 <p>Standard Operating Procedure</p>	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

1.0 Purpose:

- 1.1 Measure the systolic blood pressure and the heart rate of conscious mice using the tail-cuff system.

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 Leader of Cardiovascular Investigations.
- 2.3 Any deviances from this protocol must be reported to the Leader of Cardiovascular Investigations


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.

4.0 Associated Documents:

5.0 Notes:

- 5.1 The validity of results obtained from cardiovascular phenotyping 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 is familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.

 Standard Operating Procedure	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08


- 5.2 The majority of mouse cardiovascular studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.
- 5.3 Environmental factors may contribute to the level of stress of the mice. Noise levels in the experimental room must be kept to a minimum and several parameters such as temperature, moisture and ventilation must be controlled according to regulations of animal welfare. These conditions should be maintained throughout the procedure (5 days).
- 5.4 Due to circadian variations of haemodynamic parameters, it is recommended that all cardiovascular tests be carried out at approximately the same time of day. It is recommended that all cardiovascular investigations be undertaken in the morning.
- 5.5 It is recommended that the same operator complete experimentation during these five days.
- 5.6 Cuffed mouse tails are immobilized with tape during data acquisition. To ensure proper fixation, a minimal tail length of 6-7 cm is required.

6.0 Quality Control:

- 6.1 In order to obtain reproducible results, the system used should be inspected every day for leakages, following the procedure described in users manual.
- 6.2 To obtain accurate systolic blood pressure measurements with the equipment must be calibrated on a regular basis according to manufacturer recommendations.
- 6.3 It is essential that the control unit is turned on at least 30 minutes prior to placing mice on the subject platform in order to reach the correct temperature (38°C). Warming the mice is essential to obtain adequate blood flow in the tail artery.
- 6.4 If possible, measurements should be repeated if less than 7 of 10 cycles are successful. At least 20 successful measurements are necessary to complete the experiment

7.0 Equipment:

- 7.1 The Blood Pressure Analysis Systems consisting of four main parts:
specimen platform

 <p>Standard Operating Procedure</p>	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

control unit
computer
data acquisition PCMCIA card (on notebook systems).


Examples of these systems are:
The BP-2000 Blood Pressure Analysis System - Visitech Systems:



The MC4000 Blood Pressure Analysis System - Hatteras Instruments:

8.0 Supplies:

- 8.1 Elastic cuff band material
- 8.2 End cap used to secure the cuff elastic to the cuff housing

 <p>Standard Operating Procedure</p>	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

8.3 Masking tape

8.4 Alcohol 50%

9.0 Procedure:

9.1 The cuffs should be changed every Monday morning, prior to the start of testing.

9.2 BP-2000 Blood Pressure Analysis set-up and data collection:

9.2.1 Switch on the power (at the back of the control unit) to the 'ON' position.

9.2.2 Turn the heater control button on the front of the control unit to approximately the 11 o'clock position. You should now observe the following:

9.2.2.1 The power indicator light on the front of the control unit should be illuminated.

9.2.2.2 The red LEDs on the mouse-tail covers should be illuminated on each of the four channels.

9.2.2.3 The surface of the specimen platform should be warming. It should take approximately 30 minutes to reach approximately 38°C.

9.3 Computer set-up:

9.3.1 Switch on the computer. Double click on the 'Blood Pressure Analysis' program icon to start the program, and then, click on the 'OK' button of the program's 'about box' to dismiss dialog. Proceed with the following:


9.3.1.1 Click on 'Configuration' to display the configuration drop down menu.

9.3.1.2 Select 'Options' to display the 'Options' dialog.

9.3.1.3 Select 'Measurements' to define the number of 'Preliminary Measurements' (5 preliminary cycles are recommended). Define the number of 'Actual Measurements' (15 cycles of measurement).

9.3.1.4 Click on the 'OK' button to dismiss the dialog

9.3.1.5 Click on 'Analysis' to display the 'Analysis' drop down menu.

 Standard Operating Procedure	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

- 9.3.1.6 Click on 'Register Specimens' to display the 'Specimen Registration' dialog.
- 9.3.1.7 Enter values for each of the four subject identifications and, click on the 'OK' button to dismiss the dialog.
- 9.3.1.8 Click on 'Analyse' to begin an analysis session.

9.3.2 Four analysis windows should be displayed. Each window corresponds to one of the four channels. It displays the waveform received by each channel.

9.3.3 To confirm that the software records the signal coming from each channel correctly pick up each channels tail cover and move your finger over the tail slot. The screen should display the effect of the finger moving on the platform. If the waveform does not signal the movement of your finger, you must complete the following:

- 9.3.3.1 Check all of the connections between the subject platforms, the control unit and the computer.
- 9.3.3.2 Make sure that the data acquisition PCMCIA card is firmly inserted into PCMCIA slot. If necessary, insert the PCMCIA card into a different slot and test again.

9.3.4 The system is now ready for experimentation.

9.3.5 Following completion of the experimental session, click on 'Data', to display Data drop down menu. Select 'Reports' and report wizard appears.


9.3.6 Select the appropriate experiment for which the data must be seen and click on the 'Next' button.

9.3.7 Select the group(s) to see the corresponding data and click on the 'Next' button.

9.3.8 Select the specific report required: 'statistics for measurements sets' or 'individual measurements' and click on the 'Next' button.

9.3.9 Select the data columns for the report and click on the 'Finish' button.

9.3.10 The appropriate report should appear according to the specific report selected.

 Standard Operating Procedure	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

9.3.11 Click on 'Report', to display Report and drop down menu. Select 'Export to Excel' and give the worksheet a file name, then click on the 'Save' button.

9.3.12 Close down the database and turn off the computer once all data is saved.

9.3.13 Drop the temperature of the platform unit by turning to zero position the heater control knob at the front of the control unit.

The Blood Pressure Analysis set-up for the MC4000 is very similar (please follow the detailed instructions of the manufacturer).

9.4 Measurement process:

Summary of measurement steps:

- Switch the control unit on
- Turn on the computer and start the Blood Pressure Analysis program.
- Calibrate the BP-2000's pressure transducer.
- Check for pressure leaks.
- Set analysis parameters.
- Place one or more mice in the mouse holders on the platform. Ensure that their tails are correctly placed in their tail cuffs.
- Register the Identification code for each animal in the program.
- Begin the measurements process via the program.
- Save the results to file, or print them.
- Remove the mice from the platform.


These steps are more detailed in the following sections (9.3.1 - .

9.4.1 Switch on the control unit located at the back. In order to increase blood flow to the tail, restrain the mice on a heated platform.

Note:

The platform must be preheated to at least 38°C (maximum temperature of 40°C) 30 minutes prior to placing the mice in the measurement chambers. Turn the heater control button on the front of the control unit to approximately the 11 o'clock position.

9.4.2 Turn the computer on and start the Blood Pressure Analysis program:


 Standard Operating Procedure	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

The computer should be turned on. All power management functions on the computer should disappear. Start the program by clicking on “Blood PressureAnalysis”. Enter your login in the appearing window and then click on “OK”.

Calibrate the BP-2000 pressure transducer using a mercury manometer. To obtain accurate systolic blood pressure measurements, the calibration must be performed on a regular basis. The system is calibrated using a mercury manometer. Procedure:

9.4.3

- 9.4.3.1 Disconnect the pressure tube attached to the subject platform and connect it to a manometer.
- 9.4.3.2 Access the ‘Configuration’ drop down menu from the main menu bar, and click on ‘calibrate pressure’. Connect the manometer and click on ‘OK’. The ‘Pressure calibration’ dialogue window appears.
- 9.4.3.3 Click the ‘Pressurise’ button to turn on the air pump. When the desired pressure is reached on the manometer, click the stop button to turn off the pump.
- 9.4.3.4 Click the set button. A window that allows you to enter the value from the manometer appears. Enter the manometer pressure, and click on ‘OK’.
- 9.4.3.5 Repeat the previous two steps at least four times for different pressure values. For mice, pressure values of approximately 50, 100, 150 and 200 mmHg would be appropriate.
- 9.4.3.6 Click ‘Finished’ when complete.
- 9.4.3.7 Once the procedure described above is completed the calibration should be verified. Open the ‘Pressure Calibration’ window and set a random pressure value. Confirm that the current measured pressure displayed on the window, corresponds to the actual pressure as read on the manometer. Repeat this for various other values.

