

**Metabolism and Phenotyping Research
Platform Instructions
- From an User Standpoint -**

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4 Instructions

1. Introduction

The purpose of this document is to provide a hand to hand introduction method to the PhenoMaster/ LabMaster-Phenotyping Research Platform from TSE-Systems. It aims to provide simplified information and clues to set up and run an analysis. For more detailed information, users will be oriented to other referenced manual.

The TSE LabMaster System represents a modular research platform allowing metabolic and behavioural investigations. It combines a series of sensors and integrates a vast amount of information for each animal. It does allow an automated, non-invasive long-term simultaneous monitoring for a large numbers of animals (rats and mice) of physiological and behavioural parameters in a stress-free home cage environment. Those parameters are:

- Drinking and feeding (amounts and patterns).
- Spontaneous locomotors activities (Total, Fine and Ambulatory)
- Metabolic performance (O2 consumption, CO2 production, RQ)

Each of those parameters will be reviewed through the uses of the equipment for your experiments, the implementation (setup/calibration), point's limits, trick, and useful spreadsheet. It will be reviewed equipment to modulate the environment such as temperature and light schedule.

2. Principles of metabolic performance analysis

This document we will not attempt to provide a review of open-circuit indirect calorimetry methods, equipment, mathematical and physiological interpretation. Excellent reviews and articles provide such information (see **Reference** section).

Since the mid 90's, we are facing a large interest in understanding the physiological mechanisms regulating energy metabolism and food behaviours. Indirect calorimetry is the main way to investigate how compounds, regimen, genetics and environmental manipulation affects energy metabolism, thus energy consumption, body weight and animals composition.

In direct calorimetry, the heat or energy produced by a living organism is measured directly by placing the subject in a calorimeter and the amount of heat given is measured.

Complete Weir formula:

$$EE=[3,941(VO_2) +1,106(VCO_2)].1.44 -2,17 (UN)$$

Abbreviated Weir formula:

$$EE=[3,941(VO_2) +1,106(VCO_2)].1.44$$

Where VO_2 = Volume oxygen consumption (ml/h/kg)

VCO_2 = Volume Carbon dioxide production (ml/h/kg)

UN = Urinary nitrogen (g/h/kg)

EE = energy expenditure (kcal/h/kg)

Kg of either total body weight or lean body mass (recommended).

Tab 1: Indirect Calorimetry equations

Indirect calorimetry, by contrast, estimates energy production or expenditure (EE) from the organism's oxygen (O2) consumption and carbon dioxide (CO2) production.

Not only is indirect calorimetry easier to perform than direct calorimetry, but also, if both gases are monitored (and preferably if nitrogen excretion is measured as well), it allows a more reliable estimate of EE than O2 consumption alone. Energy expenditure can be estimated by various formulas made out of the Weir equation.

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$RQ = VCO_2 / VO_2$

1 ethanol + 6 O₂ → 4 CO₂ + H₂O

1 palmitate + 230 O₂ → 160 CO₂ + 16 H₂O

1 Amino Acide + 5,1 O₂ → 4,1 CO₂ + 2,8 H₂O + 0,7 Urea

1 glucose + 6 O₂ → 6 CO₂ + 6 H₂O

Substrate utilization (RQ)

Ethanol (only)	0,67
Fat Oxydation (only)	0,71
Protein oxydation (only)	0,82
Carbohydrate Oxydation (only)	1
Mixed substrate oxydation	0,85
Lipogenesis / overfeeding	1-1,2

Tab 2: Interpreting respiratory quotient (RQ)

It is possible to calculate the proportion of fat and carbohydrate fuels, extracted from the Respiratory Quotient (RQ) or Respiratory Exchange Rate (RER). The RQ is calculated by dividing the volume of CO₂ produced by the volume of O₂ consumed ($RQ = VCO_2 / VO_2$) on the assumption that there is no net inter-conversion of fuels within the organism, RQ provides valuable information about substrate utilization

Unlike humans' studies, resting energy metabolism (REE) is more complicate to evaluate with animals, as animals are unrestrained. Nevertheless, several approaches have been described in the literature and we will also make some suggestions.

3. Reference

- **Tschop M. et al. (2012).** A guide to analysis of mouse energy metabolism. *Nature Methods* **9(1): 57–63. *The most up-to-date guidance, authored by a consortium of leaders in the field. Explains the importance of analyzing data by covariance, rather than comparing mean values.***
- **Patrick. C. Even and Nachiket A. Nadkarni (2012).** Indirect Calorimetry in laboratory mice and rats: Principles, practical considerations, interpretation and perspectives. *Am. J. Physiol. Regul. Integr. Comp. Physiol* **303: R459-R476. *Exhaustive review and selective methods to how normalize the energy expenditure by the weigh the lean and fat mass.***
- **Kenneth A. et al. (2010).** The 24-hour respiratory quotient predicts energy intake and changes in body mass. *Am. J. Physiol. Regu. Integr. Comp. Physiol.* **298:R747-R754. *Tests acute effect of high fat diet. Describes an approach to adjusting data for weight loss.***
- **Andrew A. Butler and Leslie P. Kozak (2010).** A Recurring Problem With the Analysis of Energy Expenditure in Genetic Models Expressing Lean and Obese Phenotypes. *Diabetes* **59, February, pp 323-329. *Discusses the issue of whether to normalize metabolic values to total body weight or to lean mass.***
- **Bruss MD et al. (2010).** Calorie restriction increases fatty acid synthesis and whole body fat oxidation rates. *AmJ Physiol Endocrinol Metab.* **2010 Jan; 298(1):E108-16.** *Tests effects of calorie restriction on FA oxidation and FA synthesis with indirect open-circuit calorimeters*
- **JRS Arch, D Hislop, SJY Wang & JR Speakman (2006).** Some mathematical and technical issues in the measurement and interpretation of open-circuit indirect calorimetry in small animals. *International Journal of Obesity* **30:1322-1331.** *Discusses normalizing data's by analysis of covariance. Also explains the importance of air flow rate.*
- **Patrick C. Even, Asghar Mokhtarian and Agnes Pele (1994).** Practical Aspects of Indirect Calorimetry in Laboratory Animals. *Neuroscience and Biobehavioral Reviews, Vol. 18, No. 3, pp. 435-447.* *Operating principles of open-circuit calorimetry and discussion of principles for discriminating between maintenance metabolism and activity-related metabolism.*

4. Setting up an experiment

4.1. Chronology

An experiment can be schematically resumed by the following steps:

One week before

One day before

Start the measurement

Monitoring of the experiment

Stop the measurement

Export data

Analysing data

Running a complete and integrate calorimetric study including the measure of metabolic (EE and RQ), and behavioural (locomotion activities, drink and feed) performance in mice or rats need a precise planning. The following example is based on a **TSE Phenomaster/labmaster** system comprising:

- A calorimeter components used for recording automatically metabolic parameters of the animals in the CaloCages (O₂ consumption, CO₂ production, Respiratory exchange rate, total energy expenditure).
- Measurements of spontaneous locomotors activities using specific frames equipped with infrared light-beams in the basic plan (X or X,Y) and rearing (Z). Recording of the beam break will allow us to access total activity (XT+ YT), the fine movement (XF, YF) and ambulatory movement (XA, YA).
- 12 CaloCages with adapted lid to adjust 3 sensors and one temperature sensor.
- Dual sensors for drink and feed mounted on the lid of the cage.
- An automated food access.
- Environmental control climatic chamber housing the CaloCages for thermo-neutral conditions or temperature challenge experiment.
- O₂ and CO₂ High Speed sensors unit.
- One set of 2 calibrations gas bottles and on dry-air bottle

Each step will also imply preparing the material and calibrating the differences sensors.

In this example, we assume that the system is well mounted and that the users went through a training sessions with a TSE representative.

See PhenoMaster/ LabMaster – Software Operating Instructions - Basic modules.

See PhenoMaster/ LabMaster – Hardware Operating Instructions - Description of Standard Components.

4.2. A week before

- All animals should be individualised, as stress of separation may eventually modify their body composition. (*At the time of the experiment animals should have recover their initial body weight*)

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- Animals should be carefully weighted and if available their body composition monitored by a non invasive methods (Dual X-ray or EchoMRI) at this time and prior running the calorimetric analysis.
- During this period, animals would be housed in CaloCage. It is recommended that the animals are trained on the special drinking nipple before the first test is carried out. This can be done by using a training bottle equipped with the special nipple that can be inserted in the feed rack of the standard home cage or the CaloCage.



4.3 A day before

- The system of calorimetry must be switch ON to warm up all the following module:
 - Air pump
 - Calorimetry Process control Master and Slave unit
 - Air Drying unit.
 - High Speed Sensor Unit.
 - Sample switch unit.
 - MoTiI2 process control unit.
 - The computer
 - Click the icon of the appropriate software; LabMaster (rat) or LabMaster (mice). (See section **Launch a program**)

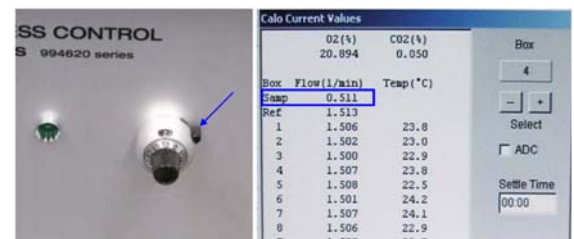


A green light is displayed when the module are switch ON.
(See section **Tips** for the connection)

See PhenoMaster/ LabMaster – Hardware Operating Instructions – calorimetry measurements-P40.

The regulation knob on the front panel of the Mass Flow Controller unit is used for setting the sampling flow toward the O₂ and CO₂ sensors. It should be set between 0.35 and 0,5 L/min.

- The sample flow set on the Mass Flow Controller knob can be read from the calorimetry status (**Status/ Calo**) menu in the line “**Samp**”.
- Open the dry air bottle connected to the High Speed Sensor Unit. (4-5 bar).



See PhenoMaster/ LabMaster – Operating Instructions – O₂ & CO₂ Measuring Module.

- Connect the Sample In/Sample out connections to the appropriate reference cage.
- Empty the glass bottle beneath the Air Drying Unit.

If an Environmental Control Cabinet or climatic chamber for CaloCages is available. Switch ON the Climatic Chamber, (See section **Environmental Control Cabinet**).

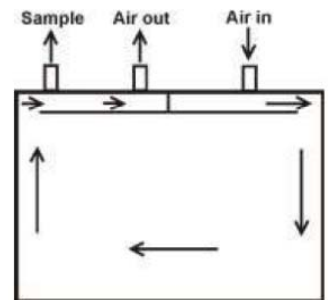
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- Set the adjustable thermostat protection for low and high temperature (black Knob).
- Set the appropriate light intensity and the day and night cycles.

Note → Environmental Control Cabinet

Once the doors of the climatic chamber are closed, the compressor should be heard as the compressor is working to reach the appropriate temperature.

- Hold all the cover to the holding hook (A cover holder is provided for each cage to hold the cover with a holding hook).
- Connect all Sample In/Sample Out to the cover.
- Choose the type of feeding basket and drinking bottle (See section **Drinking & Feeding**) and hang it.
 - If calibration has to be done, the drinking bottle and/or the food basket should be empty (See section **Calibration**).
 - If calibration were done previously, the food basket and the drink basket can be filled before the entrance of the animals.
- Connect the temperature sensor to each cover (*The temperature sensor is equipped with a bite protector to stop it from being gnawed by the animal. Always use the cages with the bite protector in position*).
- The temperature can read off from the calorimetry status (**Status/Calo**) menu in the column Temp (C) +/-0,1.
- If not set, connect the activity frames.



See PhenoMaster/ LabMaster – Hardware Operating Instructions – Activity measurements via sensor frames – P65

- Adapt each frame to the size of the animals:
 - For mice/rats X/Y frames screws at the level of the fourth hole.
 - The Z frames at the eighth hole for mice and the fourth hole from the top for the rats.
- Insert the black frames.

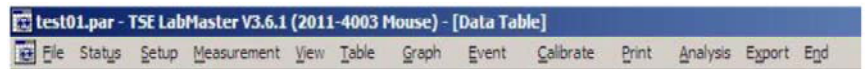
4.4. The day of experiment

- We recommend that they are being scanned via an EchoMRI or a dual x-ray (DEXA) to evaluate their lean body mass or metabolic active mass.
- All bottles and food baskets should be filled.
- CaloCages should be installed with animals with new bedding, beware of the amount of beddings and avoid adding nesting materials cellulose-based since it could interfere either with food and drink basket or the activity frames.
- Removed all possible objects (cables) between the cages and the frames which could interfere with the activity reading.
- Undertake carefully the air calibration (See section **Calibration**). Once the air calibration is done, make sure that calibration bottles are well closed and that the connecting tube out of the air drying unit is reconnected.

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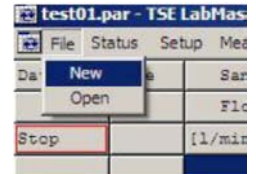
Launch LabMaster

Launch the appropriate software and then follow the following sequence.



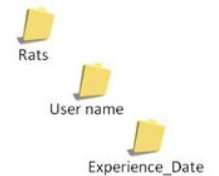
See PhenoMaster/ LabMaster – Software Operating Instructions - Basic modules – P18

A new file need to be created (names of the “*Experiment_Date*”; e.g. ObObfast_01012013; and save it to your folder).



4.4.1. Under Setup (initial file) / Experiment

- Filled the blank space with the name of the user, name of the experiment, type of animals, the date.
- Filled the Runtime [hours] Entry in hours for Auto-Stop. The measurement can be stopped at any time before, **set 240h**.
- Filled the Sample Interval [min], (it will depends of the number of cages being used + the cage reference, the O2/CO2 module, the distance of the CaloCage to the module). The time per Box (CaloCage) will be automatically calculated. (See section **Tips**).
- CheckBoxes - Activity, Calorimetry, Drink/Feed/Body Weight, Time Manager, Paired Feeding, etc... Checkboxes for activating the required measurements.



4.4.2. Under Setup (initial file) / Boxes

- Enable the number of cages being used (**Y**) or enable it (**N**).
- Filled the animal number.
- Enter the weight (it is needed to calculate the Heat production/Energy expenditure or H(1) in [kcal/h/kg body weight]).
- Filled the blank (text 1,2,3) for maximum information.

Note → Measurement activation

Only measured values from enabled checkboxes (e.g. activity, calorimetry) can be registered. If a checkbox has not been selected then the corresponding tab will not be available in the Setup menu nor the corresponding columns in the table nor the corresponding parameters in the graphics presentation.

4.4.3 Under Setup (initial file) / Activity

- Check that the sensors activity are well functioning.
- If the animals are already in their cage, red square will show under each axes (X,Y,Z).
- **Scan Rate**; Select Scan Rate $\leq 100\text{Hz}$.
- **Write binary file**; Check to register additional binary file (*.bin) that contains detailed information about the individual sensors.
- Recording interval (1-60min), applies if **Write binary file** is checked.
- Write **Raw data file**.
- **Refractory period [sec]**, Set at 1 sec.
- Select center area depending of the CaloCage size, i.e.
 - Center Area X1-X2 : 6 and 11 for mice.
 - Center area Y1-Y2 : 3 and 6 for mice.
- Enable Light beam for X, Y and Z, tick green boxes.

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See PhenoMaster/ LabMaster – Software Operating Instructions – Activity-P6

See PhenoMaster/ LabMaster – Software Operating Instructions – Activity-P14

4.4.4. Under Setup (initial file) / Calo

Energy expenditure is expressed by kcal/h, then it will be given expressed by body weight (H1), lean body weight (lean body mass = weight exponential 0.75) (H2), or by box (H3).

- Do not use the continuous mode (only 1 box), which can be used when a treadmill is connected as the first CaloCage
- **-CAUTION-** Set the appropriate air flow [L/min]. It should be set between **0.45** up to **0.6** or **1** up to **1.6** Litre/min respectively for mice and rats, depending on the average body weight of the experimental group. (See section **Tips**)
- Tick storage of binary files

See PhenoMaster/LabMaster – Software Operating Instructions – Calorimetry, section Setup, Calo (calorimetry)- P10

4.4.5. Under Setup (initial file) / Drink/Fed

- Enable the function standing on the left side.
- Set the smoothing ADC/ moving average of 5-120s, e.g. **10s**
- Max. Delta ADC (1-999): interval of valid delta within the time previously set, e.g. **40**
- Observation interval for Trial Monitor (Drink/fed), e.g. **10s**
- Tick only positive consumption (consumption is always defined as positive).
- Confirm each sensor at start (Optional).
- Start boxes separately (Optional), if checked then the drinking and feeding data acquisition for the boxes should be started separately after the start of the measurement itself.
- Check for **“Write binary data files”**.
- Min. Consumption (0.01-1): set 0.05.
- Max. Consumption (0.1-20): set to 0.5.
- Used sensors, check appropriate need.

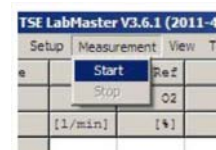
See PhenoMaster/ LabMaster – Software operating Instructions. Drinking / Feeding / Body Weight – P15

4.4.6. Under Setup (initial file) / Feeder

Set “feeders” to drinking/feeding sensors (of use when setting up a pair feeding experiments), set to feed in our case.

4.5. Start the measurement

Go through the Checklist, (See section **Appendix-Checklist**), once all steps are being fulfilled. Click **Measurement / Start**. (Alterations in the Setup tabs are no longer possible if a measured value has already been registered, i.e. if a line has been written in the table).



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4.6. Monitoring of the experiment

To control the good development of the experiment, open the **Status/Calo** and **Status/Trial drink and feed monitor**.

4.6.1. Status / Trial Monitor Drink / Feed

See **PhenoMaster/ LabMaster – Software operating Instructions. Drinking / Feeding / Body Weight - Trial Monitor Drink / Feed – P10**

In this panel, the user controls the level of food and water. We recommend paying particular attention at the level of drink intake as a principle marker of animals well being. Compare to other animals, a lower drink intake might be interpreting either by an alteration of the well being of the animals and/or the bottle being obstructed.

- Feed and drink might be refilled in this panel.
- Right Click on the wanted cage
- Click to **Pause boxes**

Note → No registration during “pause” (Yellow tag)

*Not only the drinking and feeding measured values will cease to be registered, **all** other measured values (e.g. activity data) will no longer be registered as well.*

- Refilled bottles or baskets
- Click to **Continue boxes** and the filling process is confirmed by pressing OK

Note → Caution

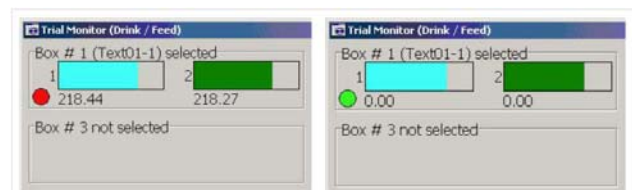
Do not stop another box if the previous one is not properly back to normal (Green buttons). If not, fault measurement might occur and measurement of the ongoing boxes being stopped.

4.6.2. Status / Calo

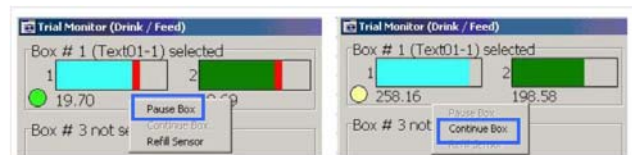
In this panel, user can read the current values of %O₂ and %CO₂ being measured, which cages is being measured, the air flow in (Box ref, 1, 2, 3....) and the sample out (Samp).

- “Samp” airflow must be lower or equal than other airflow and must correspond to the value set to the knob on the control unit.
- Reference and box airflow should be all equal and as set in the Setup/Calo/Airflow.
- % O₂ and %CO₂ should match with the value displayed on the O₂/CO₂ sensor Unit.
- On the **Table**, Ref %O₂ and %CO₂ should be equal to O₂ =20,89 +/-0.01, CO₂ = 0,045 +/- 0.005, depending of air pressure, presence in the room, temperature etc...

See **PhenoMaster/LabMaster – Software Operating Instructions – Calorimetry, section Setup, Calo (calorimetry) –P6**



Trial Monitor (Drink/Feed) window – Prior to or after start



Trial Monitor (Drink/Feed) window – Context menus

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4.6.3. Parameters to observe on the Table

- Click to **View** and set on the **Select View Parameters** panel the following option
 - Select boxes/ Set all
 - H(3)
 - RER
 - Drink
 - Feed
 - dO₂ and dCO₂
- Check that RER/RQ oscillate between 0,7 (Fasted animals, high fat diet, daytime) and 1 - 1,1 (normal chow diet, night time and high lipogenesis).
- An appropriate air flow can be seen if the dO₂ and dCO₂ oscillate between 0,3 and 0,7 with an absolute % of CO₂ < to 1%.
- Check that the animals are drinking normally.

Note → Caution

Errors can occurs when the on sample flow In/out is badly connected, it can be seen at the H(3) values, dO₂ and dCO₂ or if the cover cages is improperly positioned.

Note → Caution

If an animal is not drinking normally (low water and food intake coupled with a RQ around 0,75), we recommend checking if the nipple is not blocked or change the bottle or ultimately add a cup with water. In the event the animal continues to not drink, remove it from the study.

4.7. Stop the measurement

- At the end of the experiments: Click **Measurements/ Stop**. Airflow is still ON, but the measurements stopped, no parameters are being recorded.
- At this time, animals can be removed out of their CaloCages and the material removed to be cleaned.

See PhenoMaster/ LabMaster – Cleaning and Decontamination Instructions.

- Hook all cover.
- Remove the animals, we recommend that all animals should be weighed and scanned for their body weight composition (EchoMRI/ DEXA).
- Disconnect all Samples in/out.
- Removed food and drink baskets.
- **-CAUTION-** Laid the sensors on their side not on their hook.
- Empty the bottle below the air-drying unit
- Stopped the software (End/ Exit Program? / Yes)
- In the case that no other experiment are being run within the next 48h, we recommend that you switch off all equipment and close the compressed dry air bottle

Note → Data storage

Even if data have not been exported, they are safely stored in the set folders and can be retrieved and exported once the software and the protocol (Experiment_Date .par) are being re opened.

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See PhenoMaster/LabMaster – Software Operating Instructions – Basic Modules, section Selective storage of measuring data and Data export –P46-P49

4.8. Export the data

The Table menu can be used to export the measuring data that can be seen in the table view

- Under View/ Select View Parameters, select all boxes, set the desired parameters: i.e. XT +YT, Z, CenA, CenF, H(3), VCO2(3), VO2(3), Drink, Feed.
- Depending of the matrices of analysis select the type of format.

Note → Export Table

Format 1 - In the export file the boxes (= animal cages) are listed one below the other.

Format 2 - In the export file the boxes (= animal cages) are listed side by side.

Format 3 - In the export file the boxes (= animal cages), the measured parameters and the measured values are listed one after the other in one line.

- In the file type dropdown field a selection can be made between two export formats, save under *Experiment_Date .csv or txt* files types.
 - *.csv: These tables are read in directly by MS Excel without any inquiry.
 - *.txt: Text files with tabulator characters as separators for other programs.
 - Activity and drinking-feeding data recorded at smaller time intervals (the tabs labeled “Act” and “DFT”)

4.9. Analysing data

Under a tabulator (i.e.: excel) we recommend separating each parameters and subsequently separate each groups.

First we recommend graphing activity (Total activity) with respect to time. Different strains may display slightly different circadian rhythms, so we recommend first graphing the raw activity data vs time to see what the curve looks like for your animals. That will help you choose time intervals to analyze. Activity and feeding are typically higher at night, with the mice resting during the day. Many mice show peaks of activity at dawn and dusk.

- The most sensitive energy expenditure readings are obtained when activity (XT) is lowest.
- Apply basic statistic to extract the mean +/-SEM or SD per groups to create graphic representation.
- Reorganize values to performed statistical analysis.

The parameters to take account are multiple and depend mainly of the design of the experiment, however, it is recommended to take into account the following variables for each parameter:

- Time (per hour, per 12h, day/light schedule) for each parameter.
- Body weight and/or lean body weight for the expression of energy expenditure, O2 consumption, CO2 production and food intake.
- Days, as the first 24h transcribes the behaviour of the animal placed in a new environment for all parameters and each day display variances.

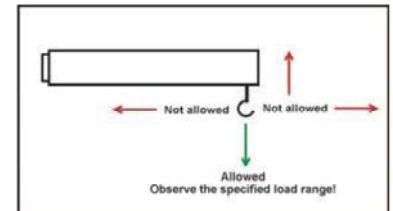
Several reviews discuss how to approach and analyze the parameters extracted from indirect calorimetric measurement (See Section **Reference**).

5. Drinking & Feeding

See PhenoMaster/ LabMaster – Hardware operating Instructions. Description of Standard Components - Drinking & Feeding measurements P78.

See PhenoMaster/ LabMaster – Software operating Instructions. Drinking / Feeding / Body Weight

First, we would advise to go through the following sections (PhenoMaster/ LabMaster – Phenotyping Research Platform. Hardware Operating Instruction – Drinking & Feeding measurements (p 78). From the point of view of the user, high caution should be apply in the handling of the sensors, hooks are highly sensitive and should be stored carefully.



Caution

-Make sure at each experiments that the right cable is well screwed to his sensors and the cable to the drink/fed modules (This verification should be done once). It is recommended that you mark the sensors and the sensor cable with corresponding numbers.

-When using the **drink/fed modules** it is recommended that you use odd numbers for one sensor type, e.g. feeding sensors, and even numbers for the other type, e.g. drinking sensors.

- Make sure that the bottle have a little hole on the cap to allow the water to run.

TSE-Systems provides two sets of feeding and drinking baskets, some are container and glass bottles with protection cap and the other is the recently designed feeding container with food hoppers beneath which allow collection of spillage.

We would strongly advise to use the latest development as it will reduce the variability between animals regarding their food behaviours,

especially when animals are fed with a palatable and friable diet and/or if the measurements are being made in the Urine/Faeces modules (sack of food contamination of the urine and faeces). Also glass bottles are subject to breakage during washing but also when being use with rats.

With mice, in the case you are using of the feeding baskets with no closing tops. We recommend blocking the access of the basket by adding an object forbidding the mice to rest in top of the basket.

Depending of your **PhenoMaster/LabMaster** version, several combinations can be made, e.g. two food baskets and one drinking bottle, and combined the drinking access unit/ food access unit to allow paired feeding measurements. We would recommend going through the following sections:



PhenoMaster/ LabMaster – Phenotyping Research Platform. Hardware Operating Instruction – Paired feeding measurements (p 92).

See PhenoMaster/ LabMaster – Software Operating Instruction. Drinking / Feeding / Body Weight- Drink/feed/Urine/Body Weight (p15).

Several protocols can be made; nevertheless, we will recommend performing always a trial test to access that the experiment is running as you wish:

- Duration of food/drink access
- Order of the events,
- Cables not connected
- Height of the food baskets

6. Activity measurements via sensor frames

See PhenoMaster/ LabMaster – Hardware Operating Instructions – Activity measurements via sensor frames.

See PhenoMaster/ LabMaster – Software Operating Instructions – Activity

We would recommend reading through the sections cited above. The activity system (MoTiI2 Process control Unit) uses a separate process control unit. The number of connectors for the sensor frames is custom-configured; in the extended system configuration a maximum of 16 frames can be connected to one process control unit.

