Standard Operating Procedure Draft GSF/ICS	Title: Energy expenditure and food monitoring	
	Doc.Number: ESLIM_003_001	Date Issued: 26/05/08

1.0 Purpose:

1.1 The energy expenditure is evaluated through indirect calorimetry by measuring oxygen consumption with an open flow respirometric system.
Very precise CO_2 and O_2 sensors measure the difference in CO_2 and O_2 concentrations in air volumes flowing through control or animal cages. The amount of oxygen consumed over a given period of time can thus be calculated, as far as the air flow through the cage is known. Data are expressed as ml O_2 $h^{-1}animal^{-1}$. The system also monitors CO_2 production, therefore, the respiratory exchange ratio (RER) and finally, heat production can be calculated. An activity and food intake monitoring system can also be integrated to the set up for the measurement of activity e.g. to ensure that periods of resting can be identified.

2.0 Scope:

2.1 This procedure must be followed by individuals who have been trained and are competent in performing the procedures described herein.

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, hematology and metabolism department leader.

2.3 Any deviances from this protocol must be reported to the clinical chemistry, hematology and metabolism department leader.

3.0 Safety Requirements:

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

4.0 Associated Documents:

4.1 Manufacturer apparatus manual.

5.0 Notes:

5.1 The validity of the results obtained from metabolic phenotyping is largely dependent on methods of animal husbandry. It is of vital importance 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 studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

5.3 It is essential that all phenotyping experimentation is conducted at the same time of day because physiological and biochemical parameters e.g. metabolic rate, body temperature and activity are subject to circadian and ultradian rhythms. In the indirect calorimetry module measurements begin five hours before lights-off (lights off = TO) and are finished around T16 i.e. fours hours after lights- the next morning (see Figure 1).

6.0 Quality control

6.1 Calibrate and run the system according to the manufacturer‘s specifications (see operator manual).

7.0 Equipment:

7.1 Respirometer: Multi-channel respirometric system with varying number of metabolic chambers (7-12 depending on centre) equipped with drinking bottles and feeding grids.

7.2 Animal activity monitoring system.

7.3 Water and food intake monitoring system

7.4 Computer with the apparatus software.

8.0 Supplies:

8.1 Disinfectant (75% alcohol solution)

9.0 Procedure:

9.1 The test is performed at regular room temperature of the facility (around 22°C depending on centre), bedding material is provided (paper tissue or wood shavings).

9.2 Prepare and calibrate the indirect calorimetry apparatus according to the manual. The system requires periodic calibration of the gas sensors and flow meters to insure precise measurements. The calibration procedure consists of the application of a gas of known composition and adjusting control knobs in the front of the oxygen and carbon dioxide sensors to obtain readings that reflect the contents of the calibration gas. It is recommended that the system be calibrated prior to the start of each experiment. The analysers should not be shut down if not urgently required for maintenance. If this has to be done a warm up time of at least 90 minutes is required for the gas sensors for calibration (refer to manufacturer's manual). Calibrations and shut downs should be recorded in the laboratory journal.

9.3 Weigh each mouse individually and record its weight before the start of the measurement (the mouse is weighed again after the end of the measurements, see section 9.9).

9.4 Place each mouse individually in the chamber for a period of about 21 hours (from T-5 to T16 next day) with free access to food and water. Label each chamber with the mouse identification number; close the chambers and check for adequate air flow.

9.5 Start the measurement of the indirect calorimetry system. The file menu enables the user to setup a new experiment to be run or load a previous experiment file. Total duration of the experiment is approximately 21 hours. There is a 12:12 hours light/dark cycle in the room. Ensure that nobody enters into the experimental room during the tests and, that the analysis of the air flow through the metabolic chambers is not confounded by gases diffusing in an uncontrolled way into the measuring system. Ideally, metabolic chambers are set up in a ventilated cabinet continuously supplied with an overflow of fresh air from outside.

9.6 O_2, CO_2, and activity measurements are realized at regular time intervals depending on the number of channels and delay time for collecting data from each channel.

9.7 Print the results.

9.8 Upload all gas analysis data and calculated heat production in combination with the time point when measurements were conducted (standardised to lights off = T0, see Figure 1) and which animal and channel was monitored. Data for VO_2 and VCO_2 are provided in the units ml h^{-1} $animal^{-1}$. Heat production is uploaded in the unit kJ h^{-1} $animal^{-1}$(provide deviating units as meta data so data can be converted to SI units). The respiratory exchange ratio (RER) is calculated as the ratio VCO_2/VO_2.

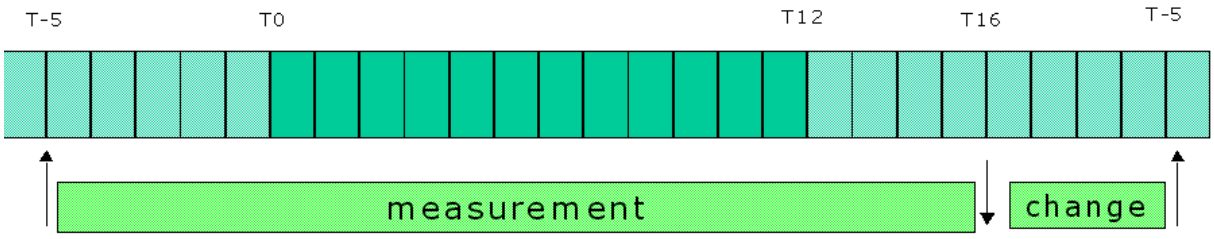

Figure 1: Daily workflow indirect calorimetry

9.9 Upload all gas analysis

9.9.1 Activity measurement: Depending on the calorimetry system two activity parameters are provided:

- Ambulatory activity (*xamb*) a count of beam breaks during the interval.
- Total activity (*xtot*) total counts of fine movement (e.g. grooming) and ambulatory activity.

For *xamb* and *xtot* the hourly means of the data measured between T-5 and T16 are calculated and uploaded.

9.9.2 Water and food intake monitoring: Depending on the centre different methods are available for food intake monitoring. Measure hourly intake if possible or record total cumulative intake between T-5 and T16.

9.9 At the end of the experiment, weigh the mice again and return them to their cages and ensure that the animals exhibit normal behaviour and have free access to food and water.

9.10 Wash and disinfect the chambers.

10.0 Data Records and Reports:

11.0 Emergency Procedures:

12.0 History Review: