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STANDARD OPERATING PROCEDURE PACKET

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: MC4000 Blood Pressure Analysis System Calibration

SOP Number: SOP0036	**To be reviewed:**
Author(s): Signed: Date:	
Editor: Signed: Date:	
Date Modified:	

INTRODUCTION:
The purpose of this procedure is to calibrate the MC4000 Blood Pressure Analysis System as part of SOP0038 – Non-Invasive Blood Pressure.

ABBREVIATIONS:
- **NIBP** = Non-Invasive Blood Pressure
- **PPL** = Project License
- **SOP** = Standard Operating Procedure

HEALTH & SAFETY:
- **RA003** – Hazardous Substances; *Section RA003.36*
- **RA007** - Musculoskeletal; *Section RA007.6*

RESPONSIBILITIES:
All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) has been read, understood and where applicable is followed in accordance with the relevant Project License (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure.

RESOURCES:

Equipment:
1. Manometer
2. MC4000 Blood Pressure Analysis System
3. Non-Invasive Blood Pressure (NIBP) Lab Book
4. Pen

Associated SOPs:
- **SOP0038** - Non-Invasive Blood Pressure

Staff Required: This procedure requires 1 person.

NOTE:
As this test is to be performed as part of the SOP0038 - NIBP procedure, you should look at that SOP and move to this one at the indicated step.

All platforms that are to be used must be calibrated on the first day of NIBP as part of the procedure.

PROCEDURE:

1. Remove the blue tubing that connects the air supply to the platform from the back of the first platform.

2. Connect the short blue tubing that is attached to the T-piece, which is attached to the manometer, to the back of the platform where the blue tubing was removed from.

3. Connect the blue tubing to the T-piece, which is attached to the manometer.

4. Select the calibration icon from the toolbar at the top of the main program window.

5. Select ‘Next’ and then ‘Begin’.

6. Read the value on the manometer and enter the value into the ‘Low Pressure Mark’ field. (Wait for the manometer to reach a steady value as after it has initially increased as it decreases slightly). Note down the value in the NIBP Lab Book.

7. Select ‘Set LOW’.

8. Read the value on the manometer and enter the value into the ‘High Pressure Mark’ field. (Wait for the manometer to reach a steady value as after it has initially increased as it decreases slightly). Note down the value in the NIBP Lab Book.

9. Select ‘Set HIGH’.

10. Check that the values in the ‘Low Pressure Mark’ and ‘High Pressure Mark’ fields match the values noted down in the NIBP Lab Book and then select ‘Accept’.

11. Disconnect the blue tubing that is attached to the T-piece, which is attached to the manometer.

12. Disconnect the short blue tubing that is connected to the T-piece, which is attached to the manometer, from the back of the platform.

13. Connect the blue tubing that connects the air supply to the platform to the back of the first platform.

14. Repeat steps 1-13 until all platforms that are due to be used have been calibrated.

15. Return to SOP0038 – Non-Invasive Blood Pressure.

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Non-Invasive Blood Pressure – V1

SOP Number: SOP0038	**To be reviewed:**
Author(s): Signed: Date:	
Editor: Signed: Date:	
Date Modified:	

INTRODUCTION:
The purpose of this procedure is to assess the cardiovascular parameters of a mouse including systolic arterial pressure, diastolic arterial pressure and heart rate using the tail-cuff method.

ABBREVIATIONS:
- **NACWO** = Named Animal Care and Welfare Officer
- **NIBP** = Non-Invasive Blood Pressure
- **PPL** = Project License
- **SOP** = Standard Operating Procedure

HEALTH & SAFETY:
- **RA001** – Laboratory Animal Allergens; *Section RA001.2*
- **RA003** – Hazardous Substances; *Section RA003.2*
- **RA004** – Physical Hazards; *Section RA004.6*
- **RA007** – Musculoskeletal; *Sections RA007.5, RA007.6 & RA007.10*

RESPONSIBILITIES:
All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) has been read, understood and where applicable is followed in accordance with the relevant Project License (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure.

RESOURCES:

Equipment:
1. The Hatteras MC4000 Blood Pressure Analysis System consisting of 4 main parts: 2 specimen platforms, power/utility unit, computer and MC4000 Analysis Software
2. Weight scale & mouse container (1st day only)
3. Surgical tape
4. Scissors
5. Mobile transport rack
6. 70% Ethanol & tissues
7. Non-Invasive Blood Pressure (NIBP) Lab Book
8. Pen

9. NIBP Record Sheet (final day only)
10. Diet (as defined by pipeline)
11. One clean nestlet per cage tested (final day only)
12. One clean cage per cage tested (final day only)

Associated Documents & SOPs:

- **SOP0036** - MC4000 Blood Pressure Analysis System Calibration
- **SOP0037** - MC4000 Blood Pressure Analysis System Maintenance
- **SOP0045** - Weigh Mice

Staff Required: This test requires 1 person per set-up

NOTE:

Prior to measurements being taken all mice due to undergo the procedure should be transferred from the animal holding room to the holding rack in the cardiovascular room with the Hatteras MC4000 Blood Pressure Analysis System to allow them to acclimatise to the room before the procedure is undertaken. In addition to this, all animals that are being transferred should have a full cage clean on the day they are moved so their cages do not become too soiled during the 5 days of the procedure.

The NIBP should be undertaken in the morning within 5 hours of dawn i.e. between the hours of 8.30am and 12.30pm.

It is recommended that the same operator complete experimentation during these 5 days.

There are 3 different sizes of holders for restraining the mice on the platforms. Size 1 (1.25"x1.25"x4.00") is recommended for mice up to 30g; size 2 (1.50"x1.50"x4.00") is recommended for mice between 30-45g; size 3 (1.75"x1.75"x4.00") is recommended for mice between 45-60g. Use the appropriate sized holder for all mice dependant on the weights recorded. If the weights within the cohort vary greatly then use the size required for each individual mouse as its ok to use a larger restrainer for some mice within a cohort if they are larger than the rest. It is also ok to use different restrainers for mutants and controls if there is a weight difference. Note what size restrainer has been used for each cohort and gender in the NIBP Lab Book.

PROCEDURE:

Before performing the procedure, verify that this is the correct procedure at this point in the pipeline by consulting the cage card(s) and confirming that the procedure has not already been performed on the mice.

1. Switch the power (at the back of the control unit above the power cable) to the ‘ON’ position. You should now observe the following:
    - The power indicator light on the front of the power/utility unit should be illuminated.
    - The red LEDs on the mouse-tail covers should be illuminated on each of the 4 channels.
    - The display on the front of each platform should read “Hatteras Instruments Model MC4000” with the temperature of each channel indicated.
    - The surface of the specimen platform should be warming. It should only take a few minutes to warm to 100°F (equivalent to approx. 38°C).

2. Log on to the computer.

3. Double click on the ‘MC4000 BP Analysis’ program icon to start the program. Proceed with the following:
   - Click on ‘File’ to display the drop down menu.
   - Select ‘Comm Port Setup’ to open the ‘Comm Port Setup’ window.
   - Select ‘Dual Platform’ to allow measurements to be taken from both platforms.
   - Starting with the 1st specimen platform, select communication port 3. If the port is active and working properly, a small black icon of a rodent will appear in the window. Next, select communication port 1 for use with the 2nd platform. Again, if the port is active and working properly, a small black icon of a rodent will appear in the window. After selecting the communication ports for each platform, click ‘OK’ to return to the main program window. The toolbar should now include the rodent icons for each platform which are labelled P1 and P2.
   - Repeat steps 1-3 for the 2nd computer (if necessary).

4. **Only perform this step if it is the 1st day of the procedure**. Referring to SOP0037 - MC4000 Blood Pressure Analysis System Maintenance, replace all tail-cuff bladders before beginning the calibration.

5. **Only perform this step if it is the 1st day of the procedure.** Referring to SOP0036 - MC4000 Blood Pressure Analysis System Calibration, calibrate the MC4000 platforms.

6. Once the calibration of the MC4000 Blood Pressure Analysis System is complete, select the specimen platform 1 icon from the toolbar to make it the active platform.

7. Click on the ‘Pressure Test’ icon and the ‘Pressure Test’ window should appear.

8. Press the ‘Up’ button. The system air compressor should immediately start and the pressure at each channel should begin to increase linearly.

9. Allow the compressor to inflate the system to approx 150mmHg and then press the ‘Hold’ button. The compressor should stop and the pressure displayed at each channel will be maintained. If the pressure begins to drop significantly over time or does not increase at all, the system may have an air leak which is an indication of a defective tail-cuff bladder. If this is the case refer to SOP0037 - MC4000 Blood Pressure Analysis System Maintenance.

10. Once the pressure display remains stable at 150mmHg for each channel, press the ‘Down’ button to release the system pressure or hit the ‘Exit’ button to return to the main program window. Make a note in the NIBP Lab Book as to whether the pressure tests passed or failed and any alterations made.

11. Select the specimen platform 2 icon from the toolbar to make it the active platform and repeat steps 6-10.
    - Repeat steps 6-11 for the 2nd computer (if necessary).

12. Before beginning an experiment the initialization parameters should be checked. Click on the 'Analysis Parameters' icon to display the 'Analysis Parameters' window. The values should read:
    - Preliminary cycles: 5
    - Measurement cycles: 15
    - Minimum pulse amplitude: 3% for C57BL6/NTac & 129S5, 20% for C57BL6 c-/c-
    - Number of consecutive peaks: 70
    - Ignore peaks smaller than: 0.5%
    - Maximum pressure: 200mmHg
    - Pulse timeout: 30 seconds
    - Measurement timeout: 30 seconds
    - Time between measurements: 3.0 seconds
    - Systolic threshold: 20%
    - Diastolic threshold: 50%
    - Systolic signal criterion: 90%; 0.5 seconds
    - Diastolic signal criterion: 90%; 0.5 seconds
    - Platform temperature: 100°F (37.8°C)

    Alter any parameters that are incorrect. Once all parameters are correct press the 'OK' button to return to the main program window.

13. **Only perform this step if it is the 1st day of the procedure.** Next, enter a new experiment for the cohort being measured:
    - Click on 'Logs' to display the 'Logs' drop down menu.
    - Click on 'Experiment Log' to open the 'Experiment Log' window.
    - Click on the 'Add Experiment' button to open the 'Add Experiment' window.
    - Enter the cohort name to be measured name in the 'Experiment Name' field. For example, 'NTAC & CALB Mouse GP 20090123' (differentiate between males & females if both set-ups are in use).
    - Press the 'OK' button to return to the 'Experiment Log' window. The cohort name should now appear in the list of experiments.
    - Press the 'Done' button to return to the main program window.
    - Repeat step 13 for the 2nd computer (if necessary).

14. To begin dual platform measurements, select the rodent icon for platform 1 from the toolbar to make it the active platform. Before loading the mice onto the platform begin the procedure for the mice on the database.

15. Place a cage of mice onto the bench top.

16. Load specimen 1 platform with 4 mice using the steps below.

17. Place the specimen tray on top of the specimen platform.

18. Remove a mouse from its cage and identify it by its earmark.

19. **Only perform this step if it is the 1st day of the procedure.** Weigh the mouse referring to SOP0045 - Weigh Mice and record the weights on the NIBP Record Sheet.

20. While holding the mouse by its tail, place the holder at the rear of the mouse so that the small circular nose hole is facing the wall at a 45° angle and gently

place the holder over the animal until the magnetic feet make contact with the tray. Be careful not to pinch the animal's feet between the holder and tray.

21. Continue to hold the mouse by the tail and slide the holder backwards until it touches the edge of the tray.

22. While continuing to hold the tail, pull the tail-cuff up out of the specimen platform. Thread the tail through the cuff and push the cuff back into the hole in the platform. At the same time, lay the tail down into the 'V' groove on the sensor assembly.

23. Gently pull back on the mouse's tail until the animal's rear is against the back of the specimen holder. Part of the mouse's rear at the base of the tail will protrude through the slot in the back of the holder. This will prevent movement of the mouse when measurements are being recorded.

24. Hold the tail near the tip and place a piece of surgical tape over the tail to fasten it to the step on the sensor assembly. This will prevent the mouse from moving and pulling its tail out of the tail-cuff. Do not place the surgical tape too firmly on the mouse's tail as this may restrict blood flow to the area and result in permanent damage of its tail.
    - **If at any point during the experiment the mouse does manage to pull its tail out from under the surgical tape or the tail-cuff pause the experiment by pressing the "Pause" icon on the top menu bar and place it back into position as soon as possible whilst trying to minimise the stress caused to the animal. Once it has been repositioned press any key to continue the procedure.**

25. Repeat the steps 18-24 until all 4 mice are loaded onto the platform.

26. Now register the mice on the database.

27. Select the 'Go' icon from the toolbar to open the specimen registration window.

28. In the 'Experiment' field select the cohort name from the drop down menu. For example, B6N MGP Select 20121102.

29. Select a name in the 'Investigator' drop down menu. (Whoever is selected as the investigator at the start of the measurements must then be entered as the investigator every time a new set of measurements are taken.)

30. In the 'Technician' field select your name from the drop down menu. (The technicians name does not have to remain the same throughout the experiment.)

31. In the 'Specimen 1' field enter the mouse barcode of mouse that is present in channel 1. For example, specimen 1 = M00001234. An existing specimen can be selected from the database through the drop down menu.
    - Use the cage-card to double check that the barcode has been correctly entered. Failure to enter the correct barcode will make it impossible for the database to upload data automatically to the correct mouse.
    - The channel number is clearly visible on the front of the specimen platforms if you are unsure which channel is which.

32. Repeat step 31 until all specimen fields are complete. (If there are any empty channels then leave the specimen field blank so the software will not run the procedure on these channels.)

33. Once all specimens are entered measurements are begun by clicking on the 'Go' button in the 'Specimen Registration' window. The MC4000 will now automatically perform the measurement process based on the preset analysis parameters. Before starting the first set of readings wait approximately 2 minutes before clicking 'Go' to allow the mice to acclimatise to the equipment for the same amount of time as the next sets of mice to undergo the procedure.

- If the equipment does not start properly or there is a fault with it during the measurement process then remove the mice from the tail-cuffs until they are required as being held in the tail-cuffs for too long may damage their tails.
- **If at any point you want to stop the measurement process due to faulty equipment or you are concerned about the welfare of the animal(s), click on the 'Abort Analysis' icon on the menu bar. The measurement process will stop immediately and any results obtained up to this point will be lost. Note: if the 'Abort Analysis' button is pressed, when you restart, the process will start from the beginning therefore you cannot run the full experiment as this will expose the mice to excess rounds of measurement.**
- Comment on any faults with the equipment or health concerns in the NIBP Log Book.

If at any point you feel a mouse has a welfare issue terminate the procedure **immediately** and contact a Named Animal Care and Welfare Officer (NACWO). QC fail the remaining measurements.

34. When the 1st platform has reached 5 out of 15 actual measurements start to load the 2nd platform following steps 15-25. (Loading the mice onto the 2nd platform at this time coincides with the measurements for the 1st platform finishing, thus ensuring that all mice spend approximately the same amount of time acclimatising to the equipment.)

35. Immediately after a measurement set is complete the software displays the 'Analysis Results' table. Click on the 'Save All' button to save all data collected automatically and return to the main program window.