 <p>Standard Operating Procedure</p>	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

9.4.3.8 The BP-2000 pressure transducer is now ready for experimentation.

9.4.4 Check for air escapes:

The BP-2000 should be assessed for leakages in the pressure system before every measurement. Inflate the system to a large pressure value (225 mmHg) and wait for it to reach a steady state. Click the 'Hold' button, and observe the 'current measured pressure' on the 'Pressure Calibration' window. If the pressure drops relatively quickly, there is a gas leak in the system. In most cases, the escape is the result of a faulty elastic cuff on one or more of the tail cuffs. To determine if a given tail cuff is leaking, use a pair of forceps to seal off the pressure tubing leading to the cuff. If the leaks still persist, the forceps should be used to seal off the pressure tubing of the remaining cuffs in turn until the escape is detected. Replace the leaking cuff(s) as necessary. If no tail cuffs are leaking, check the connection between the control unit and the subject platform.


9.4.5 Placement of animals:

Carefully place each mouse on the preheated platform. Insert the tail through the appropriate tail cuff and then place the tail into its slot. Secure the tail with a piece of masking tape on the flat area specifically designed for taping down the mouse-tail. Ensure that the tail cuff is positioned at the origin of the tail. Place the mouse-tail cover over the tail slot. Place an individual measurement chamber (cover) over each mouse to restrain and calm the animal. Allow at least 2 minutes for the animals to become quiet and calm. Experimental information may be entered into the computer at this point.

9.4.6 Set analysis parameters:

Experimental information, including the number of preliminary measurements (5-10), the number of actual measurement cycles (10-12) and the specimen identifications must be entered when the mice are in place. Refer to section 9.2.

9.4.7 Click on 'Analyse' to begin an analysis session via the program. Each cycle consists of two phases. In the first phase, the BP-2000 program monitors the pulse wave in order to determine the pulse rate and its amplitude. If the signal is good the program reports an average pulse value and calculates the average amplitude at that time. The automatic sequence of measurements is now ready to begin. The program analyses the

 Standard Operating Procedure	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

incoming data for each mouse and automatically determines when arterial pressure has been reached. Note that the BP-2000 system measures blood pressure by ascertaining the cuff pressure at which blood flow to the tail is eliminated (inflation phase).

- 9.4.8 Following completion of measurements, a report summarising the results and measurement details is generated. The summarised reports include:
- 9.4.8.1 Specimen identification
 - 9.4.8.2 average arterial pressure (mmHg)
 - 9.4.8.3 arterial pressure standard deviation (mmHg)
 - 9.4.8.4 average pulse rate (beats per minute)
 - 9.4.8.5 pulse rate standard deviation (beats per minute)
 - 9.4.8.6 number of successful measurements.


The detailed report shows the arterial pressure and the pulse rate for each measurement cycle in each mouse.

Each mouse should have at least 7 out of 10 successful measurement cycles in one session in order to determine a blood pressure value. If the mouse has less than 7 out of 10 successful measurement cycles in one session, repeat the session if you are able. (Note: some centres are not able to extend the session due to their local animal welfare regulations). A blood pressure/heart rate recording takes approximately 15-25 minutes.

- 9.4.9 Removal of mice from the specimen platform:
Remove the animals carefully from the mouse platform. Clean any faeces and urine from the tail cuffs.
The next set of mice can now be tested.
- 9.4.10 Once all data is saved, you may close down the database and turn off your computer.
- 9.4.11 Drop the temperature of the platform unit by turning the heater control button to zero, located at the front of the unit once the experimental session is complete.

A similar measurement process is described for the MC4000 (please follow the detailed instructions of the manufacturer).

10.0 Supporting information:

 <p>Standard Operating Procedure</p>	Title: Non-invasive blood pressure and heart rate	
	Doc. Number: ESLIM_002_001 Rev No.	Date Issued: 30/05/08

11.0 History Review:

12.0 Emergency Procedures: