Energy Expenditure Measurements
C1042

Version: 1
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Summary:

Oxygen consumption and carbon dioxide production is measured using indirect calorimetry. The Columbus Instruments Oxymax Equal Flow System is an indirect open circuit calorimeter designed to simultaneously measure metabolic performance of multiple subjects that have similar ventilation needs. uses an open circuit calorimetry technique. This system allows sixteen animal cages to be simultaneously monitored. Variables provided by this measurement include VO2, VCO2, RQ, and HEAT.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Clorox Wipes	Office Depot	821-808
Clean-up Clorox Spray	Office Depot	115-581
Drierite 5lb 8 Mesh	Drierite	23005
Cal Gas Mixed Gas 20.5% O2, 5000ppm CO2, bal N2 Primary Standard	Praxair	NI CDOXP27-Q
Electro Chemical Cell for O2 Analyzer	Columbus Instruments	7260-D54
Oxymax Sample Max Channels 1-8	Columbus Instruments	090678-7-CH1-8
Oxymax Sample Max Channels 9-16 & reference	Columbus Instruments	090678-8-CH9-17
Oxymax Flow Max Channels 1-8	Columbus Instruments	090678-5-CH1-8
Oxymax Flow Max Channels 9-16	Columbus Instruments	090678-6-CH9-16
Oxygen Sensor	Columbus Instruments	090678-3-NHV1-WO
Carbon Dioxide Sensor	Columbus Instruments	090678-4-PPS-WO
Sample Pump	Columbus Instruments	090678-2-WO
Gas Sample Drier	Columbus Instruments	090678-SD-WO
Fresh Air Pump	Columbus Instruments	1108000802C

Protocol:

Energy Expenditure by Oxymax Protocol   (Columbus Instruments)

1. Take Body Composition via NMR before you put animals in this experiment.
2. Set up cages (this can be done at any time before starting an experiment)
   a. Weigh animals and place into Oxymax chambers
   b. Supply food and water
   c. Make sure chamber lids are clasped firmly and evenly

3. Check to see if Drierite and Lime Soda needs to be replaced (if Drierite is pink, replace with new; if Soda Lime is **not** white, replace with new.)
   a. To change Drierite or Soda Lime, remove canister from holder and unscrew the lid (you do not have to remove hoses from container).
   b. Remove spring, metal lid and cotton filter.
   c. Empty old Drierite or Soda Lime and replace with new (DO NOT FILL PAST THE TOP HOLE IN THE CANISTER).
   d. Tap bottom of canister to insure Drierite/Soda Lime is tamped down.
   e. Insert cotton filter, metal disc, spring, and then screw on the top (in that order).
   f. Push the canister back onto the holder

Running an Experiment
1. Turn on all of the surge protectors and make sure that the power lights indicate that all of the equipment is on. Make sure the PC is powered on and logged in.
2. Run Oxymax for Windows and select the appropriate hardware configuration.
▪ After selecting the appropriate hardware configuration, the Oxymax will initialize a system scan to determine if there are any errors with the equipment/hardware. If all is detected properly, the status window will automatically minimize itself. If error messages occur, check the hardware to make sure all is on and it is set up properly, then select "**Refresh**."
3. Allow approximately **2 hours** of warm up time before calibrating the system or starting an experiment. (pg.29 in User Manual)

Gas Sensor Calibration
Calibration must be performed before the start of EVERY new experiment. This can be done before or after creating a new experiment configuration. (Before: click "**Tools>>Calibration**"/ After: click "**Experiment>>Calibration**")

*Calibration Gas Concentrations should consist of: (**Primary Standard Grade or better**)
20.50% Oxygen
0.50% Carbon Dioxide
Balanced with Nitrogen

Before calibrating, make sure that the proper calibration gas concentrations are registered for the **ACTUAL** gases that will be used for calibrating. (The concentrations will be found next to the "Span" gases, the "Zero" gases should be left at 0)
- Click on the "**Cal Gas**" button next to the sensor group label. After making any changes select "**Apply**" before selecting "**Close**".
- Before calibrating any sensor, set the sample flow meter (on the front of the System Sample Pump) to about **400 mL/min** (the middle of the indicator ball should float on top of the line marked **.4 L/min**)
- * *DO NOT re-adjust the sample flow meter before starting a new experiment. If flow sample flow meter is adjusted, recalibrate.*

When turning on the tank of calibration gas, set the outlet pressure on the tank regulator to read between **5-10 PSI**. Locate the calibration sample tube on the back of the sample pump. The tube should have a T-fitting with one end open. Insert the tube from the cal gas tank and insert it into the open T-fitting end. There should be a noticeable flow of gas from the exhaust tube. (It is better to have the gas pressure too high than too low for proper calibration)

After the system has warmed up, click the "**Calibrate**" buttons under either the "O2" or "CO2" to initiate the calibration procedure.

Oxygen Sensor Calibration
1. Click the "**Calibrate**" button under the "O2" header to open the oxygen sensor calibration.
2. Make sure that the sample flow is at about **400 mL/min**.
3. Turn on the cal gas and adjust pressure so that excess gas is apparent at the exhaust tube (5-10psi)
4. Click "**NEXT**" to begin purging the sensor with the cal gas.

5. When purging is complete, the prompt will display the target O2 concentration for the cal gas.
   ▪ Use the "Course Gain" and "Fine Gain" knobs on the O2 sensor to adjust the concentration displayed on the Oxymax Sensor Calibration Window until it matches the target concentration. The values will turn <u>green</u> when the target value is reached. (*There will be a lag time when adjusting the knobs so be sure to wait a few minutes before proceeding*)
6. After the measured value matches the target concentration, click "**Next**" to display the finalization instructions.
7. Click "**Finish**" and immediately proceed to calibrating the CO2 sensor.

Carbon Dioxide Sensor Calibration
1. Click the "**Calibrate**" button under the "CO2" header to open the carbon dioxide sensor calibration.
2. Make sure cal gas is turned on and check to see that the column of Soda Lime is **WHITE.** (if Soda Lime is not white, exchange old Soda Lime with new) The Soda Lime is used for the "CO2 Zero" calibration
3. Click "**NEXT**" to begin purging the sensor with the cal gas.
   ▪ The system will measure the "Zero" point of the CO2 sensor (the "Ready" light will turn off for a few seconds which is normal)
4. After the "Zero" has been established, the system will begin purging the "Span" gas.
5. When purging is complete, the system will measure the gain necessary to calibrate the sensor.
▪ Oxymax will display a prompt listing the calculated offset and gain for the CO2 sensor.
6. Turn off the flow of cal gas and click "**Finish**" to commit the calibration to memory.

*DO NOT RECALIBRATE BETWEEN FED AND FASTING CYCLES

Starting an Experiment
It is recommended that all hardware errors have been resolved before starting an experiment.

Configuring Experiment
1. Select an experiment configuration template; "**File>>Open Experiment Configuration**" (Select a mouse configuration for mice and a rat configuration for rats)
2. Fill out the subject roster; "**Experiment>>Setup**"
   ▪ Right click the chamber ID to enable/disable the chambers in the system when disabled, the Group/Cage ID should look like ~~0109~~
   ▪ Enter in the animals body weights
   ▪ Set the fresh air flow; animals sharing a flow controller (chambers1-8 and chambers 9-16) will have identical flows
     o <u>Mice</u>: **0.50 LPM for ~20g animals; 0.60 LPM for ~30g animals**
     o <u>Rats</u>; **2.0 LPM for ~200g animals; 3.5 LPM for ~600g animals**
   ▪ Enter in any other information in the "Label=Information" field
     o Gender, color, genotype, age, treatment, etc
   ▪ Choose a location on the hard disk to store the experiment data "Data Filename"

3. Verify experiment settings; "**Experiment>>Properties**"
   o <u>Intervals</u>- The number of intervals to be recorded. Set to zero (**0**) if you want experiment to run indefinitely.
   o <u>Cage/Reference Settle</u>- The amount of time the system waits before taking another sample (both should be set at **90**)
   o <u>Cage/Reference Measure</u>: The amount of time the system measures the sample (both should be set at **30**)
   ▪ Choose reference method based on the number of cages.
     o If room air is not stable, more references are required.
   ▪ The Volume Rate Units should be set at **mL/kg/min**

Run Experiment
1. Select "**Experiment>>Run**"
2. Monitor first few readings to verify system is working properly

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- ▪ "O2in" should be around 20.94%
        - ▪ "CO2in" should be around 0.04%
        - ▪ RER should be between **0.7 and 1.0**
            - o 0.7 = fat being burned as energy
            - o 1.0 = carbohydrates being burned as energy
3. Right click on the tabs at the top of the screen (i.e.: VO2, VCO2, RER, Heat, etc…) and set the furthest decimal place (0.00000)

Stop Experiment
1. Select "**Experiment>>Stop**"
2. Fresh air will continued to be delivered to the chambers while software is running.
3. When exiting out of the Oxymax program, it will ask if you would like to turn off the fresh air. If all animals are out of the chambers, select "**Yes**," otherwise keep fresh air flowing so that the animals do not suffocate.

Exporting/Generating Data
1. Open the desired experiment data file
    - ▪ The data generated from a just-finished experiment is still open after stopping it.
    - ▪ To open a previously saved experiment, click "**File>>Open Experiment Data**" and select desired .CDTA file.
2. Click "**File>>Generate Subject CSVs**" to automatically generate CSV files for all subjects in data file.

Cleaning Chambers
1. DO **NOT** USE SANI-CLOTH PLUS WIPES OR ANY OTHER CLEANERS THAT WILL RUIN ACRYLIC TO CLEAN OUT CHAMBERS. Only use a mild bleach solution or Clorox Wipes to clean out the chambers. If you have questions about what to use, contact someone before cleaning.
2. Wipe/rinse out the chamber thoroughly, including plastic mesh grates at bottom of chambers.
3. Remove water bottles from lids
    - ▪ Loosen the nut where the water bottle is fastened to the lid and pull the water bottle up.
    - ▪ Empty water bottles and wash out. Make sure bottles are rinsed well (Do NOT use Sani-Cloths on the plastic bottles).
    - ▪ Use bleach or Sani-Cloths to wipe off metal spigots and rinse with H2O
    - ▪ Set bottles up so they can dry
4. Wipe down all lids thoroughly (if you use Sani-Cloths, do NOT let them hit the acrylic/plastic parts of the lids)
5. Allow the chambers to dry completely before placing the lids back onto them.

CLAX
1. Select the **CLAX** icon or select Start and find the CLAX program in the Program files. If CLAX is not installed on your computer, contact Dana for the installation disk
2. On the "Manage Databench" screen, select **Add Experiment**
3. Locate the experiment you want to view and select **Open.** Multiple files CAN be added to the same databench for comparison
4. When the file has been added to the databench, there will be three (3) tabs labeled "Experiments," "Subjects," and "Measurements"
    a. **Experiments** – Under the "Experiments" tab, you can view the   Hardware Configuration, Experiment Configuration and Oxymax settings for that particular experiment. This tab also allows you to set the light/dark cycle times for your experiment (light data will be in yellow/ dark data will be in pink when looking at the raw data and the graphs).
        i. **Hardware Configuration** – Selecting the Hardware Configuration will allow you to view the specifications of the Oxymax system hardware.
        ii. **Experiment Configuration** – Selecting the Experiment Configuration will allow you to view the technical information and measurement parameters for that particular experiment.

       iii. **Oxymax** – Selecting the Oxymax icon in the Manage Databench screen will also allow you to view your experiments parameters (i.e.: date of experiment, number of animals, reference time, measurement times, etc)

   b. **Subjects** – Under the "Subjects" tab, you can view the body weights and other notes for each particular chamber/animal. This is also where you can add "Tags" for grouping animals together when looking at graphs and data. Tags can be the genotype of the animal, color, gender, treatment, etc.

       i. To add tags, go to the bottom, right hand side of the "Manage Databench" page and type in the necessary tags you want to add to the animals in the experiment. After typing in the tag, select **Create Tag**

       ii. After you have added all of the tags you wish to add to the animal IDs, select an animal ID and select which tag(s) are appropriate for that animal. Do this for all animals in the experiment.

   c. **Measurements** – Under the "Measurements" tab, you can make new equations for different measurements.

5. Once you have added the experiment(s) to the databench; locate the **Data** icon at the top of the screen and select it. This will open the raw data produced be the Oxymax system.
   a. You can click on a specific parameter to view the results of all animals for that particular parameter.
   b. You can also click on an animal number to view all of the raw data for each parameter for that particular animal.
   c. You can select the ALL box to view all data for all animals.
6. To copy the data to add to another program (Excel, Prism…) select **Export** and **Copy to Clipboard**. Open the program that you want to insert the data into, right click on the page, and select **Paste** (as if you would copy and paste like anything else).
7. Next to the "Data" icon at the top of the screen, there are other ways to look at the data including Statistics, Time Graphs, Box Plot, Histogram, and Correlation. Use these tools to help visualize, understand the data, and troubleshoot if necessary.
   * NOTE: you can not use the Time Graph to compare two different experiments as of May 1, 2010. Columbus Instrument personnel are working on updating the CLAX program to allow for this in the future

Gas Measurements

Oxymax for Windows records the same type of measurements for each gas Oxymax monitors. For simplicity's sake, rates for gases that are consumed (Oxygen) and those that are produced (Carbon Dioxide, Methane, etc) are both presented as positive measurements. In the explanation of measurements below, "x" takes the place of "O2" (Oxygen), "CO2" (Carbon Dioxide) or "CH4" (Methane).

Vx – The rate of gas consumption/production normalized to animal body mass. The units for this measurement are selectable, but are typically displayed in mL/kg/hr.

xIN – The measured concentration of room air taken at the last reference. The generally excepted values for room air concentrations are:

Oxygen – 20.91%-20.97%: Readings greater than 20.97% are usually the result of an insufficiently long warm-up period or, in the case of the Fuel Cell Sensor, a significant increase in ambient temperature since calibration.

Carbon Dioxide – 0.040%-0.100%: This concentration depends highly on the air quality in the region in which the system is installed. It also depends on the turnover rate for the ventilation system and the volume of the room compared to other CO2 contributors like people. Values slightly below 0.040% are acceptable, but no environment should read 0.000% unless a chemical CO2 scrubber is being used.

xOUT (mL/kg/min)– The measurement of chamber air taken for the sample. Chamber concentrations will vary depending on room air concentration, subject contributions and fresh air flow.

Dx – The difference (delta) in concentration between the xOUT and xIN values. Controlling the fresh air flow to the chambers can be used to move these values into the optimal ranges for the sensors. If the difference between the input and output concentrations is too small, it's difficult to measure with any degree of certainty. If the difference is too high, the air will be too "thin" for the subject to breathe regularly and will have an impact on its respiration.

ACCx – The total volume (in liters or milliliters) of gas consumed or produced by the subject.

RER – "Respiratory Exchange Ratio". The unit-less ratio of the rate of Carbon Dioxide production to the rate of Oxygen consumption. When the subject is metabolizing pure fat for energy its RER is close to 0.70. When the subject is metabolizing pure carbohydrates for energy its RER is close to 1.0. The subject's RER can exceed 1.0 during intense exercise or while synthesizing fat from carbohydrates.

Heat (CAL/HR/kg) – The measure of energy expenditure for a subject. This is presented either as a whole-subject or mass-normalized rate, either in kilocalories or kilojoules.

Flow – The rate of fresh air delivery to the chamber in liters per minute (LPM). The target fresh air flow rate is based mostly on subject mass, but typical values are:
> **Mice** – 0.50-0.60 LPM
> **Rats** – 2.0-3.5 LPM

Metabolic Calculations
The metabolic data reported by Oxymax is based on five empirical measurements:

Gas concentrations measured by sensors:

Reference Oxygen Concentration	(O2i)
Reference Carbon Dioxide Concentration	(CO2i)
Sample Oxygen Concentration	(O2o)
Sample Carbon Dioxide Concentration	(CO2o)

Fresh Air Flow
The fresh air flow measured by Oxymax for Windows depends on the ventilation system used. Positive ventilation systems throttle and measure the fresh air before delivering it to the chambers (Vi). Negative ventilation systems draw fresh air into the chamber from the atmosphere around it. The measured flow in this case is not the rate of air entering the cage, but exiting it (Vo).

The calculation of Oxygen consumption (VO2) and Carbon Dioxide production (VCO2) values requires the use of both the input (Vi) and output (Vo) flows to the chamber. Despite the fact that the chamber is not pressurized and does not allow gas to build up as an experiment progresses, the chamber cannot be modeled as a perfect steady state system because of the subject's unequal effect on the respiratory gases: the subject does not exhale the same volume of gas as it inhales.
Gases in an Oxymax system are broken into three categories:

Consumed – Oxygen
Produced – Carbon Dioxide, Methane
Inert – All other gases not involved in respiration (Nitrogen, Argon, and other noble gases)

Since inert gases are not used during respiration, their rates of flow at the output (VNo) and input (VNi) are the same (Equation 1).

$$VN_i = VN_o$$

The flows of inert gases are products of the flows to and from the chamber and the concentration of inert gases (Ni and No) in each flow (Equation 2). The concentrations can be calculated and one of the flows is measured so the other (Vo for positive ventilation, Vi for negative ventilation) can be calculated.

$$V_iN_i = V_oN_o$$

The concentration of inert gases in a sample is assumed to be all gases that are not involved in respiration (Equation 3).

$$N = 100\% - O_2 - CO_2$$

Assuming a positive flow system, combining the previous equations yields the Vo for the system.

$$V_o = V_i [(100\% - O_{2i} - CO_{2i})/(100\% - O_{2o} - CO_{2o})]$$

The volumetric flow rates for consumption and production can then both be calculated. The equations are arranged such that both consumption and production are presented as positive values.

$$VO_{2, Subject} = V_i O_{2i} - V_o O_{2o}$$

$$VCO_{2, Subject} = V_o CO_{2o} - V_i CO_{2i}$$

$$VCH_{4, Subject} = V_o CH_{4o} - V_i CH_{4i}$$

Oxymax for Windows always presents consumption and production rates normalized to the subject's body mass in kilograms. To convert the whole-body rates into mass-normalized rates just divide the whole-body rate by the mass (m) of the subject in kilograms.

$$V_{x, norm} = (V_{x, subject} / m)$$

The accumulated respiration figures (A) are calculated using the whole-body rate and the amount of time that has elapsed since the last measurement (t). The amount of gas consumed or produced in an interval is added to a running total of all sample volumes. Units are adjusted to match the ones specified in the experiment settings (Equation 9).

$$A_x[n] = A_x[n-1] + V_{x, subject}^{t}$$

The respiratory exchange ratio can be calculated from either set of consumption and production rates (Equation 10).

$$RER = VCO2_{subject} / VO2_{subject} = VCO2_{norm} / VO2_{norm}$$

Oxymax for Windows uses one of two possible methods for calculating energy expenditure (Heat). The first uses a "calorific value" (CV) that shows the relationship between heat and the volume of consumed Oxygen. This value is derived from empirical data from Graham Lusk's "The Elements of the Science of Nutrition" (Equation 11).

RER	Kilocalories per liter of O2
0.707	4.6862
0.75	4.7387
0.80	4.8008
0.85	4.8605
0.90	4.9226
0.95	4.9847
1.00	5.0468

$$CV = 3.815 + 1.232 * RER$$

The rate of energy expenditure can then be calculated using the subject's rate of Oxygen consumption (Equation 12).

$$Heat = CV * VO2_{subject}$$

Oxymax for Windows also allows for the definition of a custom heat calculation equation. Coefficients for Oxygen, Carbon Dioxide and Methane rates can be specified and used to calculate heat (Equation 13).

$$\text{Heat} = (K_1 * \text{VO2}_{\text{subject}}) + (K_2 * \text{VCO2}_{\text{subject}}) + (K_3 * \text{VCH}_{4,\text{ subject}})$$