Instruction Manual

MM-100

Metabolic Monitoring System

Read instructions carefully before operating this device.

1. *This device is not to be used for Human Life Support applications.*
2. *To avoid possible electrical shock, do not operate this device if is wet or has had liquids spilled onto it.*
3. *Service or calibration procedures should only be performed by qualified personnel familiar with the electrical hazards of line-powered devices.*

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IF THIS INSTRUMENT FAILS WITHIN A PERIOD OF ONE YEAR FROM THE DATE OF DELIVERY OR INSTALLATION, CWE, INC. WILL, AT ITS OPTION, REPAIR OR REPLACE IT FREE OF CHARGE TO THE PURCHASER. THIS WARRANTY EXCLUDES DAMAGE INCURRED THROUGH ABUSE OR ACCIDENT AND CONSUMABLE ITEMS OR COMPONENTS SUCH AS BATTERIES. CWE, INC. DOES NOT ASSUME ANY LIABILITY FOR ANY CONSEQUENTIAL DAMAGES RESULTING FROM THE USE OR MISUSE OF THIS INSTRUMENT. THIS WARRANTY IS APPLICABLE ONLY TO THE ORIGINAL PURCHASER OF THE INSTRUMENT AND IS NON-TRANSFERRABLE.

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Instruments manufactured by CWE, Incorporated are not authorized for use as critical components in human life support devices or systems. "Life support devices or systems", as used herein, are devices or systems whose failure to perform, whether through misuse, failure, or proper operation, can reasonably be expected to result in significant injury to the operator or subject persons.

USER INFORMATION:

Base Unit Serial Number: _____ Expansion Unit:_____

Internal MM-100 Software Revision: _____

MM-CommVS Software Revision: _____

Media supplied: CD_____ USB Drive_____

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Figure 1: MM-100 Metabolic Monitor front panel

1.0 INTRODUCTION

The **MM-100 Metabolic Monitor System** is an accurate, easy-to-use instrument for measuring oxygen consumption (VO_2), carbon dioxide production (VCO_2), and Respiratory Exchange Ratio (RER) in small animals. The device is completely self-contained, with all the required sensors, sampling pump, and electronics housed in one unit. The only required external equipment is an air source, the measurement chamber, and a monitoring computer.

When expanded to multi-animal monitoring (using the MMX-8 expansion unit), the gas measurements are still made by the host MM-100. Air flow through each cage is measured in the expansion unit. This plan ensures reproducibility and direct comparison between animals, since all measurements are made with the same set of calibrated sensors. The expansion unit switches the gas sample as required between the cages and performs the airflow measurements.

Oxygen consumption (VO_2) is measured by monitoring O_2 and CO_2 concentrations at the inlet and outlet of the sample chamber, while passing a known flow of fresh air through it. The drop in O_2 concentration and rise in CO_2 in the chamber effluent air is proportional to O_2 consumed (or CO_2 produced) by the animal. See ***Appendix A*** for a technical description of the principal calculations.

The MM-100 system uses the *dry* technique for gas measurement and subsequent computations, so water vapor must be removed from the sampled gas using a desiccant. A high-resolution paramagnetic sensor is used to measure O_2, and CO_2 is measured by a single-beam infrared analyzer. Airflow to the chamber is measured by a thermal mass flowmeter. All the measurements are digitized and sent to a computer, where the VO_2 and VCO_2 results are computed and displayed using the supplied MM-CommVS software package. Analog signals for all the measurements are also available on rear panel jacks.

The MM-100 features an External Control Interface that allows a computer to control the sequencing of measurements and collect the resultant analog data measurements. Windows software (MM-CommVS) is provided to display the metabolic measurements and save the data to disk files. The generated data files are in a form (CSV) that can be easily pasted into a spreadsheet for further analysis.

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2.0 QUICK START GUIDE

We recommend reading through this entire manual before attempting to use this instrument. However, this section provides an abbreviated guide to setting up an MM-100 system. Steps marked with an asterisk (*) and in *italics* apply to multi-chamber setups (designated here MMX). Please refer to later sections of this manual for details on any procedure.

1. Equipment set-up: Place MM-100 unit, air pump, and animal measurement chamber in a convenient location. The monitoring computer should also be nearby.

** Place MMX expansion unit next to, or underneath, MM-100. Locate measurement chambers within 6' of expansion unit.*

2. Connect the tubing: Connect air pump outlet to **FRESH AIR INLET** on MM-100 rear panel; connect cage **INLET** to **FLOW TO CAGE** port on MM-100 rear panel; connect cage sample port (side arm of "T") to **SAMPLE INLET ON** MM-100 rear panel

** Connect air pump to **FRESH AIR INLET** on MMX rear panel; connect chamber **INLETS** to corresponding **FLOW TO CAGE** ports on MMX rear panel; connect chamber sample ports to corresponding **SAMPLE INLETS** on MMX rear panel; connect MMX **SAMPLE OUTLET** to MM-100 **SAMPLE INLET**.*

3. Connect Dri-Tech Desiccant Chamber or desiccant tube:

Connect CWE's Dri-Tech Desiccant Chamber to the MM-100 using the enclosed Dri-Tech Instruction Manual. Alternatively, connect a fresh desiccant tube on the side of the MM-100. Be sure that the small Acrodisk hydrophobic filter is connected between the desiccant chamber or tube and the side panel tubing port (near the rear panel).

> ***CAUTION:* NEVER operate the MM-100 without this filter in place as it protects the internal gas sensors from liquid water or particulates.**

4. Connect the cables: Connect the 9-pin M/F cable between the **RS232** port and your computer serial port. If your PC has no serial port, use the supplied USB-Serial adapter. Plug the thermistor probe into rear-panel jack and insert its tip into the holder on top of the measurement cage.

** Connect DB25 cable between **EXPANSION** ports on MM-100 and MMX. Connect thermistor probe to any of the chambers in use.*

5. Install the software: Insert the MM-CommVS USB Drive in your computer. If the install program does not auto-start, then browse to setup.exe on the supplied media. Follow the prompts to install the program.

6. Start MM-CommVS: From the start menu, choose programs, and locate MM-CommVS. Select it to run the program.

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7. Configure COM port: Locate the COM PORT textbox and select the appropriate COM PORT number. The textbox dropdown menu will show all active COM ports on your computer, so be sure to select the one connected to the MM-100. The program will not work if the correct COM port is not selected!

8. MM-CommVS tips: You can change the vertical scaling using the Y SCALE control at the bottom of the graphic window.

9. Power up the MM-100: Turn on the MM-100 power using the rear panel switch. After a few introductory messages, the LCD will display the measurement screen. The **SENSOR NOT READY** LED will stay ON until the CO_2 and flow sensors are warmed up (about 5 - 10 minutes).

** MMX will power up automatically and begin its warmup sequence.*

10. Calibration: After the unit is warmed up, an initial automatic 5 minute calibration will occur. During this time, you can adjust **O2 HI** until O_2 reads about 20.950%, and **CO2 LOW** until CO_2 reads about 0.040%. DO NOT adjust **O2 LOW** or **CO2 HI** unless you have a suitable calibration gas.

NOTE: You can take as much time as you need during these gas calibrations, since the 5 minute timer will be paused when any of the gas calibration functions are active.

11. Ready to go! Following the auto-calibration phase, adjust the cage flow to about 650 ml/min (mouse) or 1500 ml/min (rat). Place the animal in the measurement chamber and close the lid.

** With multiple chambers set up, switch MMX to **LOCAL.** Using the **MANUAL SELECT** pushbutton, cycle through the chambers, and set airflow for each one as above.*

12. Monitoring: In the MM-CommVS program, click the START button to begin monitoring. You should see data appear in the data window. If nothing happens, check that the correct COM port is set.

