For simplified VO₂ measurement (without CO₂ values), use Application Note AS221. The MP160/MP150 System with the O2100C and DA100C modules and some airflow accessories can be used to perform precise real-time Oxygen Consumption (VO₂) and Respiratory Exchange Ratio (RER) measurements. When performing tests of this kind, many factors exist to confound the measurement. For this simplified measurement, it is assumed that the volume of expired air is always the same as inspired air. This is only true when the volume of carbon dioxide expired is equal to the volume of oxygen consumed. The inspired and expired volumes are equal only when the respiratory exchange ratio (RER) equals one.

The Respiratory Exchange Ratio (RER) is defined as VCO₂ produced divided by VO₂ consumed (where V is Volume). Accordingly, when performing these measurements, precise determination of inspired and expired volumes plus accurate gas concentration level recording is required. This application note focuses on the details of the setup and calculations to obtain precise VO₂ and RER measurements from resting or exercising subjects.

The subjects breathe through a non-rebreathing "T" valve to inspire fresh air through a pneumotach (TSD107B) and expire to a mixing chamber for gas concentration (O₂ and CO₂) monitoring. When using a mixing chamber to average CO₂ and O₂ concentrations over several breaths, there is no performance degradation (due to the module gas concentration response time) when measuring these changes for arbitrarily high breathing rates. Accordingly, a mixing chamber is typically recommended for quick, accurate and easy metabolic analysis.

Additional accessories required, but not shown, include a calibration syringe (AFT6A) and calibrated gas mixture. The calibration syringe is used to calibrate the TSD107B and to flush out the mixing chamber for ambient gas concentration level calibration. The calibrated gas mixture (BIOPAC’s GASCAL with GASREG) is used to introduce known concentrations of CO₂ and O₂ to calibrate the upper and lower ranges of the gas measurement module respectively.
Setup Procedure

Equipment Connections for VO2 and RER Measurement

The above diagram illustrates the typical connections when performing metabolic analysis. The subject breathes through the mouthpiece (AFT9) which attaches directly to the non-rebreathing "T" valve (AFT21). It’s very important that any extraneous volumes are minimized between the subject and the "T" valve. Additional volumes affect the effective expired gas concentration levels.

When the subject inspires, air is drawn into the AFT21 as shown by the "Flow In" arrow, through the TSD107B airflow transducer. The TSD107B is placed on the inspiration side to eliminate any effects associated with expired air humidity.

When the subject expires, air is directed out to the 5 liter mixing chamber (AFT15A). The O2100C module connects to the mixing chamber via an AFT20 gas sampling interface kit. The gas sampling interface kit acts to filter and dehumidify the expired gases residing within the mixing chamber.

The non-rebreathing "T" valve directs only expired air to the 5 liter mixing chamber. Because only expired air is directed to the chamber, the mixing chamber acts to average respiratory outflows. This averaging effect causes the O2 concentration to vary in accordance with the mean values resident in several expired breaths. The size of the mixing chamber determines the extent of the averaging effect. For example, assuming the subject’s expired breath volume is typically 0.5 liters, the AFT15A mixing chamber will average about 10 expired breaths.

The TSD107B airflow transducer signal is amplified by a DA100C amplifier, which is attached to the MP System along with the CO2100C and O2100C modules. Accordingly, two channels of analog data need to be acquired to properly measure oxygen consumption and the Respiratory Exchange Ratio.
Definitions

\(O_2\) Oxygen fractional concentration in expired air
\(Fi\) Inspired air flow (ATP)
\(N_2e\) Nitrogen fractional concentration in expired air
\(Vi\) Inspired air volume (ATP)
\(Vis\) Inspired air volume (STPD)
\(Ves\) Expired air volume (STPD)
\(VO_2\) Volume of oxygen consumed (STPD)

AcqKnowledge Software Setup

1. Set up AcqKnowledge to record three analog channels (A1, A2 and A3). For this application note, the input signals are assigned as follows:
   - A1 – (CO2e): Carbon dioxide concentration from CO2100C module
   - A2 – (O2e): Oxygen concentration from O2100C module
   - A3 – (Fi): Inspired flow from TSD107B via DA100C module

   [Image: Analog Input Setup for VO2 and RER Measurement]

2. Set up seven calculation channels as shown below, using parameters and expressions shown on pages 4-6.

   [Image: Calculation Channel Setup for VO2 and RER Measurement]
The calculated signals are assigned to the AcqKnowledge calculation channels as shown below:

- **C0** – \(N_{2e}\): Nitrogen fractional concentration in expired air
- **C1** – \(V_i\): Inspired air volume (ATP)
- **C2** – \(V_i\): Inspired air volume (STPD)
- **C3** – \(V_e\): Expired air volume (STPD)
- **C4** – \(VO_2\): Volume of oxygen consumed (STPD)
- **C5** – \(VCO_2\): Volume of carbon dioxide produced (STPD)
- **C6** – RER: Respiratory Exchange Ratio

**C0 - Expression:** \(N_{2e}\) Nitrogen fractional concentration in expired air

Because both the carbon dioxide and oxygen concentrations are known, it’s possible to determine the concentration of expired nitrogen using the following formula.

\[N_{2e} = 100 - (CO_{2e} + O_{2e})\]