36. Now, select the rodent icon for platform 2 from the toolbar to make it the active platform and repeat steps 26-33.

37. While the equipment & software are recording measurements for platform 2 remove the mice one at a time from the platform 1 holders and place them back in their respective cage and clean the platform 1 specimen tray and holders thoroughly with the 70% ethanol and the paper towels.

- When removing the animals from the platform and holders check the mice for injuries especially their tails and feet.

38. When the 2nd platform has reached 5 out of 15 actual measurements start to load the 1st platform following steps 15-25.

39. Repeat steps 15-35 as necessary until all mice have been measured.

40. Repeat steps 15-35 for the 2nd computer (if necessary).
- During the procedure if there are any additional noises or abnormalities that may affect the sessions results make a note of them along with the time under the 'Comments' section of the days page in the NIBP Lab Book.

41. **Only perform this step if it is the final day of the procedure.** Once all mice have been measured, select 'Report Generator' from the 'Tools' drop down menu to open the 'Report Generator' window. Select 'Experiment' from the 'Match Fields' drop down menu and select the cohort that has just been completed from the drop down menu. Click on 'Show Data' to open the 'Report Data' window. Select 'Export CSV' from the 'File' drop down menu and then select 'Save As'. From the 'Save As' window select the appropriate data folder on the team drive to save the data into. Enter the file name as the cohort name followed by raw data and select 'Save'.
- Repeat this step until the results for all cohorts that have just been completed have been exported.

42. Once all mice have been measured, click on 'File' to display the 'File' drop down menu.

43. Click on 'Exit' to exit the software.

44. Print new cage cards for the cages tested.

45. Switch the power (at the back of the control unit above the power cable) to the 'OFF' position.

46. **Only perform this step if it is the final day of the procedure.** Perform a full cage clean.

47. Log-off the computer and turn the screen off.

48. Clean equipment and surfaces. Transfer all waste to a yellow clinical waste bag or clearly labelled waste container. This is transferred to blue suite for disposal as appropriate.

49. **Only perform this step if it is the final day of the procedure.** Referring to SOP0037 - MC4000 Blood Pressure Analysis System Maintenance, remove and dispose of all tail-cuff bladders. Store end caps in the NIBP cupboard.

50. **Only perform this step if it is the final day of the procedure.** Ensure all cages display updated cage cards and return mice to animal room. Register them to their respective rack and place a 'POST PROCEDURE CHECK REQUIRED' label on cages.

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: MC4000 Blood Pressure Analysis System Maintenance – V1

SOP Number: SOP0037	**To be reviewed:**
Author(s): Signed: Date: **Editor:** Signed: Date:	
Date Modified:	

INTRODUCTION:
The purpose of this procedure is to replace tail-cuff bladders and end caps as part of SOP0038 Non-Invasive Blood Pressure (NIBP).

ABBREVIATIONS:
- **NIBP** = Non-Invasive Blood Pressure
- **PPL** = Project License
- **SOP** = Standard Operating Procedure

HEALTH & SAFETY:
- **RA004** – Physical Hazards; *Section RA004.4.2 & RA004.5.1*

RESPONSIBILITIES:
All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) has been read, understood and where applicable is followed in accordance with the relevant Project License (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure.

RESOURCES:

Equipment:
1. Latex Balloons
2. Scissors
3. 0.375” Diameter Heat-Shrink Tubing
4. Section of Brass Tubing
5. Heat Source, such as Hot Air Gun or Open Flame
6. Scalpel
7. NIBP Lab Book
8. Pen

- **Associated SOPs:SOP0038** - Non-Invasive Blood Pressure

Staff Required: This procedure requires 1 person.

NOTE:
As this test is to be performed as part of the SOP0038 - NIBP procedure, you should look at that SOP and move to this one at the indicated step(s).

All tail-cuff bladders must be replaced on the first day of the NIBP as part of the procedure.

Steps 11-16 must be performed at a functioning down flow table.

PROCEDURE:

1. If whilst conducting a pressure test the pressure begins to drop significantly over time the system may have an air leak.
    - An air leak is usually an indication of a defective tail-cuff bladder.

2. The leak can be found by pinching off the air line to each cuff one at a time and watching the 'Pressure Test' window to see if the pressure is dropping. If the pressure now remains stable then you've found the leak.
    - If not, go to the next cuff and perform the same procedure.
    - If you try all four cuffs and the pressure still does not remain stable you will have to pinch off the air to two cuffs at once to find which cuffs are leaking. For example, you can pinch off the air to cuff 1 and 2 simultaneously and then 1 and 3, then 3 and 4 etc until you have isolated the leaking cuffs.
    - If you have more than one leaking cuff it is advisable to change all tail-cuff bladders on that platform and then do a pressure test to verify that you have installed them properly and without leaks.

3. To replace the bladder, extend the tubing for each channel as far as it will go.

4. Remove the end caps on the tail-cuff.

5. Remove the bladder from the tail-cuff and use it as a gage for cutting the latex balloon with the scissors to the proper length.

6. After cutting the balloon, insert the cut piece into the tail-cuff.

7. Turn each end of the bladder back on itself thereby folding the bladder back over the end of the tail-cuff.
    - Be sure not to stretch or twist the bladder when installing as stretching or distorting the bladder by twisting can affect measurement results. The bladder should lie naturally inside the tail-cuff.
    - When you change a tail-cuff bladder, make a note of the date and the tail-cuff of which platform and channel was changed in the NIBP Lab Book.

8. Replace the end caps and place the tubing back inside the platform.

9. Repeat steps 3-8 until all tail-cuff bladders are replaced where necessary and then return to SOP0038 - NIBP.

10. If when replacing an end cap it no longer stays on make a new end cap following the steps below at a functioning down flow table.

11. Slide the 0.375” diameter heat-shrink tubing over the section of brass tubing.

12. Align the pieces of tubing on one end.

13. With a suitable heat source apply heat to a small section of the end of the tubing where the parts are aligned.
    - Try not to shrink too much of the tubing back from the end of the brass as the heat-shrink will become very tight on the brass tube.

14. Slide the heat-shrink tubing past the end of the brass so that approximately 1/16” of material extends beyond the end of the brass again heating the tubing until the end shrinks and rolls over the end of the brass.

15. To make a sharp crease in the fold immediately after heating gently press the end of the tubing against a hard, flat surface.

16. After cooling, use a scalpel to cut around the tubing approximately 3/16” back from the end.

17. Repeat steps 11-16 until all end caps are replaced where necessary and return to SOP0038 - NIBP.

NIBP Data Analysis

Equipment:

1. NIBP Record Sheet

2. The Hatteras MC4000 Blood Pressure Analysis System computer

Results Analysis (Part I):

1. Switch on the computer screen and log-in. Double click on the 'MC4000 BP Analysis' program icon to start the program.

2. Click on 'Logs' to display the 'Logs' drop down menu.

3. Click on 'Session Log' to open the 'Session Log' window.

4. Open today's session.

*4.1. Select the cohort name from the experiment list.

*4.2. Select today's date and the time of the experiment.

Note: the measurements from the first test session will have the earliest time with today's date and the measurements from the last test session will have the latest time with today's date.

*4.3. Click on the 'Open Session' button to open the 'Analysis Results' window.

5. Now open the specimens results

*5.1. Highlight the results of an individual specimen.

*5.2. Click on the 'Details' button to open the 'Specimen Data' window.

6. Now go through the results and reject any erroneous measurements. For example, measurements where it appears the mouse has moved during the reading, no measurement for either systolic or diastolic pressure or extremely low/high systolic/diastolic readings that are not in context with the other results obtained. Note: If the mouse moves between taking the diastolic and the systolic reading the systolic reading will often be too high. Also if the mouse has a very active period when the heart rate is determined the diastolic pressure is often estimated to be too low.

*6.1. Highlight the result that you want to reject.

*6.2. Click on the 'Reject' button. This then recalculates the results for the mean and standard deviation for that measurement set.

Note: The data for that measurement is maintained within the software database and can be reinstated at any time.

*6.3. Once you have checked all 15 results click on the 'Done' button to return to the 'Analysis Results' window.

*6.4. Make a note under the 'Number of Measurements' column on the NIBP Record Sheet depending on which day of recorded measurements you are on for that specimen.

7. Repeat steps 3.3 to 3.6.4 for all of the results from today's session.

8. Once all results from today's session have been edited click on the 'Done' button in the 'Analysis Results' window to return to the 'Session Log' window.

9. Now click on the 'Done' button in the 'Session Log' to return to the main program window.

10. Repeat steps 3.2.1 to 3.2.9 for the second computer if necessary.

11. Go to steps 5.3 to 5.5 below if this is not the final day of the procedure. If it is the final day of the procedure go to steps 4.1 to 5.5.

Results Analysis (Part II):

On the final day of the procedure after all the results have been analysed export the results to an excel document.

1. Click on 'Tools' to display the 'Tools' drop down menu.

2. Click on 'Report Generator' to open the 'Report Generator' window.

3. In the top left 'Match Fields' field select 'Experiment' from the drop down menu.

4. In the top right 'Match Fields' field select the cohort that has just been measured name from the drop down menu.

5. Click on the 'Show Data' button to open the 'Report Data' window.

6. Click on 'File' to display the 'File' drop down menu.

7. Click on 'Export CSV' to open up the 'Save As' window.

8. From the 'Save As' window, select the appropriate folder.

9. Click on 'Save'. You should now return to the 'Report Data' window.

Turning Off The MC4000 Blood Pressure Analysis Equipment:

1. Exit the 'Report Data' window by clicking on the red square cross in the top right of the window. You should now return to the 'Report Generator' window.

2. Exit the 'Report Generator' window by clicking on the 'Done' button. You should now return to the main program window.

3. Click on 'File' to display the 'File' drop down menu.

4. Click on 'Exit' to exit the software.

5. Log-off the computer and turn the screen off.

Results Analysis (Part III):

1. Open the CSV raw data file from its location on the team drive.

2. Delete the columns containing the Specimen Type, Investigator and Technician information as this is not required.

3. Sort the Date/Time column from oldest to newest and expand the selection so that it includes all data next to your selection.

4. Check that there are 5 days of measurements and then delete all rows containing data from the first measurement day. This is the training day data which is not required.

5. Sort the Status column from A to Z and delete any rows containing measurements that were unsuccessful. These are represented as BPTO (Blood Pressure Time Out), PTO (Pulse Time Out) and MPE (Maximum Pressure Exceeded).

6. Sort the Specimen column from A to Z. This will group all of the data from one mouse together in date order.

7. Open 2 copies of the NIBP template v1.1. Save one copy for females and one copy for males (using the cohort name) in the relevant location on the team drive.

8. Using the database, copy and paste all data for one mouse into either the WT controls, Mutant hets or Mutant homs sheet in the male or female document. This will automatically average the Systolic, Diastolic and Heart Rate readings and Count the number of measurements and display it above the raw data (these values are also displayed in the Data Summary sheet).

9. Repeat step 8 for all mice from the cohort that have data present.

10. Once all data has been analysed, using the values in the data summary sheet, enter the Systolic, Diastolic and Heart Rate values into the data capture form.

- IF THE MOUSE HAS LESS THAN 20 SUCCESSFUL MEASUREMENTS, DO NOT ENTER THE DATA ONTO THE DCF. QC FAIL ALL PARAMETERS ON THE DCF WITH ‘FAILED TO OBTAIN 20 SUCCESSFUL MEASUREMENTS’

11. The DCF and procedure can now be completed.

12. Save all changes to the excel templates and close.

13. Close the CSV file but do not save any changes.