13. Operating tips: 1. Adjust the cage flow to achieve a CO2 reading of about 0.25–0.35%. Allow a few minutes for gas readings to stabilize after adjusting flow. In single-chamber mode, the unit will automatically calibrate itself periodically.

** When using the MMX expansion unit, the unit will automatically calibrate itself after cycling through all active chambers.*

2. You can adjust gas calibrations at any time by pressing the **CAL/MONITOR** switch. Calibration mode switches the valves, so the MM-100 reads gas from the **REFERENCE AIR INLET** (normally open to room air). Whatever cage flows have been set up will not be disturbed during calibration. If you change any of the gas calibrations, allow a minute or so for the

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reading to stabilize before exiting calibration mode. The O_2 and CO_2 values are recorded at the end of calibration and used as “ambient air” values for the metabolic calculations.

3.0 DETAILED SETUP (SINGLE CHAMBER SYSTEM)

The following instructions apply to a single-chamber system, with data being displayed and recorded on a PC.

1. Install the MM-CommVS software on your PC. Insert the media (CD or USB Drive) into your computer and run Setup.exe. We recommend accepting the default directories. The program will thus be installed in: C:\Users\”name”\Source\Repos\MM-CommVS. The same folder is where your data and configuration files will be stored.

2. Plug the power cord into the rear of the unit and into a suitable grounded power outlet. Connect the serial port cable from your computer (or USB-Serial adapter) to the 9-pin **RS232** port on the rear panel of the MM-100.

3. Connect your fresh air source (air pump, gas tank, etc.) to the **FRESH AIR INLET** on the rear panel. Connect the **FLOW TO CAGE** port to the **INLET** port on the measurement cage. Use 1/8” ID tubing for these connections. See Figures 2 and 4.

4. Connect a “T” connector to the cage **OUTLET** using a short piece of tubing. Attach a length of tubing between the unused leg of the “T” connector and the **SAMPLE INLET** port on the rear panel. This connection requires a male Luer adapter. Interpose a Nafion® sample line in this path. The Nafion® tubing is a membrane product that passes water vapor, thus helping to dry the sample gas. Use 1/16” ID Tygon tubing for the gas sample tubing.

5. Plug the provided YSI-402 Thermistor Probe into the **TEMPERATURE PROBE** jack on the rear panel of the MM-100. The probe tip should be inserted in its holder on the lid of the monitoring chamber. This provides ambient temperature measurement for the metabolic calculations.

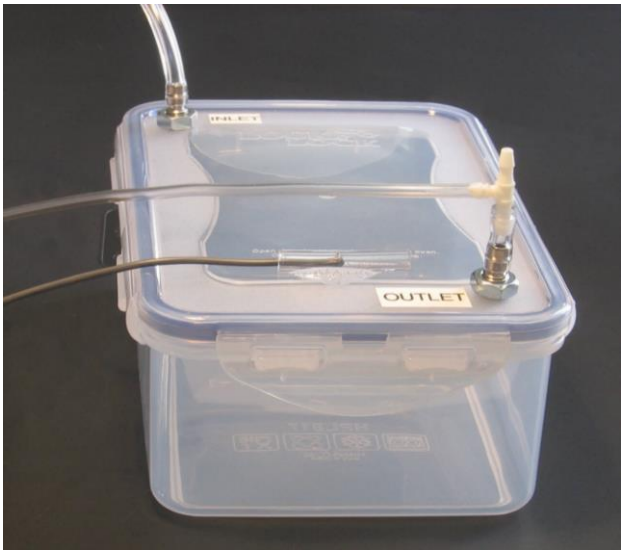

Figure 2: Mouse cage showing airflow & sample connections & thermistor probe attachment.

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6. Prepare and connect the Dri-Tech Desiccant Chamber to the MM-100 following the instructions in the manual included with the MM-100 system. OR, ***in place of*** the Dri-Tech Desiccant Chamber, you can install a fresh desiccant tube on the side of the MM-100. It is recommended that a spare desiccant tube be kept handy for replacement as needed.

Figure 3: Side view of MM-100 showing desiccant tube. Required in-line filter to the right. The Dri-Tech Desiccant Chamber connects in the same manner to the two ports.

7. When everything has been connected, turn on **POWER** using the rear-panel switch.

8. After the unit has warmed up and is in its initial calibration mode (blue **CALIBRATING** LED blinking), you can adjust the **O2 HIGH** so the display reads about 20.950%, and the **CO2 LOW** adjustment so CO_2 reads about 0.040%.

8. After the calibration ends, adjust the **CAGE FLOW** appropriately (about 650ml/min for mouse, 1500ml/min for rat).

9. With the animal in the cage, check that the CO_2 reading is in the range 0.25 – 0.35%. Adjust cage flow as necessary to get a steady CO_2 reading within this range. Note that it will take up to several minutes for the reading to stabilize after changing the cage flow because of the time it takes to flush out the sample tubing path.

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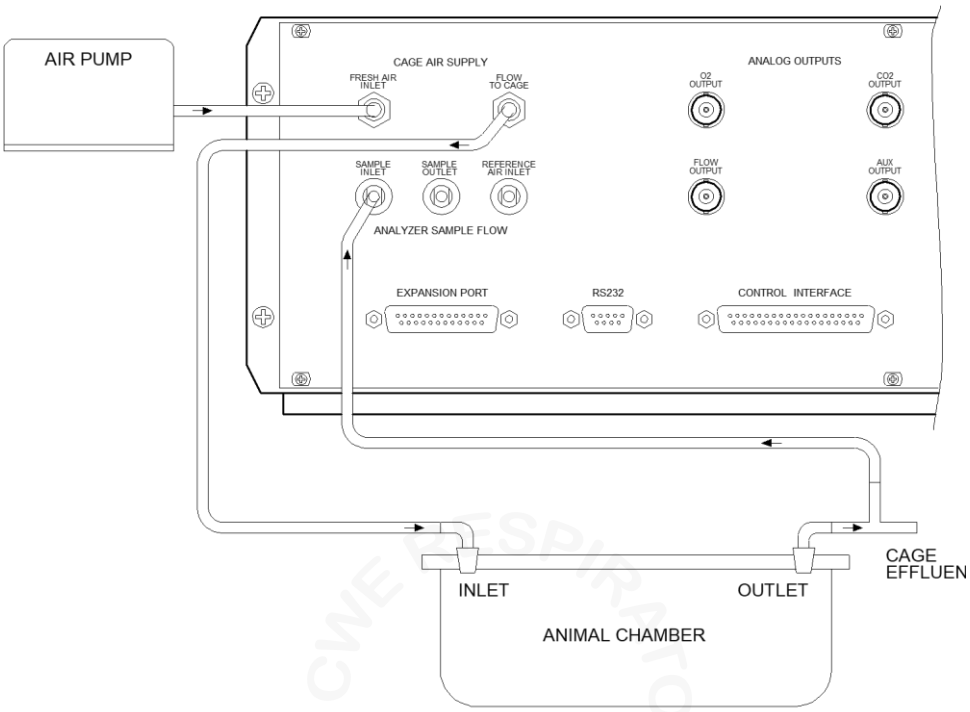

Figure 4: MM-100 single-animal tubing connection diagram

3.1 DETAILED SETUP (MULTI CHAMBER SYSTEM)

Setting up the MMX-8 is similar to the single-chamber setup but uses more tubing. The connections to the measurement chambers are the same but are multiplied by the number of chambers in use. The following instructions serve as an addendum to the single-chamber instructions above.

1. Install the MM-CommVS software as above.

2. Connect the cables as above. Additionally, connect the supplied DB25 cable between the expansion port connectors on the MM-100 unit and MMX.

3. Connect the cage airflow tubing and sample tubing between the MMX and the measurement chambers as shown in Figure 5. Repeat for all the measurement chambers being used.

4. Connect the **SAMPLE OUTLET** on the MMX to the **SAMPLE INLET** of the MM-100 using at least one 2' length of Nafion® sample tubing. Male Luer connectors are required at each end of this tubing set.

5. Connect the thermistor probe as above and insert the probe tip into the holder on any of the chambers in use (all are assumed to be at the same temperature).

6. Be sure that a fresh Dri-Tech Desiccant Chamber OR a fresh desiccant tube is connected on the side of the MM-100. It is recommended that a spare desiccant tube be kept handy for replacement as needed.

7. When everything has been connected, turn on **POWER** using the rear-panel switch.

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8. After the unit has warmed up and completed its initial calibration, adjust the **CAGE FLOW** (about 650ml/min for mouse, 1500ml/min for rat) for each animal being monitored. This can be accomplished by switching the MMX to **MANUAL** and using the **SELECT** switch to step through the cages. Adjust the cage flow individually for each animal using the front panel **CAGE FLOW ADJUST** knobs. When finished adjusting all cage flows, switch the **MANUAL/AUTO SWITCH** back to **AUTO**. The MMX will automatically resume monitoring where it left off.

9. The instrument can now be “fine-tuned” to your particular configuration and animals. If monitoring fewer than the available cages (e.g., 4 animals on an MMX-8 Eight Cage Expansion Unit), use the **#CAGES** function to enter the appropriate number. Use the **MON TIME** function to select the monitoring time for each cage, as well as the calibration time (see detailed descriptions of each of these functions).

10. Check calibration as required. See instructions for using the MM-CommVS software to begin collecting data.

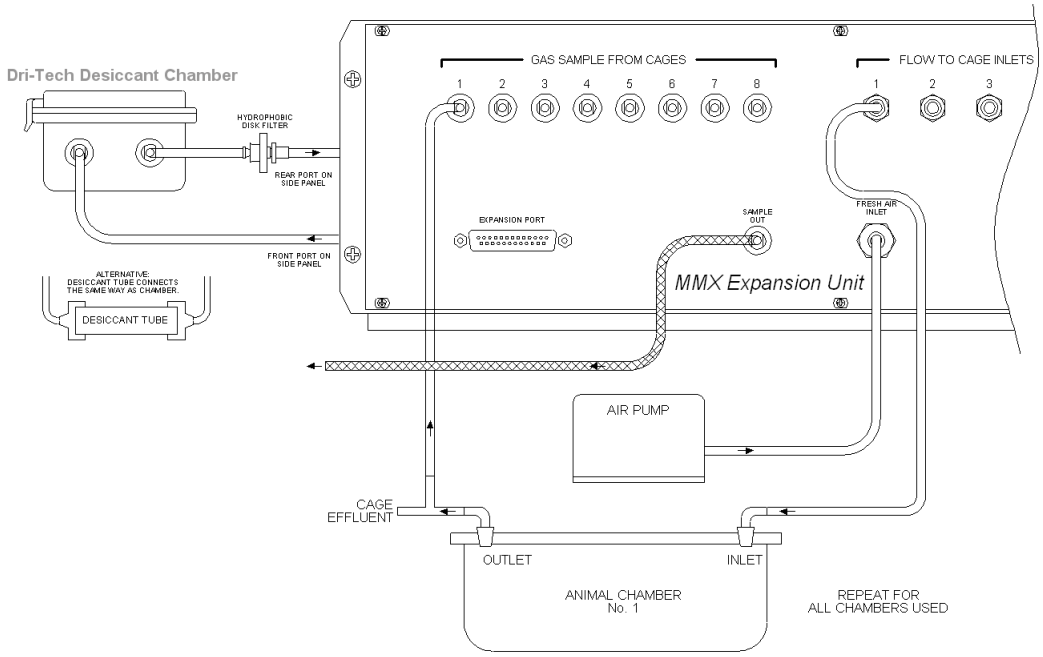

Figure 5: Expansion unit (MMX-8 shown) tubing connection diagram.

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4.0 OVERALL OPERATION

Here is a narrative description of how the MM-100 Metabolic Monitor System operates. This assumes a multi-cage system, but the same principles apply to a single-cage setup. Details of any given procedure can be found elsewhere in this manual.

The MM-100 uses one set of high-performance sensors for O_2, CO_2, and airflow. These are the basic measurements required for oxygen consumption calculations. In addition, cage temperature and barometric pressure are measured, which are used to correct and standardize the measurements. These sensors are multiplexed; i.e., they are sequentially connected to each cage in turn. Cage airflow is always maintained to each cage and is never interrupted during the multiplexing process.

Startup:

After the MM-100 has just been powered on, it will automatically wait for the flow and gas sensors to warm up and stabilize. This normally takes between five and ten minutes. After the sensors are stabilized, an automatic five-minute calibration begins. During this period the user can make minor adjustments to the gas calibrations so the readings are correct for atmospheric O_2 and CO_2. At the end of this auto-calibration, the current O_2 and CO_2 readings are saved, and considered to be the “ambient air” values. Monitoring of the measurement cages begins.

Cage Cycling:

Each cage is monitored for a specific period, the **MON TIME**. After this time has elapsed, the next cage is selected for monitoring. It takes a few minutes for the sensor flow path to flush out before a new reading is stable.

After all selected cages have been monitored, an automatic calibration occurs. As with the initial auto-calibration, this allows the sensors to read and save the room air values. This technique allows the MM-100 to automatically correct for any drift in sensor readings over the long term, since the readings and calculations are always referenced to the recently recorded “ambient air” values. The user does not need to do anything during these auto-calibrations, although the gas calibrations can be adjusted if required.

> NOTE: After pressing **MON TIME** pushbutton and setting the *monitoring dwell time* for each cage, you can press the **TEST** pushbutton which will display and adjust the *calibration time*, which should normally be set to be 60 or 120 seconds longer than the monitor time.

Calibration:

Auto-calibrations, as the name implies, occur automatically without any user intervention. As described above, these occur after a cold start and also after the complete set of cages have been monitored. The calibration time for the inter-cage calibrations is adjustable and should be made just long enough for a complete

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flushing of the sample path, and to achieve stable room air readings.

Manual calibration can be initiated by the user at any time during normal monitoring by pressing the **CAL/MONITOR** button. When this mode is selected, the user can perform major gas calibrations, changes in setup, etc. The instrument stays in this mode until the user presses the **ADJUST** knob to exit.

At the time calibration ends (whether auto or manual), the current O_2 and CO_2 values are stored and considered as “ambient air” values for metabolic calculations. For this reason, the user should always take care that stable room air values are displayed before exiting calibration.

General considerations:

The MM-100 displays the main measurements screen at most times. This shows all the current measurements for gasses, flow, temperature, and barometric pressure. When any particular function is active, such as a gas calibration, the active function will be indicated by the small LED next to the pushbutton. In addition, a user prompt will appear on the display advising the user to exit that function after it is completed. During normal monitoring operation, NO small LED’s should be on!

Error checking:

The MM-100 continuously evaluates its current state and will warn the user if attention is required. For the safety of your animals, the **LOW CAGE FLOW** warning is most critical. This warning light will blink, and the instrument will beep to alert the user that the fresh air flow to the cage is too low. This condition should be corrected immediately.

If there is an obstruction in the gas sample flow path, the **CHECK SENSOR FLOW** light will blink. The most common cause for this is a blocked hydrophobic disk filter. It is a good idea to have some spare disk filters available when the MM-100 is in operation.

Software:

The supplied MM-CommVS software is used to monitor current values of the gasses, air flow, temperature, etc., that are sent once-per-second from the MM-100. This software application also performs the metabolic calculations, graphically displays them, and can save data to disk for further analysis. Each saved data set is time-stamped and marked with the cage number providing the measurements. See Section 11.0 for a complete description of this software.

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5.0 MAINTAINING THE DESICCANT (CHAMBER OR TUBE)

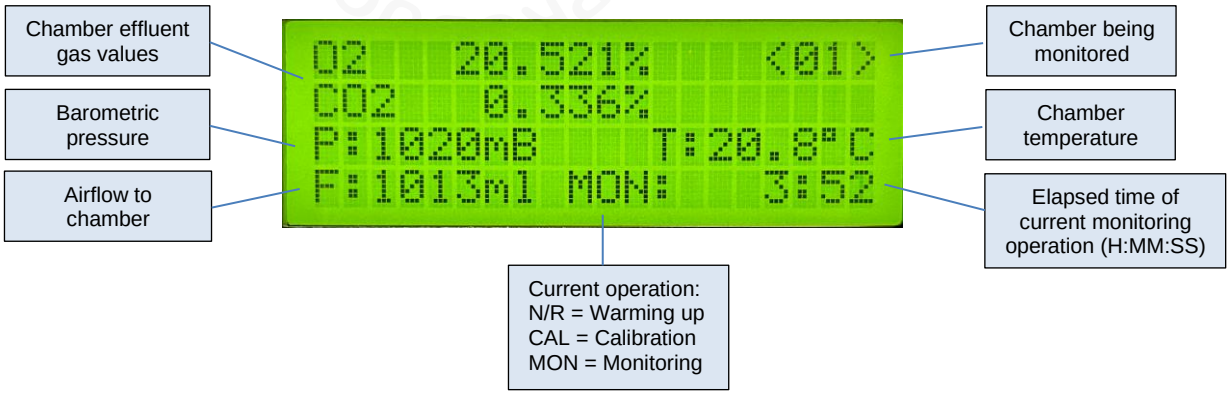
For accurate measurements, it is essential that the gas sample be dry. This is accomplished with the external Dri-Tech Desiccant Chamber (or a desiccant drying tube) that is connected to the side of the instrument. One Dri-Tech Desiccant Chamber with fresh desiccant medium will typically last approximately 10 – 18 hours (or more) before it needs to be changed. If using Drierite™ note that it starts out blue and turns pink when it is saturated with water. The Drierite™ material can be reactivated repeatedly by heating in a warm oven. See ***Appendix C*** for details.

Another desiccant medium besides Drierite™ that we recommend is *Molecular Sieve*. This material has very strong hydrophilic properties and does not interfere with gas measurements. This material is available as a granular form with an indicator that reveals water saturation.

6.0 FRONT PANEL DISPLAY AND CONTROLS

6.1 LCD PANEL

The LCD display is the primary indicator of current measurements and system status. This display during normal **MONITORING** is shown below:



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The first two lines display the current O_2 and CO_2 values. The third line shows the barometric pressure in millibars and the chamber temperature in degrees Centigrade. The bracketed number at top right shows the measurement chamber being monitored. The elapsed time (H:MM:SS) of the current operation is shown at bottom right. Airflow is displayed at bottom left.

During **calibration**, the chamber number will be shown as <00> and **CAL** will appear at lower right showing that ambient air is being sampled. During user-initiated setup or calibration procedures, the display will be modified from that shown above.

6.2 INDICATOR LIGHTS

The row of indicator lights on the front panel calls attention to several conditions:

POWER indicates whether or not the instrument is powered on.

LOW CAGE FLOW indicates that fresh airflow through the measurement chamber has dropped below 100 ml/min. This should be corrected immediately as the animal can be harmed by insufficient fresh air flow.

CHECK SENSOR FLOW indicates that there may be a blockage in the sample flow through the analyzer cells. This flow is shown on the **SAMPLE FLOWMETER** at the right of the front panel. The cause of this condition may be a kinked sample line, a saturated desiccant tube, or a plugged Acrodisk hydrophobic filter. These filter disks are located in three places: in series with the desiccant chamber or drying tube (side panel), and at the rear panel **SAMPLE INLET** and **REFERENCE INLET** ports. Replace these filters when required.

SENSOR NOT READY indicates that the sample cells have not yet reached operating temperature. This will normally go out within five minutes or so after power on.

EXPANSION UNIT IN USE shows whether an expansion module is attached. For a single-chamber system this light will normally be off.

CALIBRATING indicates that the unit is in calibration mode.

6.3 SYSTEM CONFIGURATION AND CALIBRATION

On the left side of the front panel are eight pushbutton switches, grouped according to function. These switches are used to activate specific operations and are used in conjunction with the **ADJUST** knob. For most operations, pressing the **ADJUST** knob will exit the procedure. Each of these functions is described in more detail below. When any of these functions is active, the small red LED next to the switch will be illuminated and will go off when the function is exited. Remember that for normal monitoring to occur, all of these LED’s should be off.

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6.31 O2 and CO2 CALIBRATION PUSHBUTTONS

Pushing any of these four buttons selects the labelled function: O_2 high or low calibration, CO_2 high or low calibration. Selecting any of these functions automatically sets the sampling valves to the **REFERENCE AIR** position; i.e., the instrument will be drawing room air (or calibration gas, if connected) into the **REFERENCE AIR INLET** on the rear panel. Once a function is activated, connect the appropriate calibration gas (or room air) to the **REFERENCE AIR INLET**, and rotate the **ADJUST** knob until the desired reading appears on the display. Press the **ADJUST** knob to exit.

NOTE: When switching between CALIBRATION MODE and MONITOR MODE, or vice-versa, remember to allow several minutes for the readings to stabilize with the new gas. This is due to the flushing time required by the sample tubing, drying tube, etc.

CAUTION: NEVER apply pressurized gasses directly to the REFERENCE AIR INLET. This will result in erroneous readings and can permanently damage the gas sensors. ALWAYS allow the MM-100 to draw in the calibration gas at its own rate from a stream of gas. This can easily be accomplished with a simple "T" arrangement at the sample port. Just supply an excess of calibration gas past the "T" connected to THE REFERENCE AIR INLET.

This display during normal **CALIBRATION** is shown below. Note that you must allow enough time for the room gas readings to stabilize before making any adjustments.

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6.311 CO2 LOW CALIBRATION

CO2 LOW is used to set the low calibration point for CO_2 measurement. Typically, this is set to about 0.044% when sampling atmospheric air.

6.312 CO2 HIGH CALIBRATION

CO2 HIGH is used to set the high calibration point for CO_2 measurement. A calibration gas in the range of 0.25 – 0.5% CO_2 is required for this. Connect this gas to the **REFERENCE AIR INLET** port on the rear panel. Do not apply pressurized gas directly to this input (see ***CAUTION*** above).

6.313 O2 HIGH CALIBRATION

O2 HIGH is used to set the high calibration point for O_2 measurement. Allow the **REFERENCE AIR INLET** to sample room air and adjust the reading to approximately 20.950%.

6.314 O2 LOW CALIBRATION

O2 LOW is used to set the low calibration point for O_2 measurement. This requires a calibration gas with zero O_2 concentration such as nitrogen. Allow the **REFERENCE AIR INLET** to sample this gas and adjust the reading to approximately 0.001 - 0.010% (i.e., slightly above zero).

