11.0 History Review:

12.0 Emergency Procedures: