WELLCOME SANGER INSTITUTE

STANDARD OPERATING PROCEDURE PACKET

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SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Indirect Calorimetry – V1

SOP Number: SOP0030	**To be reviewed:**	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Assessor:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to investigate animal metabolism, circadian pattern & behaviour (activity and to some extent exploratory) in wild-type and genetically altered mice contributing to the MCMS database. To obtain this information, up to 72 hours measurement of mouse movement, food and water consumption is performed.

ABBREVIATIONS:

DCF = Data Capture Form
IVC = Individually Ventilated Cage
LAA = Laboratory Animal Allergens
NACWO = Named Animal Care and Welfare Officer
PAF = Project Authorisation Document
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
Ref. Gas = Reference Gas
RER = Respiratory Exchange Ratio
RSF = Research Support Facility
SOP = Standard Operating Procedure
SMP = Sick Mouse Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.
If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

In-Life Procedures:

Problem / Issue	QC fail reason
At any point during the procedure the mouse is deemed sick and processed through Sick Mouse Procedure (SMP)	Fail whole DCF as 'Sick mouse' – for all tests that day
Mouse incorrectly scheduled at wrong week	Fail whole DCF as 'Scheduling Issue'
Insufficient anaesthesia level affects the whole test DCF	Fail whole DCF as 'Anaesthesia Issue'
Insufficient anaesthesia level affects specific parameter(s)	Fail parameter(s) as 'Anaesthesia issue'
A welfare issue makes it impossible to collect specific parameters	Fail parameter(s) as 'Welfare issue'
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'
A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
Parameter on the current DCF is not required for that specific test/pipeline	Fail parameter(s) as 'Not required'

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-1205
Assessment Title: Indirect Calorimetry and change of gas bottle
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or downflow tables), a respiratory mask, for which you have passed a face fit test, must be worn.

- The TSE LabMaster system is a pressurised system. Users performing calibration of the equipment using the gas bottles and/or changing a gas bottle should wear safety spectacles.
- Equipment is visually inspected before use. Any defects are reported and equipment is labelled to prevent use until it has been repaired. The machine is serviced once a year by the manufacturer and a certificate is given after the servicing.
- If moving gas bottles required for this procedure, safety gloves must be worn to increase grip and a trolley designed for moving gas bottles must be used. Staff are trained in manual handling.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project Licence (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual Licence (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

Equipment:

1. Balance
2. 70% Ethanol - **Hazardous substance: highly flammable**
3. Hydrex Pink Hand spray **- Hazardous substance: highly flammable**
4. Hydrex Hard Surface spray - **Hazardous substance: highly flammable**
5. Tissues
6. Tecniplast mobile Individually Ventilated Cage (IVC) rack
7. Diet (as defined by pipeline)
8. TSE Labmaster System.
9. Span gas cylinder *(Supplier name; Airproducts Ltd. Supplier product code; XXX – enter "PRODUCT CODE 327319; 1% CO2,20.% O2//N2 in a 10ltr cylinder" as product description)* – **Compressed gas and manual handling hazard: cylinders must be handled whilst wearing padded gloves and safety spectacles. Cylinders must be transported using specifically designed trolleys and must be restrained in place when in use or being stored.**
10. Reference gas cylinder *(Supplier name; Airproducts Ltd. Supplier product code; XXX – enter "PRODUCT CODE* 328170*; MIXTURE OF GASES, X10A 20.9% OXYGEN, 500 PPM CARBON DIOXIDE, IN NITROGEN. ALUMINIUM CYLINDER 1X 2.4KG" as product description)* – **Compressed gas and manual handling hazard: cylinders must be handled whilst wearing padded gloves and safety spectacles. Cylinders must be transported using specifically designed trolleys and must be restrained in place when in use or being stored.**
11. Labmaster operating instructions manual for the calibration of the food sensors

12. TSE LabMaster System software
13. x1 Clean calorimetry cage base for each mouse to be tested
14. x1 Clean food hopper for each mouse to be tested
15. x1 Clean water bottle for each mouse to be tested
16. Small petri dish cover if floor food/mash required
17. Water from autoclaved water bottles
18. Cage bedding material
19. Stuffer individual cage labels
20. 'Experimental mice removed for calorimetry' labels
21. x1 Clean cage base, with water bottle, nestlet for each stuffer
22. Safety spectacles
23. Safety gloves
24. Leak detection fluid for the change of the gas bottles. *(SNOOP® gas leak detector; Supplier name: Sigma-Aldricht; Supplier product code: Z273910-1EA)*
25. 'Post Procedure Check Required' labels

Associated SOPs/Documentation:

- **EQ18** – Use of Tecniplast Interactive Cage Change Stations
- **SOP0101** – Taking and Returning Cages for Procedures
- **SOP0045** – Weigh Mice
- **Calo check guidelines for phenotypers**
- **'Experimental mice removed for calorimetry' labels**

Staff: This test can be completed by one phenotyper.

NOTE:

Before you start any experiment the calorimeter needs to be calibrated. If the calorimeter has been switched off, the system must be left for 2hrs after switch on before calibration can begin.

This assay has been run both as a 21 hour test and as a 72 hour test, pipeline dependant. In the 21 hour version, steps 5 & 6 would be performed on the same day.

PROCEDURE:

Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).

1. First day of experiment: PREPARATION

1.1. Switch on all components of the TSE LabMaster system 2-3 hours before starting calibration.
Open Reference Gas (Ref. Gas) bottle according to the safety procedure (see Appendix 3: Open/Close gas bottles) to a pressure of 4 bars.

1.2. Log-on to the calorimetry computer.

1.3. Open the TSE Lab Master application by double clicking on the TSE System icon on the desktop. When the data table has loaded, open the CALO screen

under the STATUS tab. Use this screen to monitor flow rate and gas values during set-up and calibration.

1.4. Turn on the air pump.

1.5. Check all process control units are on (displaying a green light).

1.6. Prepare a sheet in which you should include columns with Box #, Mouse ID, Mouse Barcode, Earmark, Sex, Genotype, Location and 4 empty columns for Weight before, Weight after, Water before and Water after.

2. CALIBRATION

2.1. Empty the water from the small glass bottle in the air drying unit: this is important in order to obtain correct gas values.

2.2. Ref. Gas calibration:

2.2.1. **Wearing safety spectacles** open the Ref.Gas secondary circuit using the T-piece to a correct calibrating pressure.

2.2.2. Disconnect the main pump circuit from the front of the air drying unit and connect the secondary Ref. gas circuit. Leave the Ref. Gas running for a minimum of 10 minutes to let the gas values stabilise.

2.2.3. Check on the computer (status→Calo) that the flow rate is correct (0.35l/min). Remember to regularly check the actual flow on the computer screen as it may drop off after a few minutes.

2.2.4. On the LabMaster system, select O_2 (by pressing the button in front of the text) → calibration.

2.2.5. If this is the initial calibration performed after the calorimeter has been switched on, the system may ask you for a "level 1" password; on the LabMaster system press 111 and "Enter" when prompted.

2.2.6. Press zero calibration and record the "actual value" as "O_2 Before" under "Ref. Gas" column in the lab book.

2.2.7. Press "start calibration" and write down the new "actual value" as "O_2 after" in the lab book.

2.2.8. Press "Meas" button and accept modifications.

2.2.9. Repeat these steps for CO_2 and record the "actual values" as "CO_2 before" and "CO_2 after" under "Ref. Gas" column in the lab book.

2.2.10. Disconnect the secondary "Ref. Gas" circuit from the air dryer unit and close it using the T-piece. Ensure the Ref. Gas bottle is left open and never closed at this point.

2.3. Span Gas calibration:

2.3.1. **Wearing safety spectacles** open the Span Gas bottle according to the safety procedures (see Appendix 3: Open/Close gas bottles section at the end of this SOP) to a correct calibrating pressure and connect the circuit to the front of the air drying unit.

2.3.2. Check the flow rate on the computer (as previously described) and wait a minimum of 10 minutes for the gas values to stabilise and remain stable. On the LabMaster system, select O_2 → calibration.

2.3.3. If this is the initial calibration performed after the calorimeter has been switched on, the system may ask you for a "level 1" password; on the LabMaster system press 111 and "Enter" when prompted.

2.3.4. Press Span calibration. Record the "actual value" as "O_2 before" under the "Span Gas" column in the lab book.

2.3.5. Press “start calibration” and record the “actual value” as “O_2 after” under the “Span Gas” column in the lab book.
2.3.6. Press “Meas” button and accept modifications.
2.3.7. Repeat these steps for CO_2 and record the “actual values” as “CO_2 before” and “CO_2 after “under the “Span Gas” column in the lab book.
2.3.8. Disconnect the “Span Gas” circuit from the air drying unit.
2.3.9. Close the “Span Gas” bottle according to the safety procedures (see Appendix 3: Open/Close gas bottles) and reconnect the “Ref. Gas” main circuit to the air drying unit.

2.4. Food sensor calibration:
Feeding sensors have to be recalibrated monthly. This procedure will be carried out by the person responsible for the test and is detailed in the “lab master operating instruction” manual, under the “software modules” tab, page 63.

3. TEST PROCEDURE

3.1. Before moving the mice to their respective calorimetry cages, check on the computer (STATUS → CALO) or on the screen at the bottom of the Labmaster System that the O2 and CO2 values following calibration are as expected (from 20.91 to 20.94 for O_2 and from 0.04 to 0.06 for CO_2). If unsure, consult the person responsible for the test.
3.1.1. If the calorimeter needs recalibration, repeat step 2 and note any relevant comments in the calorimetry log book and calorimetry event register.

3.2. Collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (Refer to SOP0101 – Taking and Returning Cages for Procedures).

3.3. Place ‘Phenotyping in progress’ sign on the outside of the door.

3.4. Prepare calorimetry cages to receive mice:
3.4.1. Remove the lid by releasing the metal bracket across the top and lift into the holding position using the hook inbuilt into the lid.
3.4.2. Place a handful of bedding in the cage bases.
3.4.3. Fill food hoppers with pre-broken small diet pellets. Ensure that the pellets are not stuck in the food hopper and are available for the mouse. Fill the water bottles with sufficient water for the length of the experiment.
3.4.4. Weigh and record the weight of the filled water bottle (with lid attached).

3.5. Weigh each mouse (for instruction, refer to SOP0045 - Weigh Mice).

3.6. Record each mouse’s weight alongside the calorimetry cage it is to be placed in on the worksheet. Cages are numbered from top left to bottom right, starting with the rack on the left side of unit.

3.7. Seed the calorimetry cage with a handful of bedding from the mouse’s home cage

3.8. Place each mouse into its corresponding calorimetry cage. Replace calorimetry cage lids and secure with the metal braces.

3.9. Repeat steps 3.5-3.8 for all mice to be tested.

3.9.1. Where stuffers are present, they are to be individually housed at the same time as mice completing the procedure.

3.9.2. When relocating stuffers to an individual cage, give diet, water bottle, nestlet and cardboard tunnel and remember to seed the cage with a handful of bedding from the mouse's home cage.

3.9.3. Label the cage with a stuffer cage label and store on the mobile IVC rack in the calorimetry room.

3.10. Prepare home cages:

3.10.1. If the empty home cages are to be kept to return mice to after testing, these should be partially cleaned, stored on the mobile IVC rack in the calorimetry room, have their water bottle inverted so the spout faces outwards and their cage cards turned on end.

3.10.2. If however animals are to remain individually house after completing indirect calorimetry as part of secondary phenotyping, clean individual cages should be prepared.

3.11. Once mice are housed in individual calorimetry cages, give a visual check to ensure all mice (including stuffers) have access to food and water, that no water bottles are leaking, that there are no obstructions to the sensors measuring mouse activity, or any other issue that could be detrimental to the animals' welfare or affect the conduct of the experiment.

3.12. Dim lights to 'LOW' and exit the room.

3.13. Display the cautious "Calorimetry running….Please do not disturb the mice" sign on the outside of the door to the room.

3.14. On the computer start a new experiment:

3.14.1. Select NEW under the FILE tab on the LabMaster software at the top of the screen. This will open the "Save As" window.

3.14.2. Enter the experiment name.

3.14.3. Save this file to the desktop.

3.15. When a new file is generated the set-up window will open automatically and the settings for the experiment can be entered. Tabs; Experiment, Boxes, Activity, Calo and Drink/Feed are located at the top of this window and all contain parameters requiring input as follows:

3.15.1. LOCATED UNDER 'EXPERIMENT' TAB

3.15.1.1. FILE NAME FILE NAME – Name of experiment – This is automatically entered using the file name entered (see above).

3.15.1.2. EXPERIMENT NUMBER, USER, TEXT1, TEXT2 AND TEXT3 – For the addition of further details. – Leave blank.

3.15.1.3. STARTED AT – The time at which the experiment begins. - This field is updated by the software when the experiment begins.

3.15.1.4. SAMPLE INTERVAL – Entered in minutes (1-120min). All enabled boxes and reference cage will be measured within this. For example, if set at 1, all activated cages will record measurements within that minute resulting in the time over which the measurements are taken to be very small. – Set so the time per cage is **at least 1.50mins**. This will be dependent on the number of

cages active per run. Select cages to be active before changing this parameter (see below).

3.15.1.5. RUN TIME – The time over which the experiment will run. Measurements will no longer be taken when this time has elapsed - Leave as 240.00hrs (default setting).

3.15.1.6. CHECKBOXES – Measurements to be taken during the experiment - Activity, Calorimetry and Drink/Feed should be checked.

3.15.1.7. TIME DISPLAY – Format of the measurement taken time, as shown on the table – Check Absolute.

3.15.2. LOCATED UNDER 'BOXES' TAB

3.15.2.1. ENABLE/DISABLE BOXES – If measurements are to be taken from any box it must be enabled on this screen. Pressing Y in the field will enable that box, N will block it. This allows experiments to be run with blank cages omitted. This must be done if empty cages are present. – Disable any empty cages.

3.15.2.2. ANIMAL NUMBER – Enter the animal identity (ID) in this column. The ID used here will be the numeric barcode of the mouse starting after the first digit which is not 0 (for example M01012345 should be entered as 1012345).

3.15.2.3. WEIGHT – Enter mouse weight (in grams) for each animal in this field. This information can be taken from daily calorimetry worksheet.

3.15.2.4. TEXT1, TEXT2 AND TEXT3 – Further text fields for identifying animals (max. 20 characters possible) – Enter genotype into Text1, sex (M for males and F for females) into Text2, & Text3 unchanged.

3.15.3. LOCATED UNDER "ACTIVITY" TAB

3.15.3.1. WRITE BINARY FILE – Do not select this option.

3.15.3.2. RECORDING INTERVAL – Applies only when 'Write binary file' is marked.

3.15.3.3. CENTER AREA – The user can define an area of the cages as central. This will allow the system to differentiate between activity in the centre versus the periphery of the cage. X1-X2 defines the first and last light beam of the central area in the X (beams running from side to side) direction and likewise for Y1-Y2 (beams running from front to back) – Set as follows: X1-X2 = 6-11, Y1-Y2 = 7-10.

3.15.3.4. REFRACTORY PERIOD – The user can define a time interval for which no count is taken following the break of a light beam. This can avoid tail flicking and scratching being included in the count. – Set to 0.8.

3.15.3.5. FLIP AXIS – Allows the user to invert the X and Y axis - Do not select this option.

3.15.3.6. ENABLED LIGHT BEAMS – The user can disable light beams by unchecking these boxes. Boxes bordered by red are those marked as peripheral, those in green, central. – Enable all.

3.15.3.7. EXPORT TABLE – Determines the orientation of the data table. Format 1 = results for each cage are displayed one below the other, Format 2 = results for each cage are displayed side by side. – Select Format 2.

3.15.4. LOCATED UNDER "CALO" TAB

3.15.4.1. CONTINUOUS MODE – When enabled, all measurements will be taken from only the first enabled box. – Do not select this option.

3.15.4.2. FLOW – Sets the gas flow into each cage - Set to 0.45l/min (This should be set to 0.1l/min greater than the flow set into the reference cage which, in turn, is set during calibration and will stay at this value during further experiments).

3.15.4.3. ALARM ON 90% FLOW – When enabled, this function sounds an alarm when the gas flow in a box drops below 90% of the set value for longer than 10 sec. - This requires personnel to be present during the entire experiment. Do not check this box.

3.15.4.4. H in W/kg and CALCULATION OF VO2/VCO2 – These values are for the measurement and calculation of heat emitted. – Leave the H in W/kg box unchecked and the values in the box as **3.941, 1.106** and **0.999**.

3.15.5. LOCATED UNDER “DRINK/FEED” TAB

3.15.5.1. SMOOTHING ADC – The time over which the average of measured values are taken where the average is the measurement recorded – Set to 10sec.

3.15.5.2. MAX. DELTA ADC – The upper limit of rejection measured in ADC counts. When a measurement exceeds the average calculated during the smoothing ADC by the figure entered in this box will be rejected as anomalous. – Set to 50.

3.15.5.3. TRIAL MON. OBSERVATION INTERVAL – Time in seconds after which the trial monitor (view used to monitor experiment) is updated – Set to 1sec.

3.15.5.4. ONLY POSITIVE CONSUMPTION –basket consumption is always defined as positive, i.e. measured weight on the hook must always decrease. Weight increases are not recorded as measurements if this box is checked. – Check this box.

3.15.5.5. CONFIRM EACH SENSOR AT START – Reminds the user to check the food and water levels at the beginning of each measurement. Do not check this box.

3.15.5.6. START BOXES SEPARATELY – Allows the measurements to be manually started for each box rather than on a continuous cycle. Do not check this box.

3.15.5.7. WRITE BINARY FILE – Allows more accurate measures of consumption as the interval time here can be set as low as 5 sec. – Do not select this option.

3.15.5.8. MIN. CONSUMPTION – Lowest value to be achieved before a removal of food by the animal is recorded as a measurement. Set to 0.01.

3.15.5.9. MAX. CONSUMPTION – Upper limit for rejecting a measurement. – Set to 0.10.

3.15.5.10. USED SENSORS – Allows the user to define which sensors are in use. And block measurement for any which are not. – Check box for feed.

3.16. When the settings have been entered, click close. Set-up is automatically saved.

3.17. Open the view menu using the VIEW tab at the top of the screen. In this menu parameters to be shown in the table and graphs can be selected by clicking the check boxes. All available parameters should be selected and:

3.17.1. All feed sensors for activated cages
3.17.2. All boxes active during the experiment.
3.17.3. Graph1 = RER
3.17.4. Graph2 = feed

3.18. When selection is complete, click close. Selections are automatically saved and will now be seen in the table on screen.

3.19. Click START under the measurement tab. Once the first measurement has been recorded, set-up options cannot be changed. **The experiment should be started no earlier than 1:30 pm and no later than 2:30 pm.**

3.20. Wait for the software to scan through calibrating all the sensors and record the time the experiment was started in the Lab book. Place the record sheet for the current run with the Lab book.

4. **CHECKS** (see Appendix 1: Monitoring and Observations for more details)

4.1. <u>First Check</u>

4.1.1. Within 30 minutes of the measurement starting, perform a check using the TABLE tab of the LabMaster software.

4.1.2. Check the reference cage gas values obtained during the first measurement are as expected and record the values on the worksheet.

4.1.3. Use the tabulated data to check the welfare of the mice paying close attention to the flow, RER, VCO2 and food intake parameters for each cage. Record observations on the worksheet.

4.1.4. Check that the food intake for each mouse is not ≥ 0.5g.
4.1.4.1. If a mouse has eaten above 0.5g, stop the measurement, close and re-open the LabMaster software and start a new experiment adding "_2" to the regular experiment's file name.
4.1.4.2. Record observations and/or actions on the worksheet.

4.1.5. Enter the room and perform a visual check of the mice; ensure that all mice (including stuffers) have access to food and water, that no water bottles are leaking, that there are no obstructions to the sensors measuring mouse activity, or any other issue that could be detrimental to the animals' welfare or affect the outcome of the experiment.

4.1.6. Take any appropriate action as required, remembering to check which cage is currently being read (STATUS → CALO) before making any cage manipulations in order to ensure there is sufficient time to avoid disrupting subsequent cage readings.

4.1.7. If in doubt about the welfare of any animal, discuss with a NACWO and take appropriate action as required.

4.1.8. Note the time and any relevant comments in the calorimetry log book and calorimetry event register.

4.2. <u>Last check of the day</u>

4.2.1. Before leaving at the end of the day AND after the experiment has been running for at least 1.5 hours, perform a final check using the TABLE tab of the LabMaster software. This can be performed remotely if absolutely unavoidable (see Appendix 2: Performing a remote check).

4.2.2. Use the tabulated data to check the welfare of the mice paying close attention to the flow, RER, VCO2 and food intake parameters for each cage.

4.2.3. At this point, do not perform a visual check of the mice in order to avoid disturbing the animals, unless a welfare issue deems it necessary to enter the room.

4.2.4. If in doubt about the welfare of any animal, discuss with a NACWO and take appropriate action as required.

4.2.5. Note the time and any relevant comments in the calorimetry log book and calorimetry event register.

5. **Subsequent day(s)**

5.1.1. Perform an initial check of the calorimeter first thing upon arriving in the morning. This should be performed before 9 am.

5.1.2. Using the GRAPH tab (and if necessary the TABLE tab) of the LabMaster software to check the welfare of the mice, paying close attention to the RER and food intake in particular.

5.1.3. At this point, do not perform a visual check of the mice in order to avoid disturbing the animals, unless a welfare issue deems it necessary to enter the room.

5.1.4. If in doubt about the welfare of any animal, discuss with a NACWO and take appropriate action as required.

5.1.5. Remember to check which cage is currently being read (STATUS → CALO) before making any cage manipulations in order to ensure there is sufficient time to avoid disruption to subsequent cage readings.

5.1.6. Remove, weigh, and return to its home cage any mice that falls into at least one of these categories:

5.1.6.1. Prolonged period (≥ 2 hrs) of RER values lower than 0.7
5.1.6.2. Food intake has stopped AND RER is lower than 0.7
5.1.6.3. No food intake for ≥ 12 hours
5.1.6.4. Total food intake <1g for the last 24 hours
5.1.6.5. No access to food and/or water

5.1.7. Note the time and any relevant comments in the calorimetry log book and calorimetry event register. If in doubt about the welfare of any animal, discuss with a NACWO, scientific team leader or an experienced phenotyper and take appropriate action.

5.1.8. If the experiment is planned to run for more than 24 hours, the animals require checking in the morning and afternoon of each subsequent day. Perform the check as you would normally AND enter the animal room to

visually assess the animals welfare (never open a cage), and check if mice have sufficient food and water.

6. **Final Day of Experiment: STOP EXPERIMENT AND EXPORT DATA**

6.1. At the end of the experiment (**not before 11:30 am**), terminate measurements by selecting STOP under the MEASUREMENT tab.

6.2. Select 'TABLE' under the EXPORT tab and save the table onto the desktop (this will be the default location displayed automatically and created in step 3.14).

6.3. From the desktop, copy the 3 files from the experiment run and paste them into the relevant folder on the team drive.

6.4. Enter the Calo room, returning the lights to the 'HIGH' setting.

6.5. Remove and weigh all water bottles from the Calorimetry cages, recording the weights on the Calo worksheet.

6.6. Prepare to remove mice from the calorimetry cages:

6.6.1. Remove the lids of the calorimetry cages by releasing metal braces across the top and lift into the holding position using the hook inbuilt into the lids.

6.7. Weigh each mouse (for instruction, refer to SOP0045 - Weigh Mice) and record the weights on the calorimetry worksheet.

6.8. When taking mice off, observe and assess each animal's clinical status. If in doubt about the welfare of any animal, discuss with a NACWO and take appropriate action as required.

6.9. Place mice back into their home cage or individual cages depending of the experiment requirements.

6.9.1. Empty the food from calorimetry hopper into home cage food hopper.
6.9.2. Top up with extra diet if required.
6.9.3. Where applicable, return stuffers to their home cage.

6.10. Remove dirty cages and wipe cage lids with 70% ethanol.

6.11. Start the DCF by uploading the calorimetry data.

6.12. Perform data QC if applicable:

6.12.1. For the mouse requiring QC, click on the "view data" button.
6.12.2. Select rows and columns to be QC failed, (this could be a specific parameter at a specific time or all parameters for the entire experiment depending on the issue).
6.12.3. Write the appropriate failure comment in the box at the top right of the page, referring to the table in the "QC DURING PROCEDURE" section for approved QC fail comments to be used during procedures.
6.12.4. Click on the "save" button and the QC failed parameters should now appear in dark red (failed and saved).
6.12.5. Repeat this procedure for all the mice that need to be QC failed.

6.13. Print the new cage cards and save this data.

6.14. **All cages must display the updated cage card. Place a 'POST PROCEDURE CHECK REQUIRED' label on all cages and register them to the correct rack whilst returning them to their destination/home rack in the animal room. (Refer to SOP0101 – Taking and Returning Cages for Procedures).**

6.15. Send dirty cages and used water bottles for cleaning.

6.16. Clean food hoppers:

6.16.1. Wash food hoppers thoroughly with hot water, wipe with 70% ethanol and allow to dry before use in next experiment.

6.16.2. If no further experiments are to be setup for 72 hrs, soak the food hoppers in a cage base of 1% Trigene solution overnight (or for at least the entire afternoon if it is a Friday), rinse with water and allow to dry.

6.17. If no more experiments are to be run immediately, turn off all components of the TSE LabMaster System:

6.17.1. Switch off the air pump

6.17.2. Close the Ref. Gas bottle

6.17.3. Shut down the computer

6.17.4. Switch off all units.

6.18. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

TROUBLESHOOTING:

1. When starting the experiment (see step 3.19), an error message appears indicating that too much food was put in a food hopper. Remove a little food from the specified hopper and click "OK" to continue.

2. When starting the experiment (see step 3.19), an error message appears indicating that there is not enough food in a food hopper. Check the hopper to see if it is not stuck in the holder or touching the edges. If this is the case, reposition the hopper correctly and click "OK" to continue. If this is not the case, add some more food to the hopper, and click "OK" to continue.

APPENDIX 1: MONITORING AND OBSERVATIONS

Mice in calorimetry experiments should be monitored every day, last thing in the evening and first thing in the morning. Care should be taken to ensure all mice have access to food and water, any leaky water bottles seen during evening checks should be tightened if possible or replaced.

1. Under the VIEW tab: make sure that all the active cages are selected.

2. Measurements can be monitored during an experiment using the following options found:

   2.1. Under the STATUS tab:
   - 2.1.1. CALO – This opens the "Calo current values" box, displaying the O2 and CO2 values for the box selected, the flow, and temperature for all other boxes. When the "Calo current values" box is first opened, the box currently being measured will be displayed. The user can change which box is displayed using the + and - buttons at the top right of the window.
   - 2.1.2. ACTIVITY – When selected, a grid representing the light barriers for all activated cages is shown. An uninterrupted beam is shown in green, interrupted in red and disabled (via the setup menu before starting the experiment) in grey. Clicking counts in the lower left of this window will display numerical values for beam break counts in each cage.
   - 2.1.3. DRINK/FEED – This opens the "Drink/Feed current values" box displaying the current values given by the food sensor.
   - 2.1.4. TRIAL MONITOR – This shows the status of the feeding containers. Green areas represent the amount of food in the basket, red: the amount removed by the animal since the start of the measurement or since the last refill, and grey: the unused volume of the basket. A circle in the left of the field basket is also given in this field.

   2.2. Under the GRAPHS tab:
   - 2.2.1. This is the most useful presentation of data and can allow the user to view many parameters at one time and monitor the progress of the experiment. Only parameters selected during the setting of the new experiment (see step 3.17) will be shown.
   - 2.2.2. To view a graph, select the graph tab at the top of the screen. To move between cages in the graph screen, press Ctrl+B.

3. When checking mice at any time, special attention should be paid to ensure all mice have eaten enough in the last 24 hours (RER near or below 0.7 for prolonged periods (2 hrs+) is an indication of fasting) and RER above 0.7. If any abnormality is observed, remove the mouse from the metabolic cage, weigh and return to its home cage.

4. Refilling the food basket during an experiment:
   - 4.1. Select SET under the EVENT tab to open the "Store event markers" window.
   - 4.2. In this window, select the food sensor to be refilled using the second dropdown menu, or select "Refill All" if all baskets need filling.
   - 4.3. When the prompt to check the container appears, before pressing OK, lift the food basket from the cage by releasing the sensor platform from its holder in a gentle upward motion. The food basket is attached via chains to the underside of this platform. **TAKE CARE** – the food basket will sway if the platform is lifted at an angle or too fiercely and may be damaged.

4.4. Refill basket, lower the platform back into place and push to secure. Now press OK.
4.5. The program will wait until the sensor has become stable and will then record the new weight value.
4.6. Finally, the confirmation that the container has been refilled will appear.

5. If the calorimeter needs recalibration:
   5.1. Stop the measurement.
   5.2. Re-do the calibration (see step 2), paying attention to the flow rate value and gas concentrations (these need to be stable).
   5.3. Start a new experiment from scratch (do not select “start measurement”, but start a new experiment adding “_2” to the regular experiment’s file name (see step 3.14)).

6. If an event has occurred during the measuring time which may affect results (e.g. loud noise, change in room conditions), these should be recorded using the SET function under the EVENT. Note the time and any relevant comments in the calorimetry log book and calorimetry event register.

APPENDIX 2: PERFORMING A REMOTE CHECK

A remote check does not replace an ‘in-person’ check

1. Remotely connect to the PC.

2. Perform the Calo check as normal using the tabulated data to check the welfare of the mice paying close attention to the flow, RER, VCO2 and food intake parameters for each cage.
   2.1. If in doubt about the welfare of any animal, enter the RSF, discuss with a NACWO and take appropriate action as required.

3. Note the time and any relevant comments in the email template ‘Remote calo check performed’ and click send.
   3.1. The responsibility of noting the time and comments in the lab book falls to the person responsible for performing the subsequent day’s check.

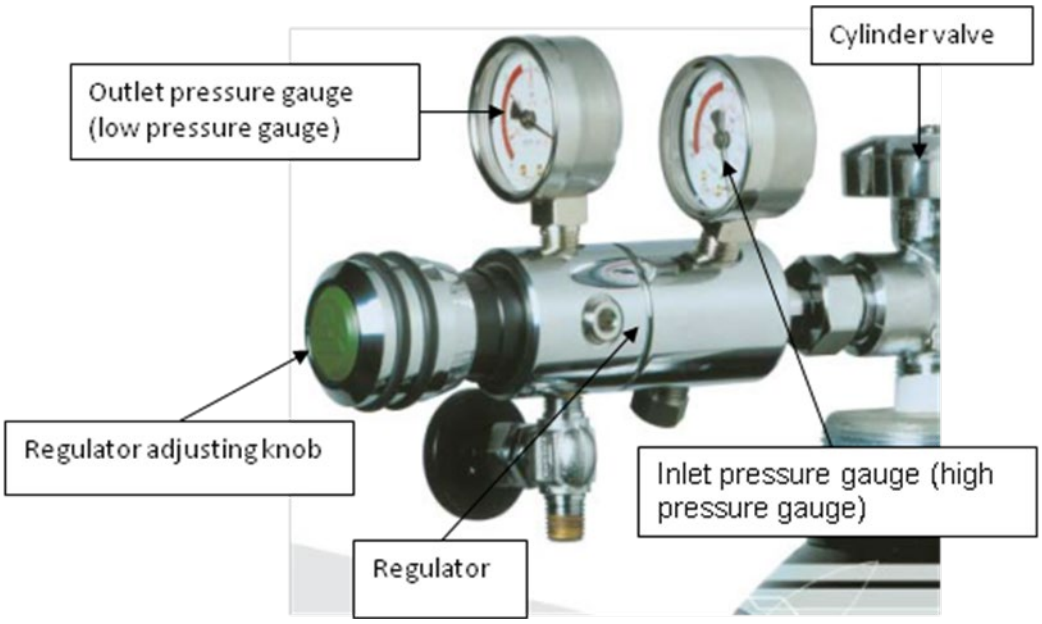
4. To finish the session, click on the cross of the new tab you can see at the centre of the screen. Take care not to close the TSE LabMaster software itself.

APPENDIX 3: OPEN/CLOSE GAS BOTTLES

1. Safety checking prior to using a gas bottle:
   1.1. **Always use safety spectacles.**
   1.2. Ensure the regulator adjusting knob is fully turned counter clockwise (i.e. Off).
   1.3. Stand at arm's length to the cylinder and where possible, with the cylinder valve between you and the regulator (if not possible, remain on the side of the regulator).
   1.4. Slowly open the cylinder valve and allow pressure to increase gradually into the regulator.
   1.5. Once the inlet pressure gauge (high pressure gauge) has levelled off, open the cylinder valve fully.

2. Adjusting the pressure:
   2.1. Turn the regulator adjusting knob clockwise to allow gas to pass through the equipment.
   2.2. Turn the adjusting knob to set your chosen (recommended) working outlet pressure by referring to the low pressure gauge.

3. Closing a gas bottle:
   3.1. Close the regulator by turning the adjusting knob counter clockwise fully (make sure it's not forced fully closed by turning the knob clockwise a little bit).
   3.2. Close the cylinder valve fully.
   3.3. Vent the gas from the regulator and system by turning the regulator adjusting knob clockwise until that both pressure gauges indicate 0.
   3.4. Close the regulator by turning the adjusting knob counter clockwise fully (make sure it's not forced fully closed by turning the knob clockwise a little bit).



APPENDIX 4: CHANGE OF A GAS BOTTLE

Before starting a new experiment, check if the pressure in the Ref. Gas or Span Gas bottles is approaching low (approximately below 30 bars). If you are unsure whether the bottles need changing, ask the staff member responsible for the calorimetry to have a look and make the decision

1. **Always use safety spectacles.**

2. Ensure that the gas bottle to be changed is closed (see Appendix 3: Open/Close gas bottles).

3. **Put safety gloves on when moving gas bottles**.

4. Remove the old bottle from the rack and unscrew the regulator from the bottle using a spanner.

5. Identify the next appropriate gas bottle according to the delivery date on the "calorimetry gas bottles" sheet and take it from the stock of gas bottles situated in the bottle rack.

6. Install the regulator on the new gas bottle and tighten it using a spanner.

7. Open the cylinder valve according to the safety procedures (see Appendix 3: Open/Close gas bottles section) and test the connection of the regulator on the cylinder for any leak using a leak detection fluid. If the bottle is not used for calibration or an experiment on the same day, close it according to the safety procedures (see Appendix 3: Open/Close gas bottle section above).

8. Place the bottle in its correct location next to the calorimeter.

9. Take the data sheets from the pocket attached to the bottle and enter the new gas set-points on the calorimeter control panel using the values written in the "actual value" column of the sheet:

    9.1. <u>For a "Ref. Gas" bottle:</u>
        9.1.1. Press the top softkey beside O_2.
        9.1.2. Select "calibration", if necessary type "111" for the code and press ENTER.
        9.1.3. Select "Setpoints for zero/span".
        9.1.4. In the "setpoint for zero", type in the reference gas value for O_2 and press ENTER.
        9.1.5. Press ESC until you're back to the main screen.
        9.1.6. Press the softkey directly beside CO_2.
        9.1.7. Select "calibration", if necessary type "111" for the code and press ENTER.
        9.1.8. Select "setpoints for zero/span".
        9.1.9. In the "setpoint for zero", type in the reference gas value for CO_2 and press ENTER.
        9.1.10. Press ESC until you're back to the main screen.

    9.2. <u>For a "Span Gas" bottle:</u>
        9.2.1. Press the top softkey beside O_2.
        9.2.2. Select "calibration", if necessary type "111" for the code and press ENTER.

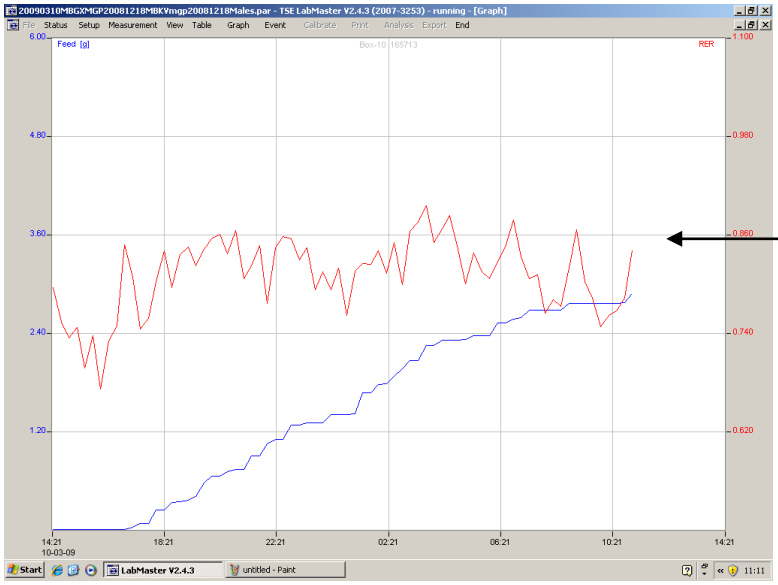
9.2.3. Select “setpoints for zero/span”.
9.2.4. Use ENTER to move the cursor from the top down to the second line (setpoint for MR1).
9.2.5. Type in the span gas value for O_2 and press ENTER.
9.2.6. Press ESC until you’re back to the main screen.
9.2.7. Press the softkey directly beside CO_2.
9.2.8. Select “calibration, if necessary type “111” for the code and press ENTER.
9.2.9. Select “setpoints for zero/span”.
9.2.10. Use ENTER to move the cursor from the top down to the second line (setpoint for MR4).
9.2.11. Type the span gas value for CO_2 and press ENTER.
9.2.12. Press ESC until you’re back to the main screen.

10. Let the staff member responsible for calorimetry know which bottle you have changed so a new one can be ordered immediately.

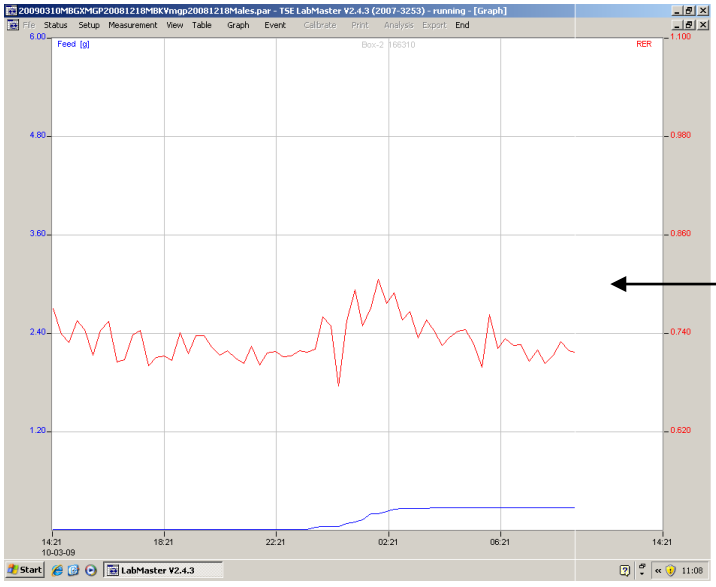
Indirect Calorimetery – Terminating a weekend procedure

Part A: AM Observations

1. Mice in calorimetry experiments should be monitored last thing in the evening and first thing in the morning. Care should be taken to ensure all mice have access to food and water by observing mice during the experiment. When entering the room for such checks, care must be taken to be quiet and lights remain dimmed.
2. Use the RER/FOODINTAKE graphs to check if mice are under fasting conditions. Mice under such conditions must be removed from their calorimetry cages and returned to their original home cage. Remove mice if:
    - Food intake is less than 1g or food intake has stopped for a period longer than 4 hours
    - **AND** RER is lower than/approaching 7.0.



Food intake good, RER good – Mouse to be left in calorimetry cage until end of procedure



Food intake below 1g **AND** stopped for prolonged period. RER low. – Mouse to be removed before end of procedure.

3. Each cage has its own graph, check each one. To move between cages in the graph screen, press Ctrl+B.
4. If mice are to be removed, make a note of these cage numbers in the Lab book located in front of the calorimetry computer. Follow steps 9 and 10 described below.

Part B: Stop experiment and export data.

5. Aim to record calorimetry measurement until 11.30am. At this time, end the experiment by selecting STOP under the Measurement tab.
6. Select ‘Table’ under the Export tab and save table onto the desktop (this will be the default location displayed automatically).
7. From the start menu located at the bottom left corner of the screen, open my computer and using the drop-down menu, select ‘DESKTOP’. From the desktop, copy (Right click mouse, select copy) all the files from the experiment run (3 in total). The file names for all 3 will begin with **yesterday’s** date, in the format ‘*yyyymmdd*’.
8. Using the drop-down menu again, select the team drive. Paste the 3 files copied earlier into this location. If more than one cohort is being run on the same day, save a copy into each cohort’s folder.

Part C: Remove mice.

9. Remove calorimetry cage lids by releasing metal braces across the top and lifting into the holding position using the hook inbuilt into the lids.
10. The ID of each mouse, its calorimetry cage number and home cage barcode is recorded on the log sheet. Using this information return each mouse to its original home cage, weighing at point of removal. Record this weight on the log sheet.
11. Remove and weigh all water bottles, recording this data on the log sheet.
12. Using a computer with internet and team drive access, log onto the MCMS database and upload the data files. If a mouse is a food cruncher (evident by recording food crumbs in the metabolic cage base) or appears to have not eaten during the experiment, select the check box to ignore Food Intake Data. Submit this data and print new cage cards which will now list this procedure.
13. Switch off all calorimetry units using the large button on the front of the UPS located below Rack 2. Switch off all sockets behind Rack 1. **CLOSE THE REFERENCE GAS BOTTLE** (located on the right of the cabinet) using the silver knob on the top of the bottle. The rear dial will reduce to zero when this bottle is closed, check this is done before leaving
14. Return cages to the animal holding room.

Guidelines for calorimetry checks

For time dependent criteria, apply these relative to “lights off” (7:30pm)

		QC action required					
Issue	**Welfare action required**	**Activity**	**Food**	**RER & gas**	**Water**	**Time points**	**QC comment**
Food intake <1g	Remove mouse	✔	✔	✔	✔	All	Welfare issue
No food intake for <4 hours AND food intake >1g	No action required^						
No food intake for ~4-8 hours AND food intake >1g	No action required^ (Consider the graph and only if necessary QC fail)	✔	✔	✔	✔	All	Erroneous data
No food intake for >8 hours AND food intake >1g	No action required^	✔	✔	✔	✔	All	Erroneous data
>2 hours of RER < 0.7	Remove mouse	✔	✔	✔	✔	All	Welfare issue
Food cruncher	No action required*+ If confirmed, QC the following data		✔			All	Erroneous data
Large food pellet fallen from hopper	No action required*+		✔			Affected time points	Erroneous data
Food hopper stuck AND food intake >1g	No action required		✔			All	Erroneous data
Gas issue	Is there a problem with the seal/kink in a tube/data entered –Yes; Resolve issue, no further action required			✔		Affected time points	Equipment error
	Is there a problem with the seal or a kink in a tube -No; Remove mouse	✔	✔	✔	✔	All	Equipment error/manual error/welfare issue
Any other issue	Seek advice from primary phenotyper responsible for test, a NACWO or a member of the senior management team	As advised					

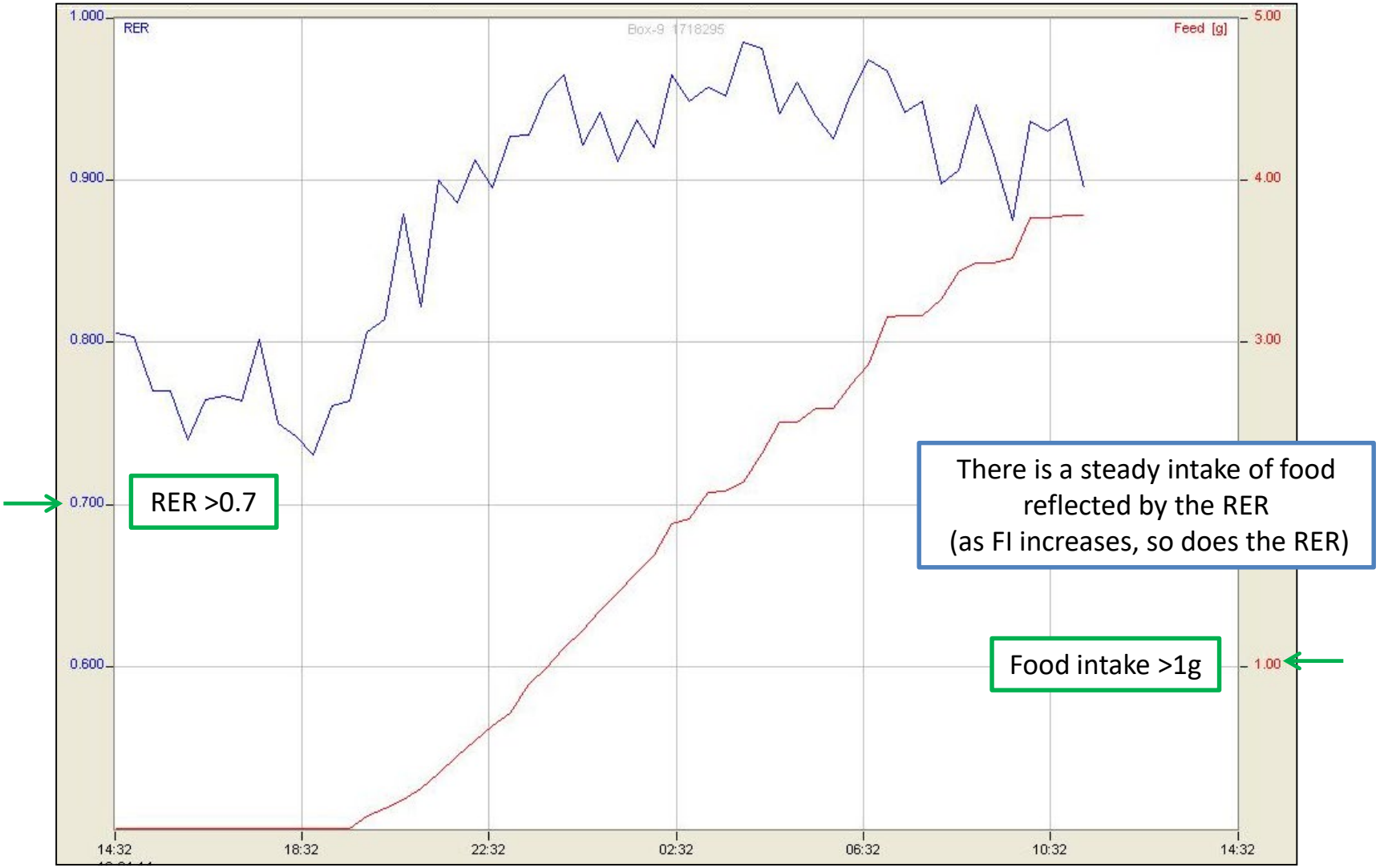
* for >24h experiment check there is sufficient food remaining.

+ QC data if food cruncher is confirmed upon checking of the cage base.

^ if the period of no FI suggests no access to food, ensure food hopper is not resting on the side of the holder.

Please record any observations on the worksheet, lab book and ‘calo event register’ if applicable.

“As expected” graph

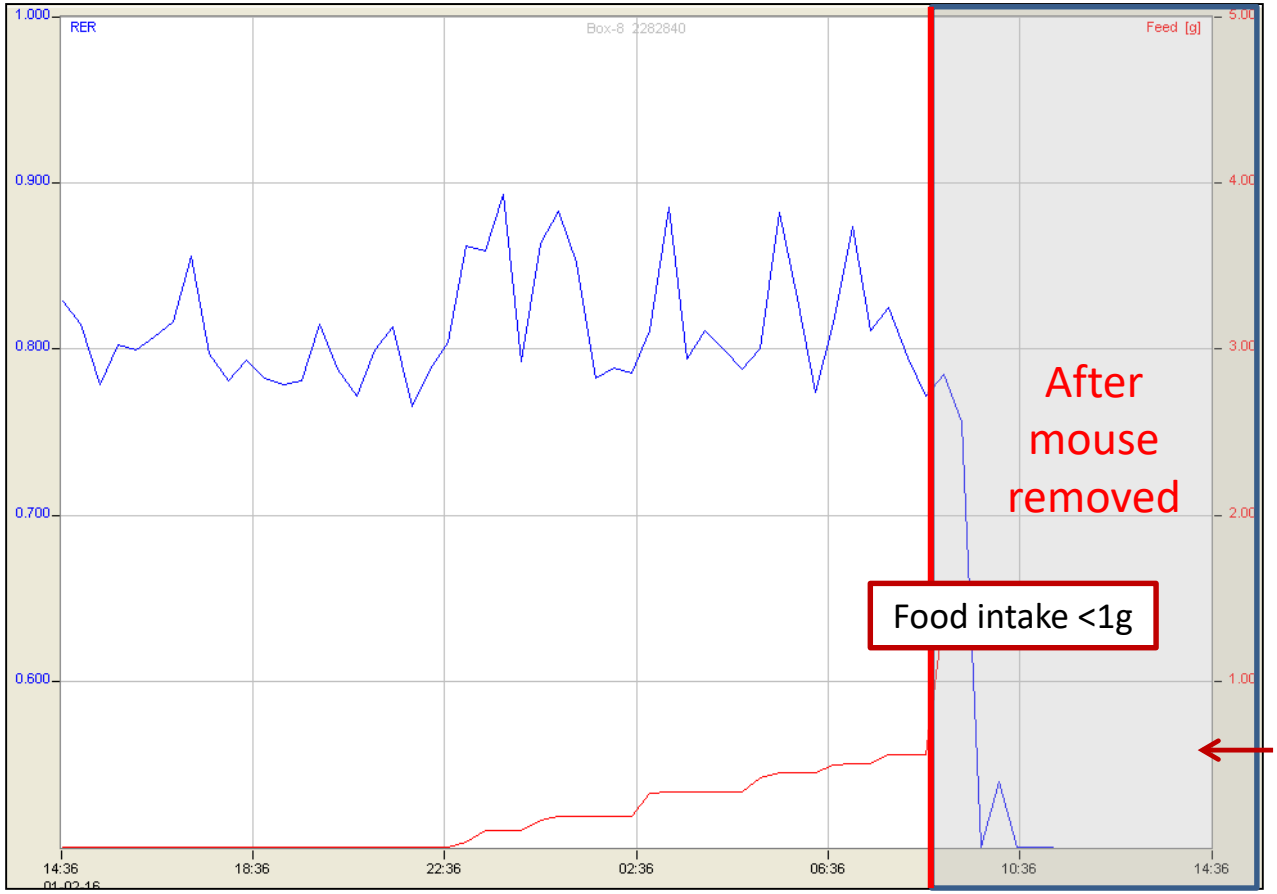


“As expected” table

Date	Time	Box-1															
		Flow	Temp	O2	CO2	dO2	dCO2	VO2(1)	VO2(2)	VO2(3)	VCO2(1)	VCO2(2)	VCO2(3)	RER	H(1)	H(2)	H
		[l/min]	[°C]	[%]	[%]	[%]	[%]	[ml/h/kg]	[ml/h/kg]	[ml/h]	[ml/h/kg]	[ml/h/kg]	[ml/h]		[kcal/h/kg]	[kcal/h/kg]	[kcal
02-11-10	14:36	0.45	21.0	20.46	0.385	0.462	0.342	4404	4389	133	3048	3037	92	0.692	20.728	20.656	0.6
	14:53	0.45	20.9	20.53	0.332	0.386	0.276	3701	3688	112	2468	2459	75	0.667	17.315	17.254	0.5
	15:10	0.45	20.8	20.48	0.371	0.441	0.317	4226	4211	128	2829	2819	86	0.669	19.783	19.714	0.5
	15:27	0.45	20.8	20.55	0.316	0.370	0.263	3553	3540	108	2345	2337	71	0.660	16.595	16.537	0.5
	15:44	0.45	20.8	20.49	0.361	0.429	0.307	4116	4101	125	2740	2730	83	0.666	19.251	19.184	0.5
	16:01	0.45	20.7	20.54	0.330	0.381	0.276	3639	3626	110	2458	2450	74	0.676	17.058	16.999	0.5
	16:18	0.45	20.7	20.54	0.327	0.386	0.275	3707	3694	112	2462	2453	75	0.664	17.333	17.273	0.5
	16:35	0.45	20.6	20.61	0.269	0.314	0.219	3029	3018	92	1960	1953	59	0.647	14.103	14.054	0.4
	16:52	0.45	20.6	20.62	0.256	0.302	0.207	2919	2908	88	1850	1843	56	0.634	13.548	13.501	0.4
	17:09	0.45	20.8	20.41	0.415	0.515	0.369	4957	4940	150	3265	3254	99	0.659	23.147	23.066	0.7
	17:26	0.45	20.7	20.57	0.295	0.354	0.245	3414	3402	103	2187	2180	66	0.641	15.872	15.817	0.4
	17:43	0.45	20.6	20.63	0.259	0.298	0.207	2878	2868	87	1846	1839	56	0.641	13.383	13.337	0.4
	18:00	0.45	20.5	20.63	0.249	0.292	0.197	2832	2822	86	1758	1752	53	0.621	13.104	13.058	0.3
	18:17	0.45	20.7	20.45	0.398	0.476	0.347	4554	4538	138	3103	3092	94	0.			
	18:34	0.45	20.8	20.51	0.368	0.415	0.316	3945	3931	120	2826	2816	86	0.			
	18:51	0.45	20.6	20.60	0.279	0.326	0.227	3146	3135	95	2027	2020	61	0.			
	19:08	0.45	20.7	20.55	0.342	0.375	0.292	3531	3519	107	2603	2594	79	0.737	16.794	16.735	0.5
	19:25	0.45	20.6	20.55	0.350	0.375	0.298	3525	3512	107	2661	2652	81	0.755	16.834	16.775	0.5
	19:42	0.45	20.7	20.43	0.459	0.490	0.407	4575	4559	139	3634	3621	110	0.794	22.048	21.971	0.6
	19:59	0.45	20.9	20.48	0.425	0.445	0.371	4140	4126	125	3311	3299	100	0.800	19.978	19.908	0.6
	20:16	0.45	20.8	20.50	0.397	0.420	0.344	3931	3918	119	3069	3058	93	0.781	18.888	18.822	0.5
	20:33	0.45	20.9	20.50	0.388	0.429	0.335	4054	4040	123	2993	2982	91	0.738	19.288	19.220	0.5
	20:50	0.45	20.8	20.53	0.378	0.397	0.325	3715	3702	113	2905	2895	88	0.782	17.855	17.793	0.5
	21:07	0.45	20.7	20.58	0.334	0.344	0.280	3215	3204	97	2500	2492	76	0.778	15.436	15.383	0.4
	21:24	0.45	20.9	20.49	0.421	0.437	0.370	4060	4046	123	3297	3286	100	0.812	19.649	19.580	0.5
	21:41	0.45	20.7	20.61	0.318	0.315	0.264	2932	2922	89	2349	2341	71	0.801	14.152	14.103	0.4
	21:58	0.45	21.0	20.50	0.412	0.421	0.359	3914	3900	119	3208	3197	97	0.820	18.972	18.905	0.5
	22:15	0.45	20.8	20.53	0.386	0.395	0.335	3678	3665	111	2993	2982	91	0.814	17.806	17.743	0.5
	22:32	0.45	20.6	20.62	0.304	0.303	0.251	2825	2815	86	2242	2234	68	0.794	13.613	13.565	0.4

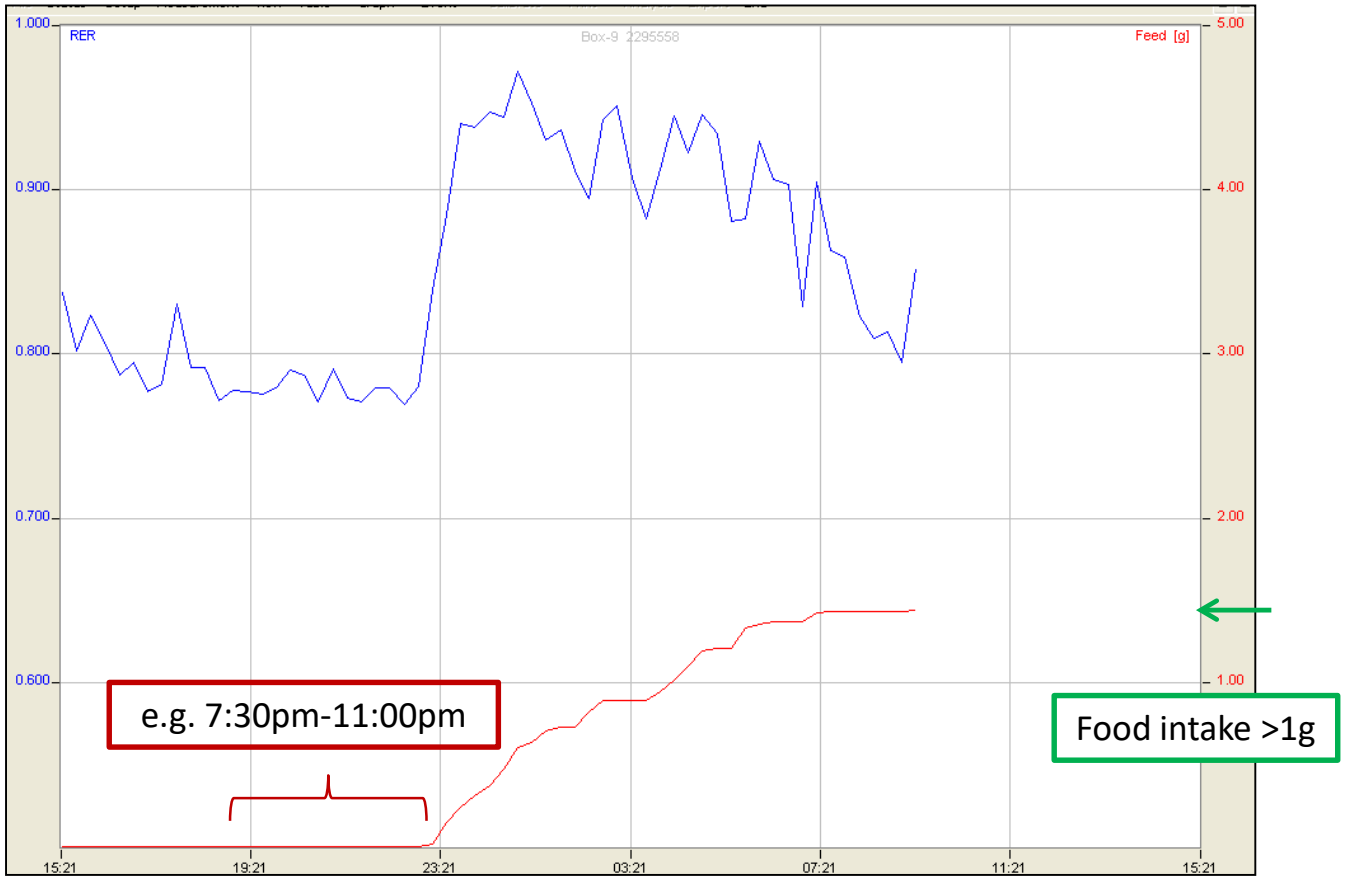
Please use the baseline values as a guide

Food intake <1g



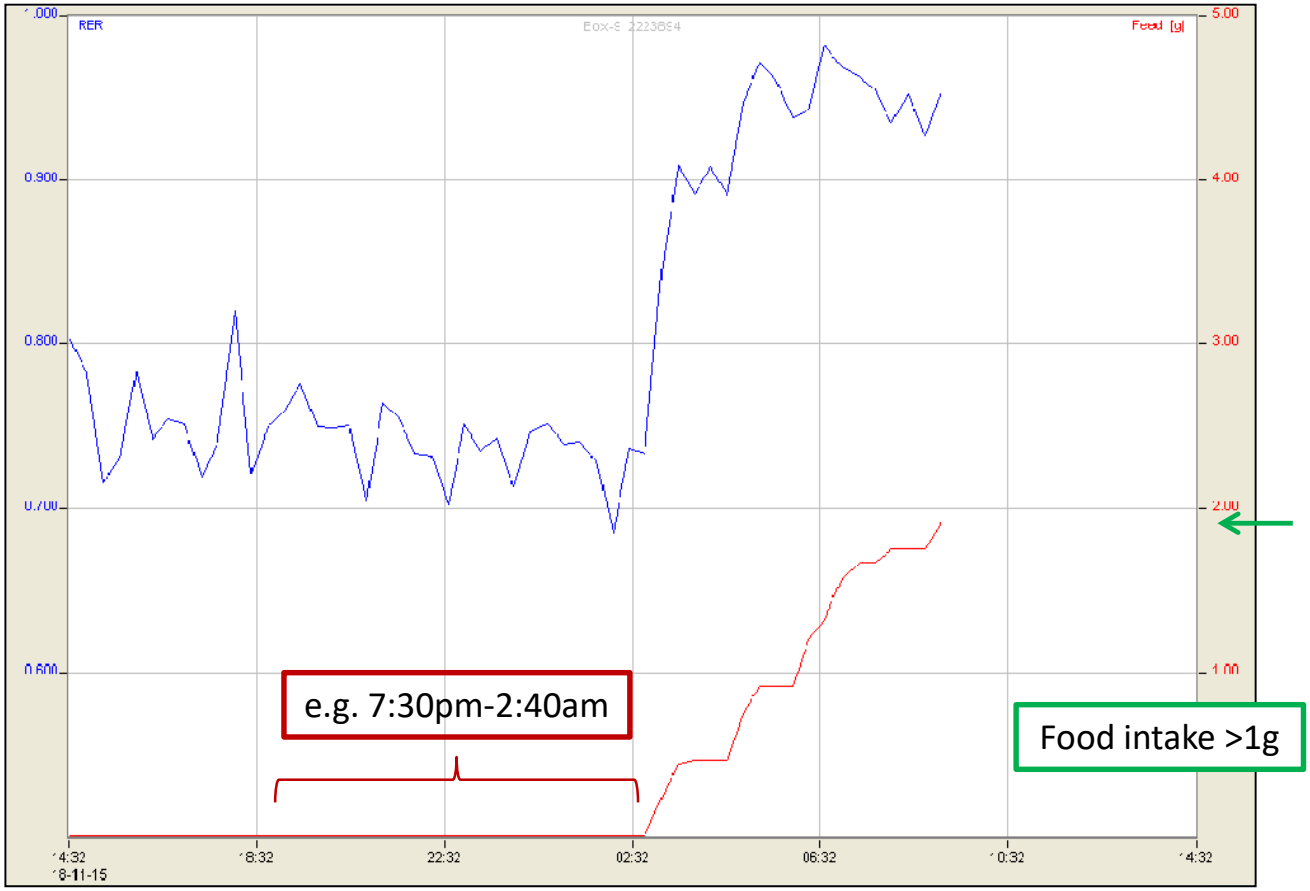
Issue	Action required	Activity	Food	Gas	Water	Weight (post exp. only)	Time points	QC comment
Food intake <1g	Remove mouse	✔	✔	✔	✔	✔	All	Welfare issue

No food intake for <4 hours, FI >1g



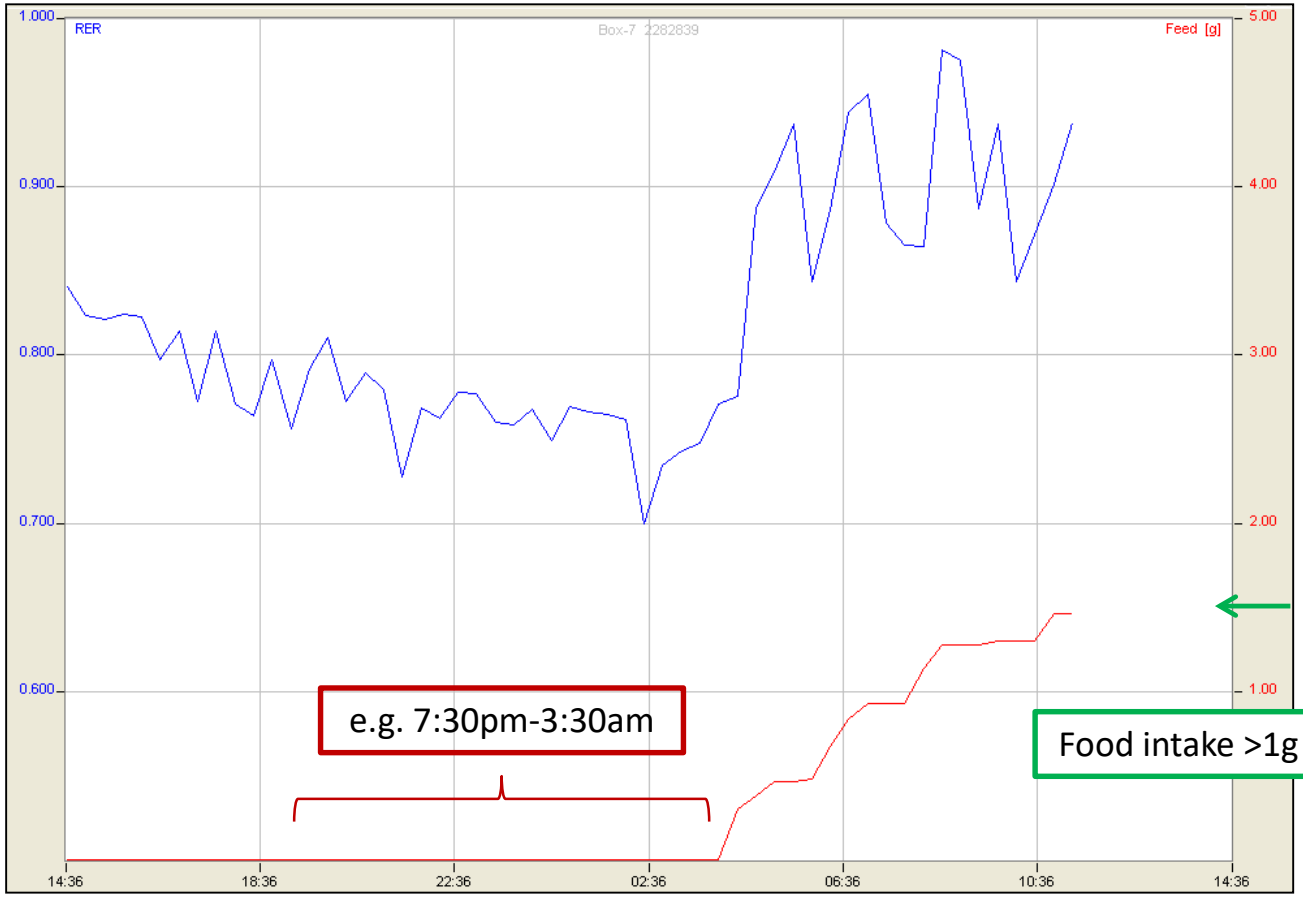
Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
No food intake for <4 hours AND food intake >1g	No action required						

No food intake for ~4-8 hours, FI >1g



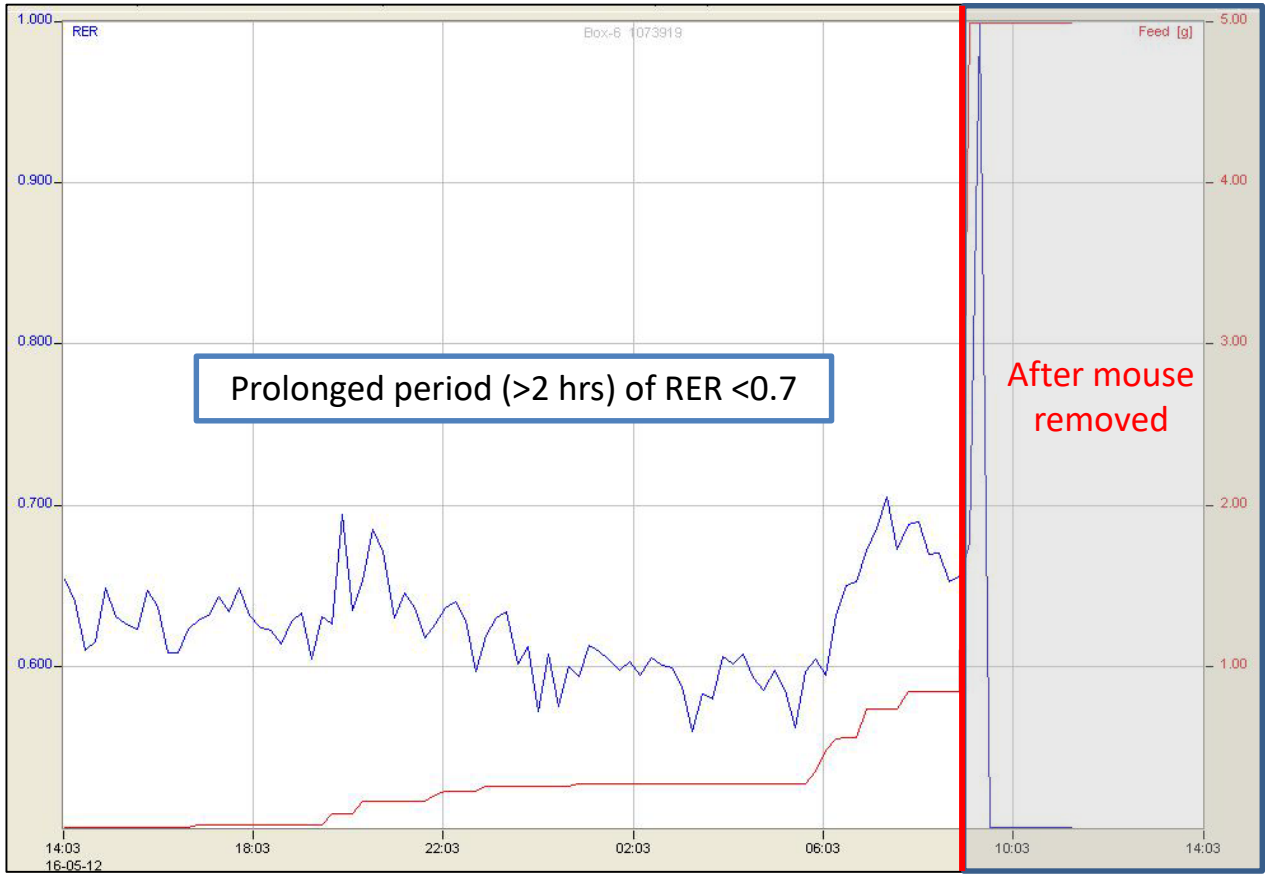
Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
No food intake for ~4-8 hours AND food intake >1g	No action required (Consider the graph and only if necessary QC fail)	✔	✔	✔	✔	All	Erroneous data

No food intake for >8 hours, FI >1g



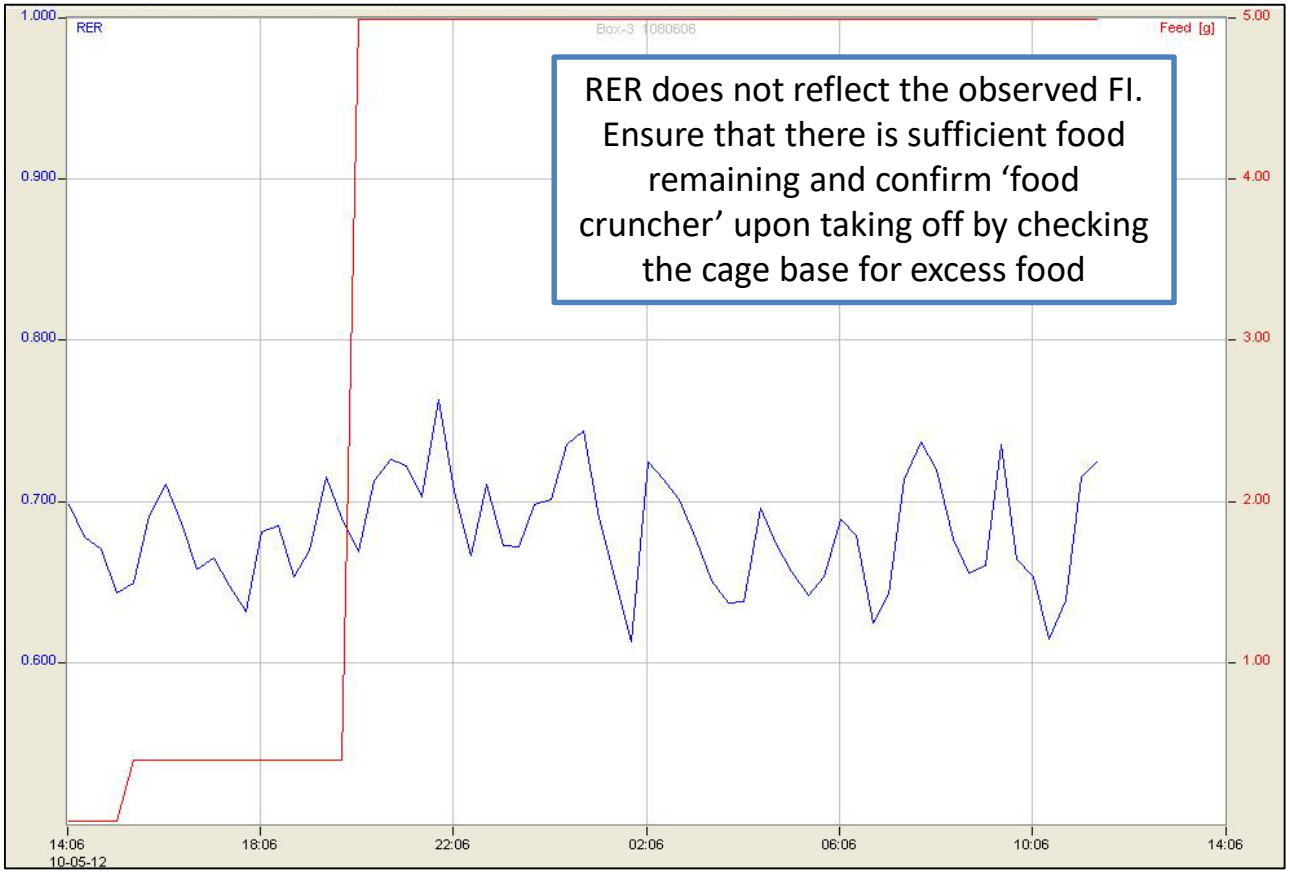
Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
No food intake for >8 hours AND food intake >1g	No action required	✔	✔	✔	✔	All	Welfare issue

RER below 0.7 for >2 hours



Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
>2 hours of RER <0.7	Remove mouse	✔	✔	✔	✔	All	Welfare issue

Food cruncher

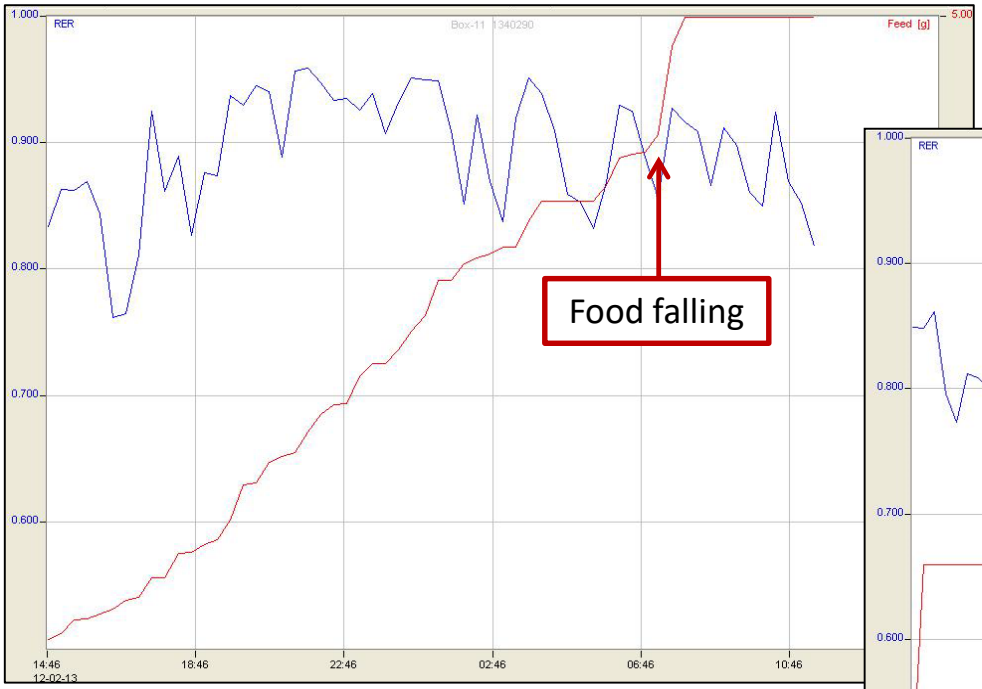


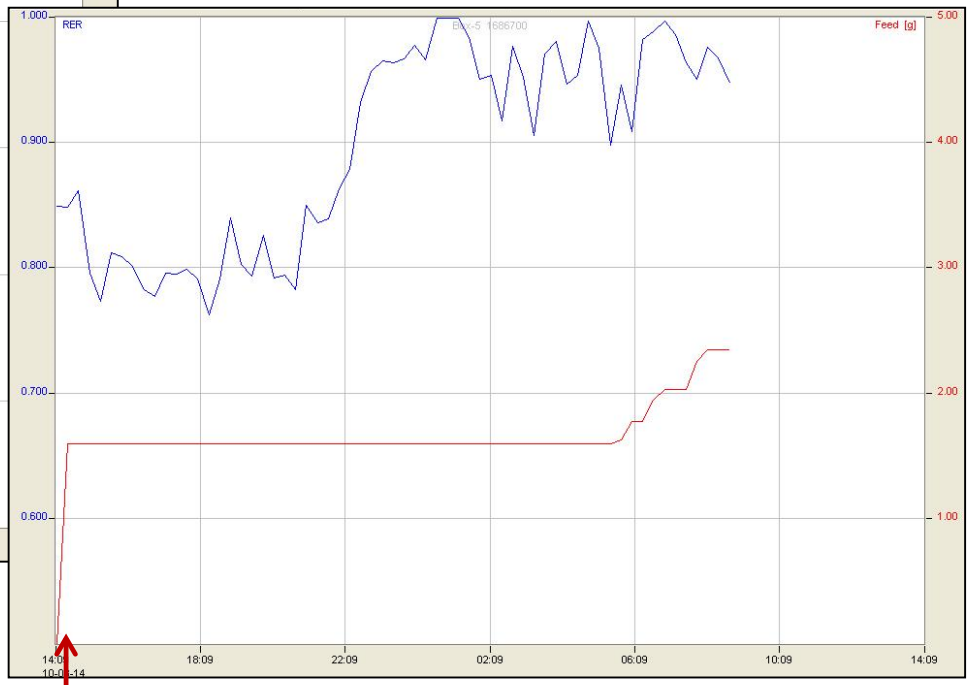
Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
Food cruncher	No action required*+		✔			All	Erroneous data

*for >24h experiment check there is sufficient food remaining.

+ QC data if food cruncher is confirmed upon checking of the cage base.

Large food pellet fallen from hopper





Sudden intake of food not reflected in the RER. RER remains steady as the mouse is eating from the cage base.

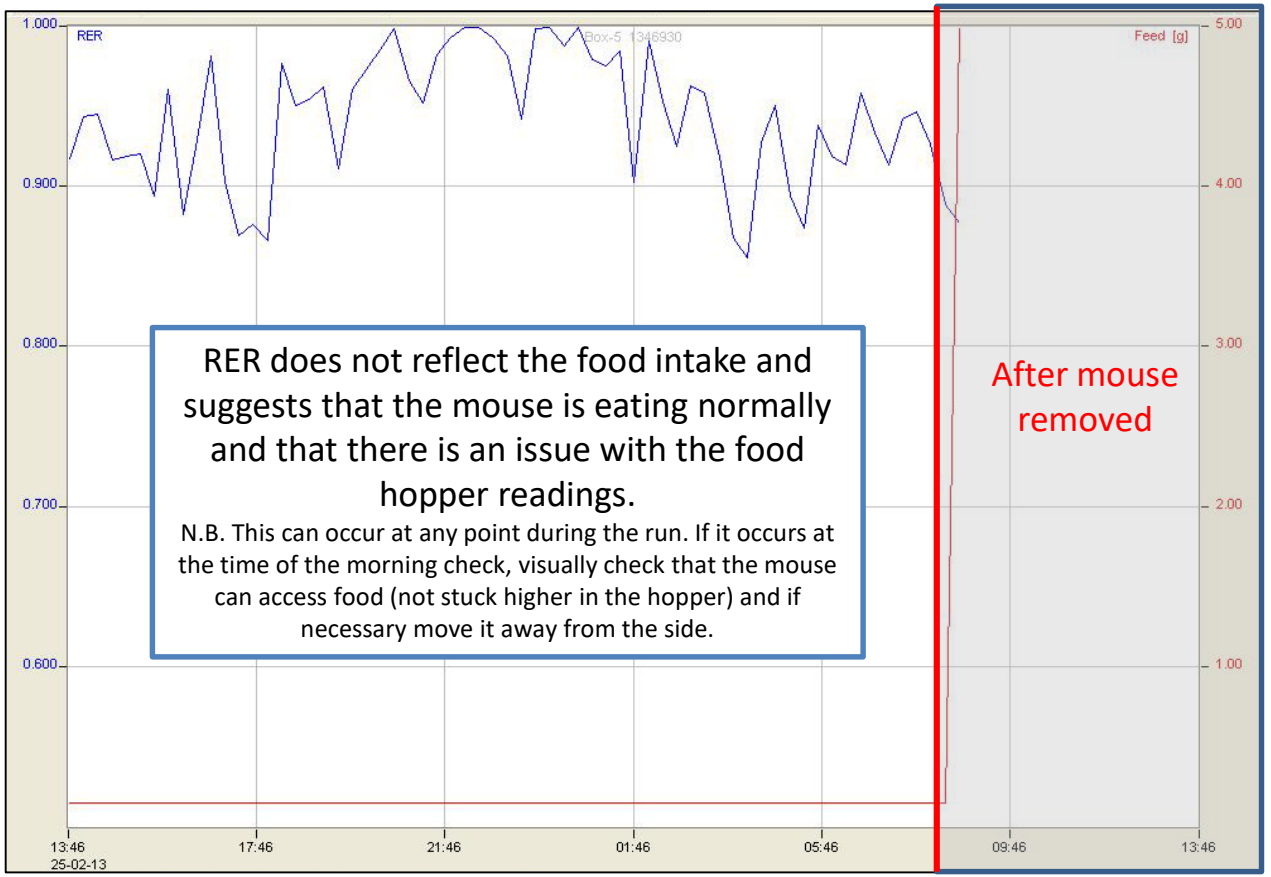
N.B. this can occur at any point during the run.

Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
Large food pellet fallen from hopper	No action required*+		✔			Affected time points	Erroneous data

*for >24h experiment check there is sufficient food remaining.

+ QC data if fallen food pellet is confirmed upon checking of the cage base.

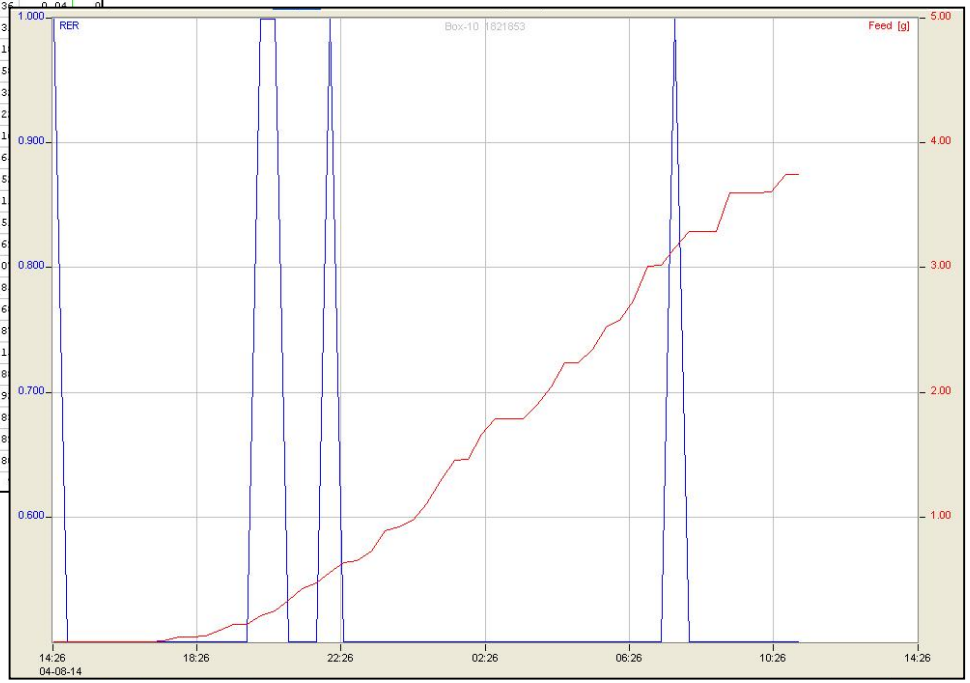
Food hopper stuck against/touching the side of holder



Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
Food hopper stuck AND food intake >1g	No action required^		✔			All	Erroneous data

Gas issue

Date	Time	VO2(1)	VO2(2)	VO2(3)	VCO2(1)	VCO2(2)	VCO2(3)	RER	H(1)	H(2)	H(3)	XT	XA	YT	YA	Feed	Bo F
							[ml/h]					[Cnts]	[Cnts]	[Cnts]	[Cnts]	[g]	[l/m
04-08-14	14:26	-246	-246	-6	4118	4102	103	-16.709	3.583	3.570	0.090	1654	1182	1078	749	0.04	0
	14:49	-838	-835	-21	3613	3600	90	-4.309	0.691	0.689	0.017	1420	997	1056	676	0.04	0
	15:12	-932	-928	-23	3395	3383	85	-3.644	0.083	0.083	0.002	815	543	626	342	0.04	0
	15:35	-748	-745	-19	3655	3642	91	-4.885	1.094	1.090	0.027	161	62	201	101	0.04	0
	15:58	-765	-763	-19	2789	2779	70	-3.644	0.069	0.068	0.002	35	15	38	15	0.04	0
	16:21	-727	-724	-18	2649	2639	66	-3.644	0.065	0.065	0.002	22	8	50	12	0.04	0
	16:44	-800	-797	-20	2917	2906	73	-3.644	0.072	0.071	0.002	542	290	472	23	0.04	0
	17:07	-727	-725	-18	2651	2641	66	-3.644	0.065	0.065	0.002	44	16	87	3		
	17:30	-647	-645	-16	2359	2350	59	-3.644	0.058	0.058	0.001	43	26	34	1		
	17:53	-730	-728	-18	2662	2652	67	-3.644	0.066	0.065	0.002	449	249	337	15		
	18:16	-844	-841	-21	3076	3065	77	-3.644	0.076	0.075	0.002	74	31	107	3		
	18:39	-785	-782	-20	2861	2850	72	-3.644	0.070	0.070	0.002	90	30	54	2		
	19:02	-762	-760	-19	2779	2769	69	-3.644	0.068	0.068	0.002	25	4	36	1		
	19:25	-757	-754	-19	2757	2747	69	-3.644	0.068	0.068	0.002	545	340	406	26		
	19:48	-582	-580	-15	3916	3901	98	-6.730	2.038	2.030	0.051	844	541	733	45		
	20:11	-958	-954	-24	3491	3478	87	-3.644	0.086	0.086	0.002	1417	1004	1112	71		
	20:34	-589	-587	-15	3883	3868	97	-6.590	1.972	1.965	0.049	987	656	711	45		
	20:57	-683	-681	-17	2489	2480	62	-3.644	0.061	0.061	0.002	1136	763	1030	66		
	21:20	-1025	-1021	-26	3735	3721	93	-3.644	0.092	0.091	0.002	653	433	490	30		
	21:43	-227	-226	-6	4484	4467	112	-19.760	4.065	4.050	0.102	1563	1118	1272	88		
	22:06	-1043	-1039	-26	4171	4155	104	-4.000	0.504	0.502	0.013	1950	1472	1617	116		
	22:29	-1066	-1062	-27	3884	3870	97	-3.644	0.095	0.095	0.002	1691	1246	1320	88		
	22:52	-1051	-1047	-26	3831	3817	96	-3.644	0.094	0.094	0.002	1554	1083	1325	91		
	23:15	-1031	-1027	-26	3756	3743	94	-3.644	0.092	0.092	0.002	720	440	531	28		
	23:38	-770	-767	-19	2805	2795	70	-3.644	0.069	0.069	0.002	206	142	153	9		
05-08-14	00:01	-897	-894	-22	3952	3937	99	-4.404	0.834	0.831	0.021	1463	1050	1273	88		
	00:24	-1048	-1044	-26	3820	3806	96	-3.644	0.094	0.094	0.002	1199	864	793	48		
	00:47	-856	-853	-21	3119	3107	78	-3.644	0.077	0.076	0.002	558	346	321	18		
	01:10	-817	-814	-20	2977	2966	74	-3.644	0.073	0.073	0.002	134	21	34			



Please use the baseline values as a guide

Issue	Action required	Activity	Food	Gas	Water	Time points	QC comment
Gas issue	Is there a problem with the seal/kink in a tube/data entered –Yes; Resolve issue, no further action required			✔		Affected time points	Equipment error
	Is there a problem with the seal or a kink in a tube -No; Remove mouse	✔	✔	✔	✔	All	Equipment error/manual error/welfare issue