> ***NOTE: Setting the O_2 low or CO_2 high calibrations REQUIRES a suitable calibration gas. A mixture that can be conveniently used for both calibrations is 0.25% or 0.50% CO_2, balance nitrogen.***

6.32 SYSTEM SETUP FUNCTIONS

The **#CAGES** and **MON TIME** pushbuttons are used to set up basic system parameters including: the number of animals being monitored, the monitoring time for each animal, and the calibration time. Each of these functions is described below. As with the gas calibration functions, these are selected using the labelled pushbutton, adjusted using the **ADJUST** knob, and exited by pressing the **ADJUST** knob.

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6.321 SETTING THE NUMBER OF CAGES

The **#CAGES** pushbutton is used to program the maximum number of animals to be monitored. This can be set from one to the maximum available on the MMX expansion unit. For example, using an MMX-8 allows 1 – 8 animals to be monitored.

6.322 MONITOR TIME SETTING

The **MON TIME** pushbutton is used to set the monitoring time applied to each cage. In multi-cage systems, each animal is monitored for this time before stepping to the next cage. After all cages have been monitored, the unit automatically enters into a calibration period (see below). In single-cage systems (no MMX expansion unit attached), **MON TIME** sets the time the animal is continuously monitored before an automatic calibration occurs.

6.323 CALIBRATION TIME SETTING

The **CALIBRATION TIME** can also be set here. With **MON TIME** selected, press the **TEST** switch. The display will prompt you to change or accept the current calibration time. Set this time using the **ADJUST** knob and press the **ADJUST** knob to exit. Press the knob again to exit the **MON TIME** screen.

> ***NOTE:* Both MON TIME *and* CAL TIME *should be long enough to flush the sample path and achieve a stable gas reading. This time depends on the sample flow rate, the amount of tubing between the measurement chambers and the instrument, and the type of desiccant used. For an in-line Drierite™ tube, allow 5-10 minutes (or until readings are stable); for the Dri-Tech desiccant chamber, about 3 minutes (180 seconds) is adequate.***

6.324 CAL / MONITOR SWITCH

The **CAL / MONITOR** switch is used to manually select calibration mode. Pressing this switch causes the MM-100 to enter calibration mode, which is indicated by the blinking blue **CALIBRATING** LED. A message is also displayed on the LCD screen to alert the user that he must press the **ADJUST** knob to exit this mode and return to automatic monitoring.

In this mode, the gas being measured enters through the **REFERENCE AIR INLET** on the rear panel. The calibration gas (or room air) is thus applied to this inlet port when calibrating. During calibration, any of the gas calibration or system setup functions can be activated.

After performing any of the gas calibrations while in this mode, allow the instrument to sample room air until a stable reading is shown before exiting

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via the **ADJUST** knob. The room air values are stored and used in the metabolic calculations as “ambient air” values.

6.4 CAGE AIRFLOW ADJUST

This knob adjusts the needle valve that controls the fresh airflow through the animal measurement chamber. The resulting flow measurement is shown on the LCD panel. Adjust this as required to maintain normal gas readings as described in Section 3.0 above. Note that on multi-chamber systems, the cage airflow is set individually using the corresponding needle valve on the expansion unit.

6.5 SAMPLE FLOWMETER

This flowmeter is used to set and monitor the sample flow through the gas measurement cells. A typical setting will be 100 ml/min. Using lower flows will extend the life of the desiccant, since less moisture-laden air will be drawn through them but increases the response time of the system.

7.0 REAR PANEL CONNECTIONS

7.1 CAGE AIR SUPPLY

The fresh air supply from the air pump is attached to the **FRESH AIR INLET** port. This port uses a quick-connect fitting designed for use with ¼” OD semi-flexible tubing. An adapter is supplied so you can use standard Tygon flexible tubing for this connection. To release this connection, push in the orange locking ring to release the tubing (or adapter).

The fresh air flow to the animal cage is attached to the **FLOW TO CAGE** port. Connections to these ports are typically made using 1/8” ID (3mm) flexible tubing.

7.2 GAS SAMPLE CONNECTIONS

Single-cage system:

The effluent gas from the animal chamber is connected to the **SAMPLE INLET** port using a Luer-lock connector and flexible tubing. The **REFERENCE AIR INLET** is sampled during calibration and is normally left open to room air. The **SAMPLE OUTLET** is where the analyzed gas exits and is also normally left open to atmosphere.

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Multi-cage system:

The effluent sample port on each cage should be connected to its corresponding **SAMPLE INLET** on the MMX expansion unit rear panel. Attach a Nafion™ tube from the **SAMPLE OUTLET** on the MMX to the **SAMPLE INLET** on the MM-100 rear panel. The gas sample path is thus from the animal cages to the MMX expansion unit, where valves direct it to the main sample inlet on the MM-100 for measurement.

7.3 ANALOG OUTPUT JACKS

The three primary measurements, O_2, CO_2, and Flow are available using the **O2 OUTPUT**, **CO2 OUTPUT**, and **FLOW OUTPUT** jacks, respectively. When using any MMX multi-chamber expansion unit, the primary flow signal is provided on the **EXTERNAL FLOW OUTPUT** jack. Additionally, temperature is available using the **AUX OUTPUT** jack. These signals can be externally monitored using a data acquisition system or other recording device. The scaling of these signals is given under the **Specifications** section.

7.4 CONTROL INTERFACE (DB37)

The required cable connector is a DB37 male. This connector is used to remotely control the MM-100 in multi-chamber configurations. It also provides the five measurements, O_2, CO_2, Flow, Temperature, and Barometric Pressure. The purpose of this interface is to provide a means for a remote data acquisition card to read the measurements while controlling the chamber being monitored. In single-chamber systems, it provides a convenient source of the three measurements using one connector. The analog and digital signals are supplied on the following pins. Note that the barometric pressure signal is ONLY available on this connector.

Control bit 0	pin 1
Control bit 1	pin 2
Control bit 2	pin 3
Control bit 3	pin 4
Control bit 4	pin 5
Flow	pin 11
O2	pin 12
CO2	pin 13
External flow	pin 14
Temperature	pin 15
Barometric pres	pin 16
+5V	pin 19
ground	pins 20-23
ground	pins 30-32

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Control bit 4 is a master remote control bit. Setting this high sets the MM-100 into remote-control mode. Control bits 0 - 3 are used to supply a binary digital number that controls which cage to monitor. For example, 0000 = cage 1, 0001 = cage 2,… 0111 = cage 8. Note that the 4-bit binary number ranges from 0 – 15, which corresponds to cage numbers 1 – 8.

7.5 EXPANSION PORT (DB25)

The required cable has a DB25 male connector on each end, with all pins wired straight through. This port is used to attach an MMX multi-chamber expansion unit to the MM-100, and supplies power and control signals.

7.6 RS232 PORT (DB9)

The required cable has a DB9 male connector on one end and a DB9 female on the other end with all pins wired straight through. This port is used to attach the MM-100 to the host PC serial port. A USB-Serial adapter is supplied for use when a serial port is not available on the host computer.

8.0 CALIBRATION CONSIDERATIONS

Because of the inherent stability of the gas measurement transducers, a full calibration need not be performed frequently. Normally, only the **O2 HIGH** and **CO2 LOW** will require periodic adjustment. These are both set using room air as the reference gas. See Section 6.31 for details.

At the beginning of a measurement session, after the initial warmup and during the following auto-calibration, adjust the **O2 HIGH** and **CO2 LOW** as required to achieve readings of approximately 20.950% and 0.040% for O_2 and CO_2, respectively. The exact readings are not too critical, since all gas measurements are made differentially with respect to room air, and the instrument automatically calibrates itself to room air values during each auto-calibration cycle.

8.1 CALIBRATION WITH 0.25% or 0.5% CO_2, BAL N_2 CAL GAS

A complete calibration requires a separate calibration gas source, preferably 0.25% or 0.5% CO_2, balance nitrogen. With this calibration gas mixture, and room air, a full gas sensor calibration can be performed. When this gas is available follow the procedures detailed in Section 6.31.

9.0 SPECIAL FUNCTIONS

The following functions are not part of the routine operation of the MM-100 system but may be used when required. Please note the exact pushbutton switch sequences needed for each function. These are purposely made a little difficult to prevent accidental actuation.

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9.1 RESTORE FACTORY SETTINGS

This function returns all the calibration and setup settings to the original factory values. This can be useful, for example, if a gas calibration has been dialed well outside its normal range. If this function is used, a complete gas calibration must be done.

To restore factory defaults, press the **ADJUST** knob and hold it in. Then press the **TEST** pushbutton and keep it pressed until you hear a beeping (5 seconds). After you hear the beeps starting, release both pushbuttons.

9.2 ENABLE GAS MEASUREMENT PRESSURE CORRECTIONS

Because the CO_2 and O_2 sensors respond to changes in air pressure inside the measurement cells, small differences in the reported gas concentrations can occur depending on whether the instrument is in *monitor* or *calibration* mode. This is due to the different gas pathways through the internal valves and tubing.

To compensate for this, the sampling pressure is continuously monitored. During calibration mode, the pressure is recorded. Then, during normal monitoring, if the pressure is different, the reported gas concentration is adjusted to compensate. ***Note: This pressure correction function is enabled by default.***

To turn **on** pressure compensation, press the **ADJUST** knob and hold it in. Then press the **O2 CAL HIGH** pushbutton and keep it pressed until you hear a beeping (5 seconds). After you hear the beeps starting, release both pushbuttons.

9.3 DISABLE GAS MEASUREMENT PRESSURE CORRECTIONS

If desired, you can turn off the pressure correction of gas readings. You might want to do this to temporarily check for gross imbalances in the *calibration* and *monitoring* tubing, but you should re-enable it for normal monitoring.

To turn **off** pressure compensation, press the **ADJUST** knob and hold it in. Then press the **O2 CAL LO** pushbutton and keep it pressed until you hear a beeping (5 seconds). After you hear the beeps starting, release both pushbuttons.

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10.0 SPECIFICATIONS

Oxygen analyzer type ..... paramagnetic
Linearity (0 - 100%) ..... ±0.1% O_2
Repeatability ..... < 0.01% O_2/hour
Zero stability ..... ±0.002% O_2/hour
Response time ..... 2.0 seconds
Operating temperature ..... 0 - 65°C
Maximum pressure ..... 15 psi
Maximum sample flow ..... 250 ml/min
Instrument measurement range ..... 0 - 24.5%O_2
Carbon Dioxide analyzer type ..... single-beam infrared
Operating range ..... 0 - 5000ppm (0.5%) CO_2 (usable range 0 – 0.550%)
Linearity (full scale) ..... 1% FS
Zero drift ..... 2% FS/week
Sensor cell temperature ..... controlled at 60°C
Warm-up time ..... <10 min
Maximum sample flow ..... 200 ml/min
Flow transducer ..... thermal mass flowmeter
Flow range ..... 0 – 10,000 ml/min
Response time ..... <2s
Operating temperature ..... -20 - 70°C
Cage temperature transducer ..... YSI-400 series thermistor
Temperature resolution ..... 0.1°C
Temperature accuracy ..... ±0.3°C
Barometric pressure measurement transducer ..... solid-state absolute
Pressure resolution 1mB
Pressure accuracy ..... ±5mB
Main unit analog outputs ..... flow, O2, CO_2
- Analog signal outputs (rear panel) .....BNC jacks
- Flow output ..... approx. 2500ml/min/V
- Flow output zero offset ..... approx. 0.1V
- External Flow output ..... approx. 1500ml/min/V
- External Flow output zero offset ..... approx. 0.2V
- O_2 output ..... 0.1V/%O_2
- CO_2 output .....1.0V/%CO_2
- Temperature output ..... 0.1V/°C
- Barometric pressure output 0.01V/mmHg (0.0133V/mB)

Serial data output format..... 9600 baud, 8 data bits, no parity, 1 stop bit
Serial data interval ..... complete set of readings sent 1 per second
Serial output connector (rear panel) ..... DB9 female
Dimensions, main unit..... 19W x 5.25H x 15D in.
Power requirements..... 120/240V universal input, 100VA

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11.0 MM-CommVS SOFTWARE

The MM-CommVS software provided with the system computes the metabolic measurements from the gas and flow values provided by the MM-100 and records the measurements to disk files for further analysis. Assuming the software has been successfully installed, the following description will outline its various functions.

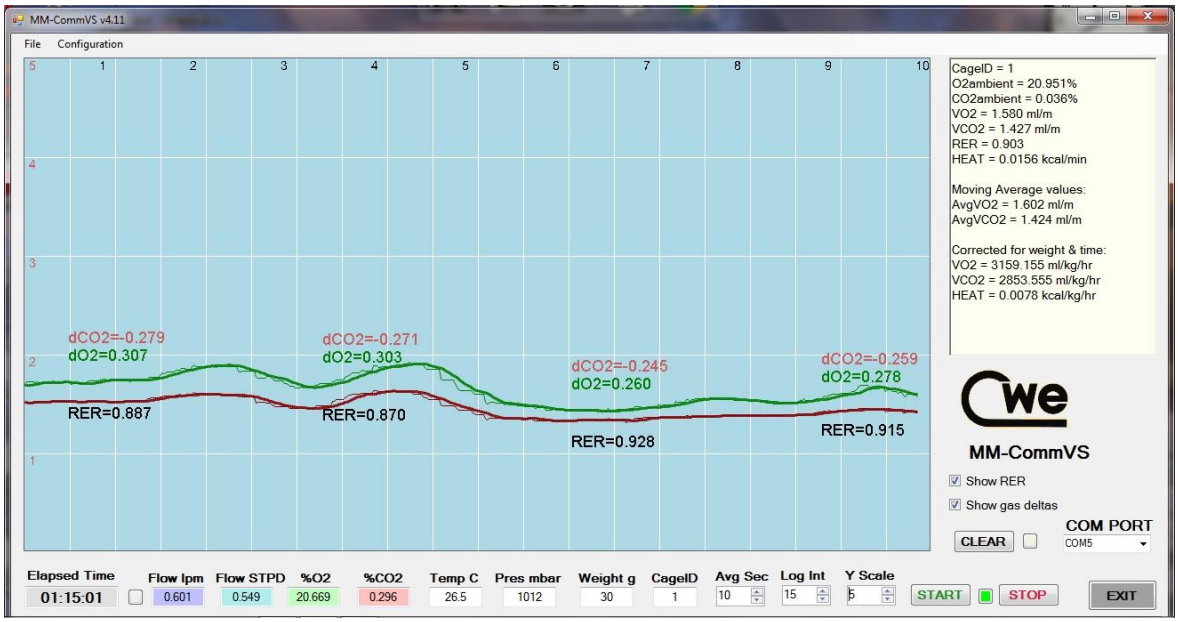

Figure 6: MM-CommVS screen during monitoring

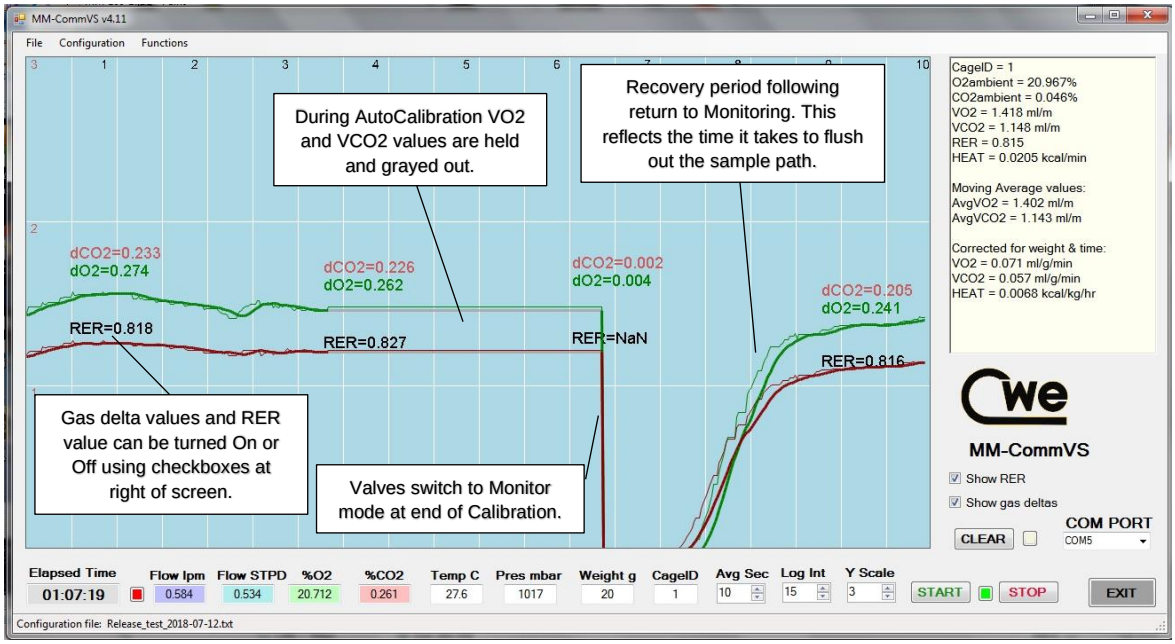

Figure 7: MM-CommVS screen showing an auto-calibration event

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Menu Commands

File menu – Use this to Open or Close a data file. Data files can be named anything you wish but will always be saved with a .txt extension. If a previously existing file is selected the new data will be appended to the end of the file. The format of the data file is as follows:

cageID, time, Flow, O2, O2ambient, CO2, CO2ambient, MAVO2, MAVCO2, RER, Heat

where:

cageID is the number of the chamber being measured (01 for a single-chamber system). A cageID of 00 indicates a calibration event.

Flow is the chamber airflow in ml/min; example: 1450

O2 is the oxygen concentration in percent; example 20.650

O2ambient is the room air reference value for oxygen in percent; example 20.948

CO2 is the carbon dioxide concentration in percent; example 0.338

CO2ambient is the room air reference value for CO_2 in percent; example 0.040

MAVO2 is average oxygen consumption in ml/min; example 1.918

MAVCO2 is average carbon dioxide production in ml/min; example 1.689

RER is the Respiratory Exchange Ratio, or VCO_2/VO_2; example 0.881

Heat is the calorific heat production in Kcal/min based on O_2 consumption and RER values; example 0.0376

The values are in standard ASCII format, separated by commas, and each record is terminated by a Carriage Return (ASCII 13).

The resultant data files can be directly copied into a spreadsheet for analysis. For example, open the file in Notepad, select all, and drop into an Excel spreadsheet. The data should automatically separate into rows and columns. Files are of the type CSV; i.e., values are comma-separated.

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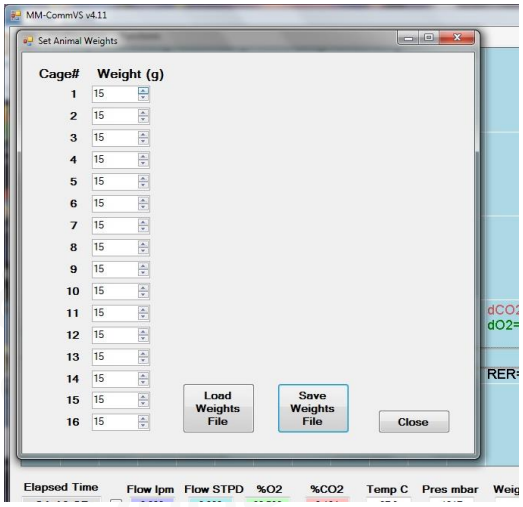
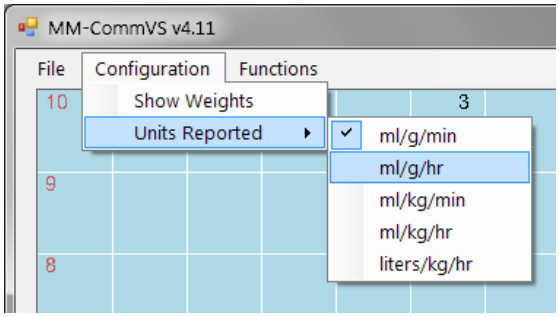
Configuration menu

Show weights – Selecting this will bring up a new window where the user can enter the weight in grams for all animals in the study (up to 8). With a single-chamber system only Animal #1 is relevant. These weights allow the program to calculate standardized values for VO_2, heat production, etc. Note that the absolute values of the various measurements are calculated correctly, regardless of the weight values entered here.



Units Reported – Use this to select the units that will be shown in the "Corrected for time and weight" section of the Data Text Box on the right of the screen. The possible selections are:



Functions Menu

Add Comment -- This allows the user to enter a time-stamped comment. This comment will be added to the output data file. Use this to enter any useful or important information or to time-mark any changes you want to highlight. You can also do this at any time via the keyboard using Ctl + Space pressed simultaneously.

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Data text boxes at bottom of screen

Elapsed Time – This is the elapsed time of the current session. This time is sent from the host MM-100 unit as: HH:MM:SS format.

Flow lpm – Current cage airflow in liters/minute.

Flow STPD – Current cage flow in liters/minute, standardized to Standard Temperature and Pressure; $0^{o}C$, 1bar.

Pct O2 – The current O_2 value of effluent air from measurement chamber.

Pct CO2 – The current CO_2 value of effluent air from measurement chamber.

Temp C – Measurement chamber temperature in ^{o}C.

Pres mBar – Current barometric pressure in mB.

Weight – Currently monitored animal weight in grams.

CageID – Cage number being monitored.

Avg Sec – Number of seconds VO2 and VCO2 Moving Average is computed.

Log Int – Interval in seconds between logged data sets to disk file.

YScale – The vertical full-scale range of the plot. Units are raw ml/min of O2 consumption (VO2) or CO2 production (VCO2).

COM PORT – This drop-down text box is used to select the COM port to be used for communicating with the MM-100 monitor. When MM-CommVS starts, all the available COM ports are identified and listed in this drop-down control. If you have more than one active COM port, be sure to select the one connected to the MM-100.

Button Controls

START – Click this to start monitoring and recording data.

STOP – Click this to stop monitoring and recording data.

CLEAR – This button causes the screen to be erased and the traces will start over at the left margin.

EXIT – This button closes the Data File and the Com Port and exits the program.

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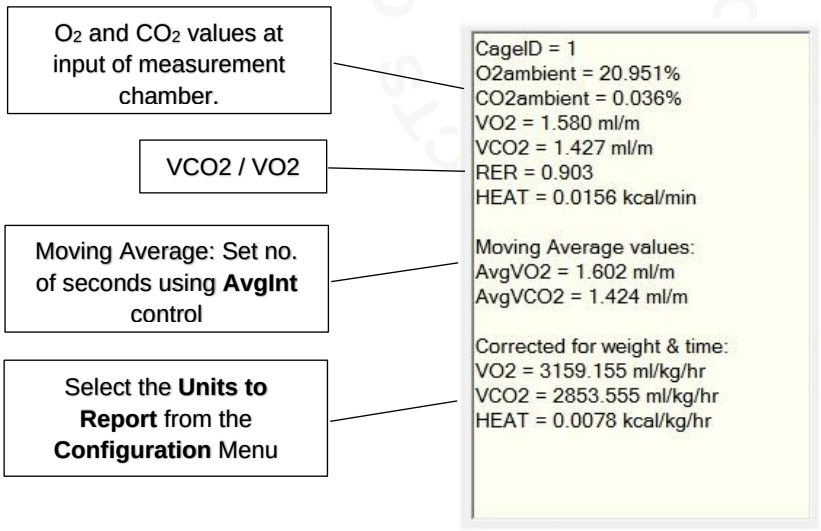
Checkbox Controls

Show RER – When checked, RER values are displayed on the graphics screen with the VO2 and VCO2 traces.

Show gas deltas – When checked, the differences between input and output gas concentrations are displayed with the VO2 and VO2 traces. This provides an indication of appropriate fresh air flow through the measurement chamber; i.e., dCO_2 should be in the range of 0.220% - 0.400%.

Log File – The data file being used to save the measurements is displayed at the bottom of the main screen. Use the File Menu to create or select the desired disk file.

Data Window – This text window to the right of the screen displays the raw and computed values as well as averaged and derived measurements:



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12.0 ORDERING INFORMATION

Part No.	Description
11-20000	MM-100 system for one animal*
11-23000	MMX-8 Eight animal expansion unit
11-20100	Mouse chamber
11-20110	Rat chamber
11-20222	Dri-Tech drying chamber kit, includes 1lb Drierite™
11-20211	Drierite™, 1lb (0.45kg) granular desiccant
11-20212	Drierite™, 5lb (2.2kg) granular desiccant
11-01103	Replacement Nafion™ tubing, 24” (61cm)

* Includes one each rat and mouse chambers and all cables, tubing, and Windows software

CWE LABORATORY PRODUCTS

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Appendix A: Oxygen Consumption Calculations

The MM-100 relies on the well-known open-circuit method of measuring oxygen consumption (VO_2) and carbon dioxide production (VCO_2). This method requires the measurement or derivation of six variables:

V_1 = fresh air flow into chamber, lpm (measured directly)

V_0 = effluent flow from chamber, lpm (derived)

X_1 = oxygen concentration at input of chamber, % (measured directly)

X_0 = oxygen concentration of effluent from chamber, % (measured directly)

Y_1 = carbon dioxide concentration at input of chamber, % (measured directly)

Y_0 = carbon dioxide concentration of effluent from chamber, % (measured directly)

V_0 is derived using the Haldane equation:

$V_0 = V_1 (N_1/N_0)$

where N_1 and N_0 are nitrogen concentrations at input and effluent, respectively

To allow for slight differences in ambient air measurement, N_1 is computed:

$N_1 = 100 – (X_1-Y_1)$

N_0 is derived by:

$N_0 = 100 – (X_0-Y_0)$

With these variables available, the main equations may be used:

$VO_2 = (V_1 X_1) – (V_0 X_0)$ for oxygen consumption in liters/min, and:

$VCO_2 = (V_0 Y_0) – (V_1 Y_1)$ for carbon dioxide production

Another derived measurement, the Respiratory Exchange Ratio (RER) may be computed:

$RER = VCO_2 / VO_2$

The VO_2 and VCO_2 measurements are reported by the MM-CommVS software as ml/min of O_2 consumption and CO_2 production. It is generally desirable to standardize the measurements to the weight of the animal, as:

VO_2 / weight and VCO_2 / weight so the measurements become ml/g/hr.

To achieve these standardized measurements, simply divide the reported results by the weight of the animal in grams.

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Appendix B: Tables

Table 1: The principal components of atmospheric air:

Constituent	Formula	Percent by volume dry air
Water vapor	H_2O	variable
Nitrogen	N_2	78.084
Oxygen	O_2	20.948
Carbon Dioxide	CO_2	0.0315
Argon	Ar	0.934
Helium	He	0.000524
Neon	Ne	0.001818

Table 2: Typical metabolic measurements for small animals:

Animal	O_2 Consumption
Mouse	1.63 – 2.17 ml/min
Rat	0.68 – 1.1 ml/g/hr

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Appendix C: Desiccant chambers and tubes

The Dri-Tech Desiccant Chamber and desiccant tubes supplied with the MM-100 system serve an important purpose: to remove water vapor from the metabolic gas sample. This process is essential for accurate metabolic measurements, which require a “dry” gas sample. The most common desiccant material is *Indicating Drierite*™ (calcium sulfate), but others, such as *Molecular Sieve* are also widely available.

Drierite™ is initially blue in color but turns pink when its capacity to absorb water is exhausted. Fortunately, this desiccant agent can easily be reconstituted to its dry state and reused. While perfectly suitable for use in the Dri-Tech desiccant chamber, Drierite™ has the disadvantage of slightly adsorbing and subsequently releasing CO_2 during use. If used in an in-line desiccant tube, this results in sluggish analyzer response, requiring longer monitor and calibration times for equilibration and best accuracy. See below for instructions on reconstituting Drierite™.

Preparing the Dri-Tech Desiccant Chamber:

The Dri-Tech chamber comes pre-assembled, and it is only necessary to fill with a charge of desiccant medium. Granular Drierite™ is convenient because it has a built-in moisture-content indicator (blue to pink when saturated), but other media can be used (e.g. molecular sieve).

To fill, unlatch the lid and pour in sufficient desiccant medium to completely cover the Nafion tubing. This typically requires about ½ pound (455g) of medium. Be careful to remove any granules that may be on the rubber seal around the lid that might otherwise prevent the airtight seal.

When not in use, insert the tethered Luer plugs into the chamber connectors to prevent humid air from entering the tubing.

Assembling a desiccant tube:

While the Dri-Tech desiccant chamber is preferred, desiccant tubes can also be used. Place a small plug of glass wool into one end of the clean and dry tube. Fill the tube with Drierite™ granules, leaving about one inch (2.5cm) of space. Place a plug of glass wool into the remaining space and place the end caps securely on the tube. If this tube is not to be used immediately, install rubber sealing caps on each end.

***CAUTION:* Use only glass wool to plug the tube end; DO NOT use cotton or other organic materials for this purpose.**

Reconstituting the Drierite:

When saturated with water, indicating Drierite will turn pink. For the regeneration of Indicating Drierite, the granules may be spread in layers one granule deep and heated for 1 hour at 210° C or 425° F. The regenerated material should be placed in the original glass or metal container and sealed while hot. The color of the Indicating Drierite may become less distinct on successive regenerations due to the

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migration of the indicator into the interior of the granule and sublimation of the indicator, but it will still retain its water-absorbing properties.

It is a good idea to have one or more freshly charged desiccant tubes handy for quick replacement of a spent tube. Desiccant tubes can be assembled and sealed against room air using the supplied flexible caps.

Components:

QTY	UNIT	DESCRIPTION	
4	ea	Desiccant tube with end caps	
1	pk	Glass wool	
8	ea	Rubber tube sealing caps	
1	ea	Drierite, 1 lb jar	

PROPRIETARY PRODUCTS CWE

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