The frames are available in different configurations which differ in both the total number of light barriers as well as in the distance of the light barriers from each other. The system shown above is provided with light-barriers in the X/Y and Z level.

Each single light barrier consists of one IR emitter and one IR receiver.

Adapt each frame to the size of the CaloCages:

- For mice/rats X/Y frames screws at the level of the fourth hole.
- The Z frames at the eighth hole for mice and the fourth hole from the top for the rats.

Special Inserts (templates) are provided for centring either mouse or rat CaloCages in the frame construction; these are inserted in the frames.



See PhenoMaster/ LabMaster – Software Operating Instructions – Activity-P14

Check that the sensors activity are well functioning or if there is connection problem.

- Open Status/activity. Status > Activity is provided for checking the functions of the light beams. It can be used for checking each individual light beam as well as whether the number and type of counts are registered correctly by the system.
- If the animals are already in their cage, red square will show under each axes (X,Y,Z). You can control it with a collaborator which could position is hand as well.
- The static test is available to test all single beams. With your fingers go from one end to another of each frame. If the sensors are functioning correctly then the status line Activity window should show a red square at the position where the light beam is interrupted. The Counts window should display the correct number and kind of interruptions.

In the software/ Setup/ Activity

- **Scan Rate**; Select Scan Rate ≤ 100 Hz.
- **Write binary file**; Check to register additional binary file (*.bin) that contains detailed information about the individual sensors.

17 Instructions

- Recording interval (1-60min), applies if **Write binary file** is checked. Enter separately adjustable sample interval [min] that allows data registration to be carried out at a higher resolution. Set to 1 min. (See section **Tips**)

(If Binary files are available, Click Export > Actimot Details, under Export Activity Table:

- Set the interval time needed
- Set the range of the sensors
- Thick the boxes and the parameter of interest
- Click “Store Table” and save it at CSV files which can be use afterward for subsequent meta-analysis.
- Write **Raw data file**, it will allow analysis with the **Special Analysis – ActiMot2 windows** or could be extracted under CSV files type for your meta-analysis.
- **Refractory period [sec]**, set at 1 sec. Applies for parameters XF, YF and Z. Enter a time-out interval [sec] for fine movements and rearing. Interruptions at the same light beam that occur in a shorter interval than that entered are not counted, e.g. to avoid scratching or tail flicking being included in the count.
- Select Center area depending of the CaloCage size, i.e.
 - Center Area X1-X2 : 6 and 11 for mice.
 - Center area Y1-Y2 : 3 and 6 for mice.
 - Enable Light beam for X, Y and Z, tick green boxes.

6.1 Definition

See PhenoMaster/ LabMaster – Software Operating Instructions – Basic Modules – P32

XT, YT

- Breaks X-beam total (XA + XF)
- Breaks Y-beam total (YA + YF)

A light barrier interruption is counted as either an ambulatory or a fine movement. XT and YT (T = total) are calculated from the sum of the ambulatory and fine movements.

XA, YA

- Breaks X-beam, ambulatory movement
- Breaks Y-beam, ambulatory movement

Record breaks of any two different X or Y-beams of light. Registers beam breaks of movements in which, for example, light barrier X_n is first interrupted followed by an interruption to a different light barrier X_m (m ≠ n). The counter registers the new interruption after a different light barrier has been interrupted first.

XF, YF

- Breaks X-beam, fine movement; breaks
- Breaks Y-beam, fine movement

Two breaks in succession of a single beam of light at the lower level X, Y. The counter registers the interruption of a light barrier that has been interrupted for the second time in succession without a different light barrier having first been interrupted.

18 Instructions

Z

- Breaks Z-beam, rearing movement, A break of a beam of light at the upper level Z.

All interruptions accruing at any light barriers will be counted. No differentiation is made between ambulatory and fine movements. A predefined refractory period is also applied to interruptions to the Z-light barriers.

CenA, CenF, PerA, PerF

- Central ambulatory movement
- Central fine movement
- Peripheral ambulatory movement
- Peripheral fine movement

The system distinguishes counts in a central area from those in the peripheral area. A count is registered as a movement in the central area if the last light barrier interruption occurs in the area that has been defined as the center. Otherwise the movement will be registered as a peripheral movement.

CenT, PerT

- Sum of central ambulatory and fine movements
- Sum of peripheral ambulatory and fine movements.

DistK [cm]

- Cumulative distance with reference to the sample interval.

To determine the distance, the “center of gravity” of an animal (i.e. of all interrupted X and Y light beams) is calculated when the status of any light beam (non-interrupted/ interrupted) alters.

DistD [cm]

- Differential distance with reference to the sample interval

To determine DistD the difference between two cumulative data in succession is calculated.

Speed [cm/s]

- Actual speed with reference to the sample interval

->NOTE – Animal size

If the animal is large enough to interrupt several light barriers then the last one to be interrupted will be counted as the currently interrupted one. This is used as a basis for taking a defined refractory period into account and for differentiating between ambulatory movements and fine movements.

7. Calorimetric studies

See PhenoMaster/ LabMaster – Hardware Operating Instructions- Calorimetry measurements

See PhenoMaster/ LabMaster – Software Operating Instructions- Basic Modules

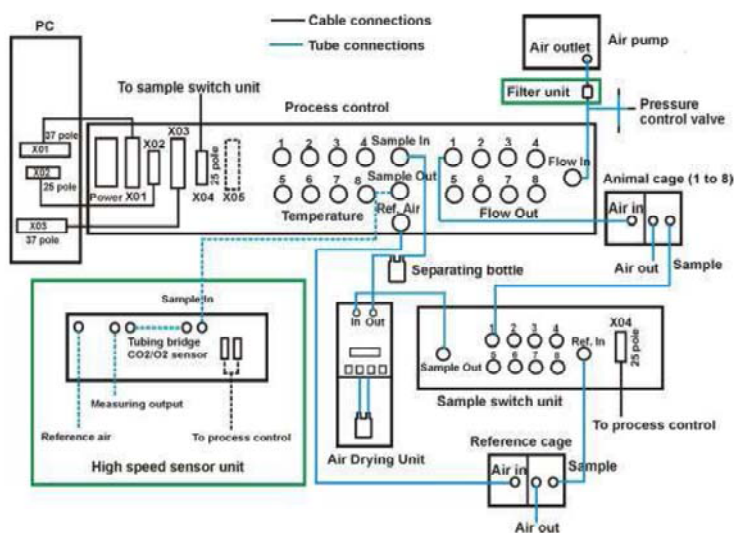
The calorimetric module is set as an open-circuit measuring system, to record and analyze automatically the animal's metabolic parameters (indirect calorimetry). During the experimental courses the animal's oxygen consumption, the carbo dioxide production and the resulting respiratory exchange rate are taken, calculated, recorded and monitored in the calosys module.

The control unit(s) provides the connection between the animal cages and the computer and the animal cages and the air pump respectively. Depending on the system configuration one or more control units may be necessary, one of which is always the master control unit.

The connections for data transfer and process control are made at the control unit(s). Up to 8 outlets are available for tubing connections to the animal cages per calorimetry control unit. An additional tubing connection is supplied for the reference cage. Depending on the system configuration it may also be possible to connect 8 temperature sensors.

Both the master and the slave control unit contain mass flow controllers for controlling and checking the air flow. The connections for exchanging data with the computer as well as the standard sensors for analyzing the O₂ and CO₂ content are contained in the master control unit. A separate high speed sensing unit is available for rapid O₂ / CO₂ measurements.

The regulation knob on the front panel of the master control unit is used for setting the flow to the O₂ and CO₂ sensors.



7.1. Definition

H in [W/kg]

- If ticked then H (Heat) will be calculated in [W/kg], otherwise the unit will be [kcal/(kg x h)].

Calculation of VO₂/CO₂

- Operator values (entries CVO₂, CVCO₂) for the calculation of H (Heat)
- Pre-set values: CVO₂ = 3.941; CVCO₂ = 1.106.

Flow

- Flow per box [l/min]
- The correct setting for the air flow depends on the animal weight and species, the type of feed and the activity of the animal; it should usually be determined in preliminary tests.
 - Standard sensors - An approximate guideline is a flow of 0.20-0.40l/min for mouse
 - High-Speed sensors (High-speed unit) - An approximate guideline is a flow of 0.35-0.50l/min for mouse

Values for dO₂ resp. for dCO₂ should be 0.3-0.7%. The ratio dO₂/dCO₂ should be about 0.7-1.0.

20 Instructions

→ NOTE – Sample flow vs flow through the boxes

Sample flow has to be smaller than flow through the boxes.

Temp

- Temperature [°C]

O₂/CO₂

- O₂ and CO₂ concentration value

dO₂/dCO₂

- Differences in O₂ and CO₂ concentration values
- ReferenceO₂ - BoxO₂ = dO₂, BoxCO₂ - referenceCO₂ = dCO₂

VO₂/VCO₂

- O₂ consumption and CO₂ production [ml/kg/h] resp. [ml/h]

RER or RQ

- Respiratory exchange rate

H or HEAT

- Heat production [kcal/h/kg] resp [W/kg] resp. [kcal/h]

(1) Expressed by body weight

(2) Expressed by lean body mass exp 0.75)

(3) Value by cages, the body weight is not considered

Measured parameters:

O₂Ref[%]

CO₂Ref[%]

Flow[l/min]

Temp[°C]

O₂[%]

CO₂[%]

Calculated parameters:

FlowML = Flow * 1000.0 * 60.0; // ml/h

dO₂ = O₂Ref - O₂

dCO₂ = CO₂ - CO₂Ref;

V₁ = N₂Ref * dO₂

V₂ = O₂Ref * (dO₂ - dCO₂)

RER = VCO₂ / VO₂

(1) Takes weight 100% into account

VO₂(1) = FlowML * (V₁ + V₂) / (N₂Ref * Animal Weight * 100.0)

VCO₂(1) = FlowML * dCO₂ / (Animal Weight * 100.0)

H(1) = (CVO₂ * VO₂ + CVCO₂ * VCO₂) / 1000

If H (Watt) then H = H * 1.16306 // Kcal/h → Watt

(2) Lean Body Mass

Animal Weight = Power (Animal Weight)

VO₂(2) = FlowML * (V₁ + V₂) / (N₂Ref * Animal Weight * 100.0)

VCO₂(2) = FlowML * dCO₂ / (Animal Weight * 100.0)

H(2) = (CVO₂ * VO₂ + CVCO₂ * VCO₂) / 1000

If H (Watt) then H = H * 1.16306 // Kcal/h → Watt

(3) The weight of the animal is not taken into account

VO₂(3) = FlowML * (V₁ + V₂) / (N₂Ref * 100.0)

VCO₂(3) = FlowML * dCO₂ / 100.0

H(3) = (CVO₂ * VO₂ + CVCO₂ * VCO₂) / 1000

If H (Watt) then H = H * 1.16306 // Kcal/h → Watt

Write binary file option group

Tick to enter sample interval [s] for calorimetry module that may be smaller than the global sample interval entered in Setup > Experiment > Sample Interval [min].

8. Environmental Control Cabinet

If available, the Environmental Control Cabinet is used to control the light intensity and the temperature, thus to perform thermo-neutral experiment or temperature challenge experiment or protocol requesting reverse light/dark cycles.

Description: It consists of several racks positioned inside the cabinet, once the doors shut, the cabinet is hermetically closed. The Cabinet is well ventilated to allow a homogenous temperature and luminosity.

On the left panel, a microprocessor control unit comprises; a temperature controller, a cooling control indicator, an adjustable safety thermostat for high and low temperature, a mains switch, a time switch (for starting the light program).



8.1. Adjustable safety thermostat

The controller displays are 2 darts knobs. We recommend setting the lower temperature at 3 C below the set temperature (min 4 C) and the high temperature at 3 C above the set temperature (Max 37 C).



8.2. Temperature control

8.2.1. Programming the Default Temperature

-CAUTION- The original or default temperature should be set first: e.g. 22,5 C:

Press "SET" -Key and adjust the set value to 22,5 C

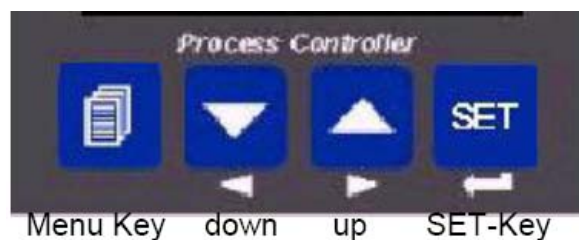
Press "SET" -Key to confirm.

"auto" should display.

This value will be memorized as the default values. The system will adjust to it when ongoing program are stopped or following an electric failure.

Note → Caution

The programming for temperature and light is completely identical; the set values are the only different. Only qualified persons should perform the setting to minimize the risk of error.



SET - key

The temperature in the chamber is controlled automatically by means of an internal controller with a connected heater and cooling machines. Thus depending on the set values the cooling machine works as a heater and as a cooler.

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- Green number = Set Value
- Red number = Actual value in the cabinet
- Mode “Ramp” = program is running
- Mode “auto” = Default value
- “Down” and “up” buttons are here to adjust value and move within program.

8.2.2. Program a temperature challenge experiment

e.g. a basal line of 20,5C for 24h (in 30 min) then a decrease of temperature (in 2hr) down to 8C for 12h and an increase to 20,5C (in 2hr) for 12h and back to default value.

- Press **Menu** Key one time and the controller is in operator Mode:

Actual value display: PASS

Set value display: oPEr (operator)

- Now the Controller is in the Operator Mode and the display goes to the sub menus for controller calibration. Press “DOWN”, up to “rnnP / SoA. You are in the programming mode and the following values will be displayed:

Actual value display: rnnP

Set value display: SoA

- With “**SET**”-Key you open the menu step by step. You will get the following display, but you confirm with “**SET**”-Key without any change.

Actual Value display: StrA

Set value display: 00.00

Actual Value display: rSto

Set value display: 000.0

- The third value must be set to 2, if not displayed: (change values with UP or DOWN)

Actual Value display: rStY

Set value display: 0002 press SET

- The first programming segment will be displayed. Here you can the set value to 20,5 with the UP or DOWN buttons:

Actual Value display: PU_1 (Process Value 1)

Set value display: 020,5

- After press “**SET**”-Key the first RAMP Time will displayed and can set to 0,3(h):

Actual Value display: tr_1

Set value display: 00.30

- Press “**SET**”-Key and the HOLD Time will be shown, set to 24 (h):

Actual Value display: tS_1

Set value display: 24.00

- Again press “**SET**”-Key and display will switch to the next program segment, set 8C:

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Actual Value display: PU_2
Set value display: 08.00

- Press “**SET**”-Key, then set Ramp Time, set to 2(h):

Actual Value display: tr_2
Set value display: 02.00

- Press “**SET**”-Key then you set the HOLD time to 12(h):

Actual Value display: tS_2
Set value display: 012.00

- Again press “**SET**”-Key and display will switch to the next program segment, set 20,5 C:

Actual Value display: PU_3
Set value display: 20.50

- Press “**SET**”-Key, then set Ramp Time, set to 2(h):

Actual Value display: tr_3
Set value display: 02.00

- Press “**SET**”-Key then you set the HOLD time, set to 12(h):

Actual Value display: tS_3
Set value display: 012.00

You can set more ramps and hold segment after that procedure (up to 8). However the program segment unused should be set to 0 (zero) and therefore they will not activate.

Note → Caution

At the end of the program, the program will be back to the default values.

The temperature controller is not link to the timer clock (24h Clock unlike the light controller) therefore the program must be launch manually.

The programming for temperature and light is completely identical; the set values are the only different.

Only qualified persons should perform the setting to minimize the risk of error.

8.2.3. Launch the program manually

The block should display “auto”

Press “menu” – Pass / oPEr display

Press “**SET**”-Key up to “Set/List” display

Press “Down” up to “run/LiSt” display

Press “**SET**”-Key up to “rSSL/ off”

Press indifferently “UP” or “DOWN” to read either “Run”, “Off” or “Hold”

Select by pressing “**SET**”-Key, if you selected “Run”, “**Ramp**” should appear

Press “Menu” to go back to the main menu.

Each time you press Menu – Key you can jump back to the beginning of the programming procedure and check the set values again.

8.3. Light control

The environmental chamber is illuminated by means of two fluorescent lamps. For the simulation of day and night cycles the light intensity is variable from 0 % to 100 %. The light intensity is controlled simultaneously for both lamps.

- **For information: 25% is equivalent to 60 Lux (recommended for animals housing).**

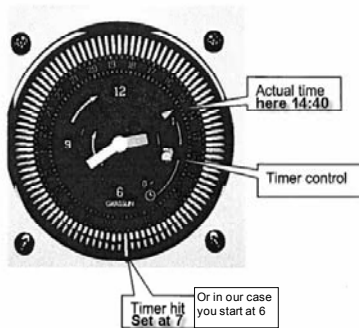
A change of the light set values can be effected by pressing the arrow keys on the keypad on the light controller.

The process value (**red display**) and the set values (**green display**) are displayed at percent. A change of the light intensity can also be programmed with ramps or dwell times.

Illumination program is connected to the Time Switch.

8.3.1. The Time switch

The timer has follow adjustment elements:



The time switch consists of a clock (12h am/pm) and a switch with day-program (0-24h) with a minimal switching time of 15 minutes. Before starting the day-program, the time has to be set by turning the switching dial (Clockwise) until the current time is opposite to the marking while arrow. Set the hand switch (inside the controller/timer control) to **Clock**, **I** is not useable and **0** stopped the program.

Activating the program. Only one segment of the switching dial must be pressed down. E.g. 6 (the program run during 24h, each time the clock hit the segment down, the program start again).

Note → Caution

The switch clocks stay ON even if the main switch is OFF, as long as the cabinet remains plugged. If unplugged, the time switch is to be set again.

8.3.2. Programming a typical light schedule

In our example, in a typical 12h/12h light schedule, day light will be on at 6h and off at 18h with a 30 min “sunset” or “dawn” and a 30 min “dusk” or “nightfall”.

The default light should be set at 0.

Press “**SET**” -Key and adjust the set value to 0

Press “**SET**” -Key to confirm.

“**auto**” should display

Note → Caution

*In a case of the system being unplugged, the controller go back to the “**auto**” mode, therefore it will be dark at all time.*

The procedure is similar as described for the temperature. (See above)

- Press **Menu** Key one time and the controller is in operator Mode:

Actual value display: *PASS*

Set value display: *oPEr (operator)*

- Press “**DOWN**”, up to “*rnnP / SoA*”.

Actual value display: *rnnP*

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Set value display: SoA

- With “**SET**”-Key you open the menu step by step. You will get the following display, but you confirm with “**SET**”-Key without any change.

Actual Value display: StrA

Set value display: 00.00

Actual Value display: rSto

Set value display: 000.0

- The third value must be set to 2, if not displayed: (change values with UP or DOWN)

Actual Value display: rStY

Set value display: 0002 press SET

- The first programming segment will be displayed. Here you can the set value to 25 with the UP or DOWN buttons:

Actual Value display: PU_1 (Process Value 1)

Set value display: 25.0

- After press “**SET**”-Key the first RAMP Time will displayed and can set to 0,5(h): (Dawn)

Actual Value display: tr_1

Set value display: 00.50

- Press “**SET**”-Key and the HOLD Time will be shown, set to 11 (h):

Actual Value display: tS_1

Set value display: 11.00

- Again press “**SET**”-Key, display will go to the next segment, set the value to 0% (Dark)

Actual Value display: PU_2

Set value display: 00.00

- Press “**SET**”-Key, then set Ramp Time, set to 0,5(h):

Actual Value display: tr_2

Set value display: 0,50

- Press “**SET**”-Key then you set the HOLD time to 11(h):

Actual Value display: tS_2

Set value display: 011.00

Once the program is conclude, the systems will be back to the set values (0%) and being restarted automatically at 6h in our case. Each time you press Menu – Key you can jump back to the beginning of the programming procedure and check the set values again.

Note → Caution

If during the day, the Light Control is in mode “auto”, it can be switch to “Ramp” using the time switch, turn the switching dial, turn slowly when the segment down, it will unclenched the program: In our example, by passing the segment of 6 hr, the light will light on and the mode “Ramp” will appear.

9. Calibration

See PhenoMaster/ LabMaster – Software Operating Instructions – Calibration

See PhenoMaster/ LabMaster –Calibrating Instructions- O2 and CO2 Measuring module – High-speed Sensor Unit for calorimetric measurements.

Calibration is a necessity for the sensors, such as CO2 and O2 sensors, Drinking/feeding and body weight sensors. Temperatures sensors are originally calibrated, nevertheless if a temperature controller is available, those sensors can be recalibrated.

Note →

The software stores the complete calibration together with all other software settings in the file **Labmaster.set** resp. **Phenomaster.set** on the hard disk. For security purposes the software writes an ASCII-file when the Calibration window is closed; this contains all the values under the name *Labmaster.cal* resp. *Phenomaster.cal* in readable text form on the hard disk.

-CAUTION-A new calibration should be carried out before a series of measurements is to be made.

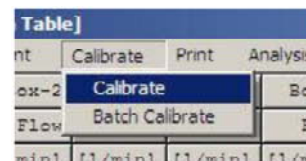
The system is delivered pre-calibrated for a first test after installation of the system. Under **Calibrate / Calibrate** menus, you will have access to the sensors that have been installed.

The calibration menus for the individual sensors are available in the following Sequence:

- O2 sensor
- CO2 sensors
- Mass flow controllers

-CAUTION- Mass flow controller parameters are set prior to delivery and should only be altered after consulting TSE service

- Temperature sensors
- Drinking sensors
- Feeding sensors
- Body weight sensors

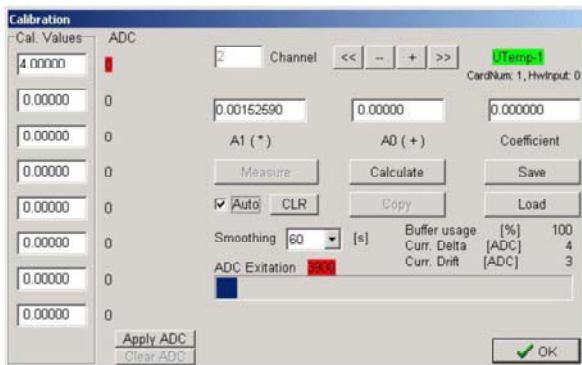


Tablej			
nt	Calibrate	Print	Analysis
ox-2	Calibrate		Bo
Flow	Batch Calibrate		F
min1	l1/min1	l1/min1	l1/w

Batch Calibration is available for calibrating Drinking/ Feeding / Body Weight sensors.

9.1. Calibration routine for Drinking/Feeding/ Body Weight sensors

Calibration is carried out by means of linear regression. At least two measurements should be carried out (minimum/maximum value). It takes approximately one day to calibrate the calorimetry sensors and the drinking sensors and feeding sensors.



NOTE-> Two Points calibration

For a calibration using only two calibration standards (e.g. two gases, two weights) the coefficient of the linear regression will always be shown as 1.00000. This means that calibration errors cannot be recognized. Variations will also have a greater effect on the accuracy than in a multipoint calibration. The more calibration standards you use, the more accurate the measurement.

A maximum of eight calibration values can be defined. The highest calibration value cannot exceed the upper ADC limit. We recommend using more calibration points for very precise measurements.

9.1.1. In a case of more than 2 points

- Select Calibrate > Calibrate to open the Calibration window.
- Press +/- to select the required sensor channel. The green status display the type of sensor allocated to that channel.
- Enter the first calibration value (the minimum value) in the first left-hand input field Cal. Values. **The calibration values should be entered in increasing order.**
- Check “**Auto**” and select the required smoothing value (60 second usually). The blue ADC bar (ADC Excitation) shows the modulation of the analog-digital converter.

->NOTE – Checking the stability

Check whether the ADC value has stabilized: If the value no longer rises or falls steadily, but varies around a particular value – can be followed by observing the Curr. Delta [ADC] values – then the calibration can be carried out with this value.

- When the buffer has filled and the ADC value has stabilized “**clear Auto**”. The ADC value will be halted and can then no longer be altered, e.g. by removing the calibration standard.
- Press **Apply ADC**, the ADC value will be accepted and appears to the right of the Cal. Values input field.
- Click in the next left-hand input field Cal. Values, and enter the next value.
- Connect the next calibration standard to the sensor.
- Check “**Auto**” and wait until the buffer has filled and the ADC value has stabilized.
- Clear **Auto**.
- Press **Apply ADC**, the ADC value will be accepted.
- Proceed as described above with all the other calibration standards.
- After measuring the last calibration value “**clear Auto**”.
- Press **Calculate**.

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- Press **Save** to save your values.

The first hardware channel, e.g. Drink1 or Temp1 is calibrated. The other channels, e.g. Drink2, Drink3, etc. should also be calibrated as described above.

->NOTE – Calibration coefficient

With more than two calibration values the Coefficient indicates the quality of the calibration. The value should be as close as possible to 1.0.

->NOTE –After calibration- Control

You can check whether the weight of a weight attached to the sensor (e.g. with a weight for drinking and feeding experiments) is shown correctly after calibration by selecting Status > Drink/Feed menu. Only slight variations should occur, otherwise a new calibration should be carried out.

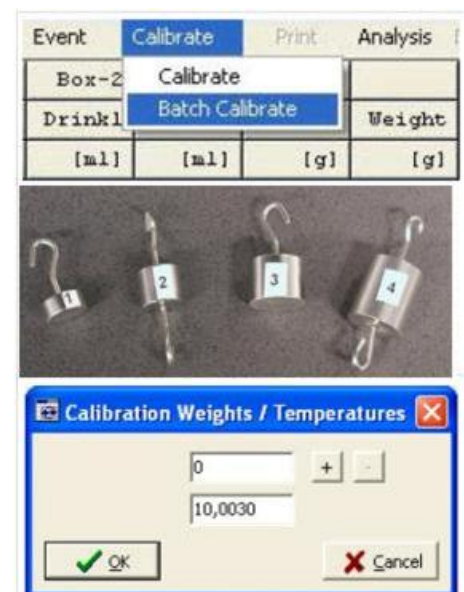
9.1.2. In a case of 2 points

We recommend using the **Batch Calibration**, it allows a time-saving handling especially with a large setup. Several calibration weights are registered simultaneously.




- A single set of calibration weights is required. Values of weights should clearly differ from each other.
- Each weight is attached once in succession to each of the sensors to be calibrated.

Select **Calibrate / Batch Calibrate**

- Task: Tick Calibration
- Click Calibration or Weights values and enter 0 in the first Box and your Calibration Weights (g).
- Press Ok
- Select **Calibration Tab**. *Bottles and baskets must be attached to their sensors hook.*
- Wait that all bottles and sensors are stabilized.
- Set Smoothing to 30 s
- Set Max. Drift to 10
- Set Max. Varianz to 3
- Un-tick Auto Catch
- Click to **“Clear Buffer”**, if stabilized all bars display should be in green. If not wait few second and click again **“Clear Buffer”**.
- Click **“Catch”**, the first point is recorded, the number “1” should appear in all bars. If not, click **“Close”** and select the **Calibration tab** and restart the procedure.
- Attach the chosen weight to the first sensors (bottles usually), you will see that the green bar (once stabilized) goes up.
- Click **“Clear Buffer”** once stabilized (green bar)
- Click **“Catch”**, a red tick should appear. ✓
- Remove the weight, go the next sensors, wait it stabilized, verified that the bar level goes up.
- Click **“Clear Buffer”** once stabilized (green bar).



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- Click “**Catch**”, a red tick should appear to this second sensor.
- Repeat this procedure for each sensor.
- At the end, when all sensors have following red tick: 
- Click “**Calculate**” (regression analysis calculation between the two points measured).
- At this stage, all sensors should display a yellow tick  (if one is missing, see section **Tips**).
- Click “**Save**”, a green tick should appear  at this stage all sensors are calibrated.

-> **NOTE – Show ADC values**

*The registered ADC values can be shown on the **Calibration** tab with a tooltip on the sensor bar*

-> **NOTE – More than two points**

*It is possible to use the **Batch Calibrate** with more than two points, all weight must be added by clicking **Presets/ Weights/ +**, fill the blank. The proceeding is the same as described before, a number 2 should appear for the second (different than 0).*

-> **NOTE – Moving around Sensors**

We recommend that all sensors are being labels. If you change position or replace the sensors, a calibration must perform again.

9.2. Calibration routine of the O2 and CO2 sensors of the high-speed unit

TSE systems give several methods to perform calibration of the O2 and CO2 sensors.

See PhenoMaster/ LabMaster – Software Operating Instructions – Calibration- Calibration of the O2 and CO2 sensor – P19

See PhenoMaster/ LabMaster –Calibrating Instructions- O2 and CO2 Measuring module – High-speed Sensor Unit for calorimetric measurements.

9.2.1. General Hints

-CAUTION- This step is critical as it will condition the whole experiment and also comparison with other studies, we recommend that the calibration routine should be undertaken by trained and well-informed personnel.

-CAUTION- Before calibration, all electronic devices have to be switched ON **at least 4h** before the test or the calibration. Calorimetry devices (e.g. control unit, high speed sensor unit etc..) should only be switched OFF during long-term interruptions to measurements. If measurements are made at regular intervals, it is recommended that the instruments are being left ON all the time.

-CAUTION- O2 and CO2 sensors of the high-speed unit must be connected to a dry air bottle equipped with a fine regulation valve the pressure must be equivalent to 0 psi or about 4-5 bar. As soon the system is ON, the bottle must be open.

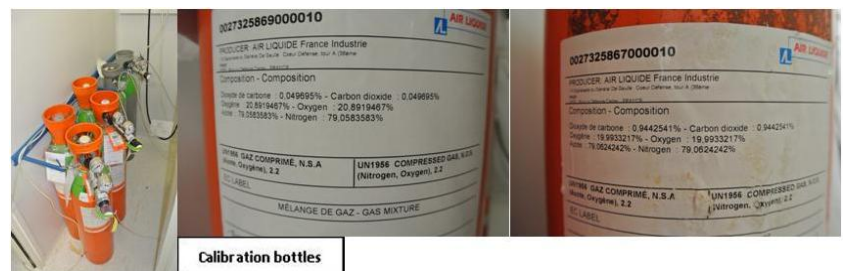
The O2 and CO2 channels are calibrated using a 2-point calibration. Check the following steps:

- Software must be ON
- The flow must be stable
- O2(%) and CO2(%) stable and reflecting the composition of breathable air
- Only use certified calibration gases bottles (Only gases whose O2 and CO2 content is known exactly will provide a correct calibration)
- The flow should be similar as use for the experiment (between from 0,2 to 4,0 L/min).

Calibration is required after the installation of the material and each time after:

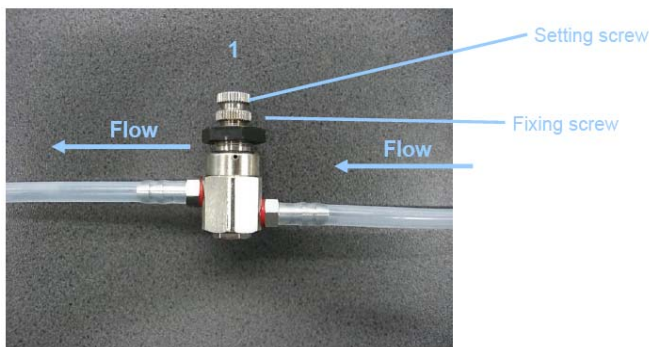
- The reference gas has been exchanged.
- Before each new advanced experiment or test series.
- If measuring values are unstable or drifting.
- If the system has not been use for longer times.
- When atmospheric pressure went through major change (form high to low pressure).

Calibration should never be carried out at the measuring limits of the sensor as this could result in calibration errors. Excessively high pressure will damage the internal sensor. Never connect a gas bottle directly to an inlet connector, calibrations bottles, both must be



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equipped with a fine pressure reducing valve set to 0,2-03 bar above atmospheric pressure. Furthermore, during calibration two manual fine regulation valves like that shown below are used to set a small calibration flow.



The connection should be made in the flow direction as shown above. The valve is closed by turning setting screw (1) counter-clockwise; the flow is increased by turning it clockwise. When the setting screw is turned counter-clockwise until it reaches the stop the valve is completely closed. The maximum flow can be limited by rotating the fixing screw clockwise or counter-clockwise. The flow is to be set in the way shown during the installation procedure.

The calibration of the O₂ and CO₂ sensor is first carried out with the reference gas, i.e. the gas with the higher O₂ content, e.g. 20.901% and the lower CO₂ content, e.g. 0.050% – this is **the zero calibration**. Calibration is then carried out with the second calibration gas – this is **the span calibration**.

9.2.2. Step to perform with new Calibration bottle

On the high speed unit:

- Press “O₂”; “Calibration”; “Setpoints for Zero/span”
- Enter the value of [%O₂] (the high value; e.g. 20,900), “Enter”
- Press “Enter” up to “Setpoints for MR4”
- Enter the [%O₂] of the second bottle (lower value; e.g. 19,500); “Enter”
- Press “Meas”; “Yes”

-> NOTE –

In case the system requires setting of codes during the calibration press:

Code1 = 111 “Enter”

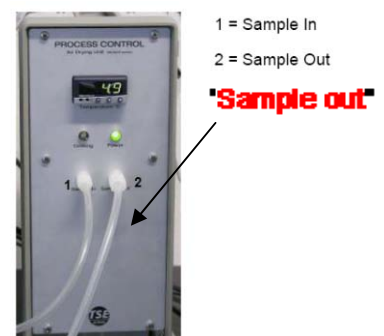
Code2 = 222 “Enter”

On the high speed unit:

- Press “CO₂”; “Calibration”; “Setpoints for Zero/span”
- Enter the value of [%CO₂] (the low value; e.g. 0,050), “Enter”
- Press “Enter” up to “Setpoints for MR4”
- Enter the [%CO₂] of the second bottle (high value; e.g. 0,950); “Enter”
- Press “Meas”; “Yes”

9.2.3. Calibration

- Remove the tubing that is connected to the **Sample In** inlet of the CaloSys control unit. This comes from the “**Sample out**” of the air drying unit which is not involved in the calibration process as the air from the cylinder is already dry.



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- Use the manual fine regulation valve to set a small gas flow – check the flow with the help of the **Status > Calo > Calo Current Values** window of the PhenoMaster/LabMaster software. Ensure that there is only a small gas flow; **this should be the same as the sample flow during the experiment as otherwise you could damage the system.**
- Wait until the sample flow is stable and adjust it if necessary.

-> NOTE –

If the air flow is not sufficient, the display of unit will flash.

9.2.4. ZERO Calibration

Connect the bottle with the higher [%O₂] and lower [%CO₂].

- Press “**O₂**”; “**Calibration**”; “**Zero Calibration**”
- Wait 5 min to have a good stability of [%air concentration]
- Press “**Start Calibration**”; “**Calibration OK**” will light up”
- Press “**Meas**”; “**Yes**”

(Do not disconnect)

- Press “**CO₂**”; “**Calibration**”; “**Zero Calibration**”
- You should have a good stability of [%air concentration]
- Press “**Start Calibration**”; “**Calibration OK**” will light up”
- Press “**Meas**”; “**Yes**”

Disconnect the bottle, close it well, and connect the other bottle.

9.2.5. SPAN Calibration

- Press “**O₂**”; “**Calibration**”; “**Span Calibration**”
- Wait 5 min to have a good stability of [%air concentration]
- Press “**Start Calibration**”; “**Calibration OK**” will light up”
- Press “**Meas**”; “**Yes**”

Do not disconnect

- Press “**CO₂**”; “**Calibration**”; “**Span Calibration**”
- You should have a good stability of [%air concentration]
- Press “**Start Calibration**”; “**Calibration OK**” will light up”
- Press “**Meas**”; “**Yes**”

Disconnect the bottle, close them well and reconnect the sample in of the CaloSys control unit to the “**Sample out**” of the air drying unit.

10. Tips

10.1. Sample Interval [min]

Set the sample interval time per cage is an important step, during the installation of the system, it will be calculated with TSE technician. It depends of your setup (type of O₂ and CO₂ sensors), the numbers of cages and thus the length of the tubes.

However, you can assess the Sample Interval time yourself. It can be understood as the sum of time spent for each cage + the reference cage.

- All apparatus are ON and the O₂/CO₂ sensors is well warmed up
- Launch Labmaster (rats/mice)
- Open Status/ Calo
- On the top you can read the %O₂ and %CO₂ being read (usually the cage ref)
- Install an animal in cage 1 and wait 30 min.
- Have a chronometer/clock nearby.
- Click on the “+” to move the reading from the reference cage to cage 1 and observe the %O₂ and %CO₂.
- % should move (O₂ decreasing and CO₂ increasing). Look at the time when the concentration stabilized (usually between 1 min up to 1 min depending of your system).
- This time will be your sample times per cage. e.g. 1.5 min
- E.g for 10 cages + 1 reference cage, your Sample interval time will be 16.5 min, enter 17
The system will spent 1,55 min per box.

10.2. Adjusting the air flow

Open the panel Setup/ Setup (Initial file) / Calo. The flow (L/min) must be entered.

- It should be higher than the Sample flow.
- It depends of the animal body weight and the species.
- For a mice of 30 g, the air flow is set at 0.55L/min
- For a rat of 250 g, the air flow is set at 1.2 L/min

For the rat, we recommend performing an initial study with a group of animal with heterogeneous body weight. E.g. from 250 g up to 500 g. Monitor the dCO₂ and the %CO₂. Adjust the airflow such as, for each body weight, %CO₂ should remain within the calibration points (below 0,950%) and the dCO₂ around 0.3-0.4.

From there you can plot a relation between body weight and air flow.

10.3. Activity/ Actimot

10.3.1. If Binary files are available, Click Export > Actimot Details, under Export Activity Table:

- Set the interval time needed
- Set the range of the sensors
- Thick the boxes and the parameter of interest
- Click “Store Table” and save it at CSV files which can be use afterward for subsequent meta-analysis.

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10.3.2. Filling with litter

- When setting the height of the X, Y sensor frame please remember that if litter is added then the frame must be set at a higher position so that the light barriers are not permanently interrupted.

10.3.3. Adjustment Z frame

- The sensor test (see section **Activity measurements via sensor frames**) should be used to ensure that the correct position of the frame has been selected.
- We recommend taking a time to observe the animals. Does the rearing frame reliably detect rearing movements? Are the Z light-beams interrupted whenever the animal rears?
- Light barriers that are permanently interrupted, e.g. by drinking or feeding containers, should be deactivated by using the software (see Software Instructions, “Setup Activity” chapter). Permanently interrupted light barriers are not counted, but are displayed in the Status menu.

10.4. Sensor cable connection

The plug head must be completely screwed in for proper operation.



10.5. Installation / Plugging modules

- All modules and computer/screen are connected to an electric power strip, which allow to switch them ON or OFF at the same time.

-CAUTION- The computer need to be started manually and the software must be ON to allow the air going through the circuit.

- We recommend that the main plug is connected to a differential circuit breaker to avoid electric overload.
- Upstream the power strip and after the main plug, we recommend to install a high frequency UPS/electrical inverter 1000 VA / 600 W including lightning protection and a battery backup. It will allow the system/ air pump to run several hours without power.



10.6. Monitoring an experiment from distance

The system is aimed to run night and days including the week-end. For convenience and for the well being of the animals, it is recommended to survey the calorimetric analysis each day. Thus we recommend installing between the computer and the main user, a VPN type connection or remote access software to allow monitoring and control of the computer from outside the laboratory. Do not forget to protect it by a password.

10.7. Cleaning and Decontamination

To carry out bio-decontamination efficiently, all adhering dirt must first be removed by a preliminary cleaning process. If detergents or chemicals are used on the components then these must be completely removed before further decontamination steps, e.g. autoclaving, are carrying out. There must be no residues to any irritations.

A special care must taking in account regarding sanitary status of the animals between experiments and thus it might be advised to gasses with H₂O₂ between experiments. Beware of the long desorption time as the animals can be stressed by H₂O₂ residues.

See PhenoMaster/ LabMaster – Cleaning and Decontamination Instructions

10.8 How to approach data

- You may find it useful to calculate Resting Metabolic Rate. Since most mice are variably active during daylight, you may use the filtering capabilities of Excel spreadsheet to select data points where activity is below a certain value.
- Energy balance can also be defines using excel spreadsheet by the differences between cumulative H₃ and daily food intake (kcal).
- We recommend discarding the first day and night parameters as during this period; the mice are hyperactive due to new environment syndrome.
- Graph a time course of food and water consumption for individual mice to make sure each started eating and drinking within a reasonable period of time, and that there are no anomalies (such as a leaky water bottle or stuck food bottle) that would throw off your group averages.
- Before taking multiple day averages, calculate each period separately to make sure values aren't progressively rising or falling. Some mice acclimate over several days and you may end up using only the last full period.
- Another quality check is to graph a time course for energy expenditure and RER to see if there are discrepancies in the circadian rhythm from day to day.

11. Appendix

Check List LabMaster

Check List Calibration

Checklist - Calorimeter

User : _____

Name of experiments : _____

Date : _____

Species : _____ Temperature _____

Before stating make sure that:

- *Animals have been habituated to the drinking nipple*
- *Animals Individualised for at least one week*
- *The system had been switch on for at least 24h*
- *All sensors must be calibrated*

Checkpoints

Tick

- *Compressed dry air bottle must be open between 4 and 5 bars.*
- *Temperature sensor connected to all cover*
- *Remove cable or object which could interfere with the activity frames*
- *Refill bottles and food baskets (check for leakage)*
- *Install the animals*
- *Check the tightness of cages (fixation, inserts etc)*
- *Check that the tubes (air in and out) are well connected to the covers*
- *Close the Environmental Control Cabinet*
- *Temperature displayed in green (set) should be ____ (mode "auto")*
- *The light-cycle must show 25% (60 Lux equivalent), with "Ramp" displayed*
- *Control the connection (sample in/out) of the reference box (rat / mice)*
- *Empty regularly the bottle below the air drying unit*
- *Open the appropriate software (rats or mice)*
- *Adapt the air flow in (SETUP / CALO) to the mean body weight of the animals*
- *Set the proper cycles and duration of experiment*
- *Fill all information needed in "SETUP / Boxes"*
- *Enable all cages being used (Yes or No)*
- *Set the air flow sample (samp = 0.45 L / min)*
- *Perform calibration (3 min per points)*
- *After calibration, reconnected all the systems and close the calibration bottle*
- *Press "MEASUREMENT" and "START"*

Within the experiment

- *Check that RQ oscillate between 0,7 (Fasted animals, high fat diet, daytime) and 1 - 1,1 (normal chow diet, night time and high lipogenesis).*
- *The air flow should be the same between all cages.*
- *An appropriate air flow can be seen if the dO2 and dCo2 oscillate between 0,3 and 0,7 with an absolute % of CO2 < to 1%.*
- *Reference cage should display: O2 =20,89 +/-0.01, CO2 = 0,045 +/- 0.005.*
- *Check that the animals are drinking normally.*
- *Empty the water regularly from the bottle below the air drying unit.*

Checklist – Calibration – High Speed Unit

User : _____ Date : _____

Reference bottle 1: %O2: _____ %CO2: _____

Reference bottle 2: %O2: _____ %CO2: _____

Before stating make sure that:

- **All system must be switch ON for at least 4 hrs, (the best being overnight)**
- **The bottle of dry air is open with a air pressure between 4-5 bars**
- **Launch a software, the airflow “Samp” must be between 0,35-0,5 L/min.**

Step to perform with new Calibration Bottle

Press "O2"; "Calibration"; "Setpoints for Zero/span"

Enter the value of [%O2] (the high value; e.g. 20,900), Press "Enter"

Press "Enter" up to "Setpoints for MR4"

Enter the [%O2] of the second bottle (lower value; e.g. 19,500); Press "Enter"

Press "Meas" ; "Yes"

Press "CO2"; "Calibration"; "Setpoints for Zero/span"

Enter the value of [%CO2] (the low value; e.g. 0,050), Press "Enter"

Press "Enter" up to "Setpoints for MR4"

Enter the [%CO2] of the second bottle (high value; e.g. 0,950); Press "Enter"

Press "Meas" ; "Yes"

In case the system requires setting of codes during the calibration press: Code1 = 111 "Enter" / Code2 = 222 "Enter"

Calibration

ZERO Calibration

Connect the bottle with the higher [%O2] and lower [%CO2].

- Press "O2"; "Calibration"; "Zero Calibration"
- Wait 5 min to have a good stability of [%air concentration]
- Press "Start Calibration" ; "Calibration OK" will light up" . Press "Meas" ; "Yes"

Do not disconnect

- Press "CO2"; "Calibration"; "Zero Calibration"
- You should have a good stability of [%air concentration]
- Press "Start Calibration" ; "Calibration OK" will light up", Press "Meas" ; "Yes"

Disconnect the bottle, close it well.

SPAN Calibration

Connect the bottle with lower [%O2] and higher [%CO2].

- Press "O2"; "Calibration"; "Span Calibration"
- Wait 5 min to have a good stability of [%air concentration]
- Press "Start Calibration" ; "Calibration OK" will light up", Press "Meas" ; "Yes"

Do not disconnect

Press "CO2"; "Calibration"; "Span Calibration"

- You should have a good stability of [%air concentration]
- Press "Start Calibration" ; "Calibration OK" will light up", Press "Meas" ; "Yes"

Disconnect the bottle, close it well and reconnect the tubing to the air drying Unit.