**C1 - Integrate:** \(V_i\) Inspired air volume (ATP)

Since the TSD107B is placed in the "Flow In" line, the flow of inspired air can be determined over a running 30 second interval, by integrating the flow signal using the expression (30 seconds equals 6000 samples at a 200 Hz sampling rate):

\[V_i = \text{Integrate}(Fi) \text{ over 30 sec period}\]
C2 - Expression: Vis Inspired air volume (STPD)

Convert gas at Ambient Pressure and Temperature (APT) to Standard Temperature and Pressure, Dry (STPD):

\[ \text{Vis} = \text{Vi} \times \frac{273}{(273+\text{Ta})} \times \frac{((\text{Pb}-\text{PH20})/760)} \]

where
- \( \text{Ta} \) is ambient temperature (e.g. 24 deg C)
- \( \text{Pb} \) is ambient barometric pressure (e.g. 745 mmHg)
- \( \text{PH20} \) is ambient pressure of water vapor (e.g. 22.4 mmHg)

Vis calculation for VO2 and RER Measurement

C3 - Expression: Ves Expired air volume (STPD)

Calculate the expired volume from the inspired volume by using the Haldane transformation (Nitrogen factor); because of the assumption for CO2 in this setup, \( \text{Ves} = \text{Vis} \).

\[ \text{Ves} = \frac{(\text{Vis} \times 79.03)}{\text{N}_2 \text{e}} \]

where the value 79.03 is the percent N2 in ambient air.

Ves calculation for VO2 and RER Measurement

C4 - Expression: VO2 Volume of oxygen consumed (STPD)

Use the following expression to determine real-time oxygen consumption volume.

\[ \text{VO2} = \frac{1}{100} \times [(\text{Vis} \times 20.93) - (\text{Ves} \times \text{O}_2 \text{e})] \]

where the value 20.93 is the percent O2 in ambient air.

VO2 calculation for VO2 and RER Measurement
C5 - **Expression**: VCO₂ Volume of carbon dioxide consumed (STPD)

Use the following expression to determine real-time carbon dioxide consumption volume.

\[
VCO₂ = \frac{1}{100} \times (Ves \times CO₂e) - (Vis \times .04)
\]

where the value .04 is the percent CO₂ in ambient air.

---

C6 - **RER**: Respiratory Exchange Ratio

Finally, RER can be calculated by:

\[
RER = \frac{VCO₂}{VO₂}
\]

---

3. Set up acquisition as follows:

- Record and Save Once using Memory or Disk
- Sample rate: 200 samples/second
- Acquisition length: 5 minutes or longer

---
Calibration

After the software setup, it's very important to calibrate the system for the following three variables:

1) Carbon Dioxide concentration (calibrate in range of .04% to 4% CO₂)
2) Oxygen concentration (calibrate in range of 21% to 16% O₂)
3) Inspired Flow (calibrate within the range of 0 to 13 liters/sec)

The calibrations for these measurements are critical. Once the equipment and software setup is complete, with all the plumbing fixed and secure, gas concentration calibration can proceed. If any changes in plumbing or gas measurement pump speed are effected, it is generally necessary to repeat the gas measurement module calibrations.

Flood the mixing chamber with the appropriate gas and concentration levels to proceed with the calibration. First, flood the chamber with fresh (ambient) air. This can be accomplished by attaching the calibration syringe to the mouthpiece and cycling fresh air into the mixing chamber. Monitor the gas concentration changes using AcqKnowledge. When the levels appear stabilized at ambient, obtain the first calibration point. Now flood the chamber with your desired gas mixture (recommended, BIOPAC GASCAL: 4% CO₂, 16% O₂ and 80% N₂). When the levels appear stabilized, obtain the second calibration point.

The pneumotach (TSD107B) is calibrated by using a calibration syringe to inject a precise volume of air through the flow transducer. Determine the appropriate transducer scaling factor so the area under the recorded flow curve equals the volume injected. Perform the first calibration point by recording the transducer output (from the DA100C) at zero flow. The second point calibration is determined by adding the previously recorded scale factor to the offset recorded by the first calibration point.

For more information on gas analysis module and flow transducer calibration, please refer to the MP Hardware Guide or AcqKnowledge Software Guide.

Other factors to be recorded for the procedure are the following:

**Ta:** Ambient temperature in degrees C  
**Pb:** Ambient barometric pressure in mmHg  
**PH2O:** Pressure of water vapor at ambient temperature in mmHg

These values are important when performing the conversion of gas at Ambient Pressure and Temperature (APT) to Standard Temperature and Pressure, Dry (STPD).

The examples below illustrate typical values obtained when performing gas and flow measurement calibrations. In the following cases, the CO2100 module was set to a gain of 1% CO₂ per volt, the O2100C was set to a gain of 10% O₂ per volt, and the DA100C amplifier (connected to TSD107B) was set to a gain of 1000. Note that these are example values only. Do not set your Cal 1 and Cal 2 values to the parameters shown in the examples prior to calibrating.
Analysis

The following graph illustrates the complete real-time Oxygen Consumption and Respiratory Exchange Ratio measurement. All the raw and calculated waveforms are displayed.

Note that the VO$_2$, VCO$_2$ and RER measurements (bottom three channels) vary smoothly with time. The graphical and continuous nature of this recording and calculation method provides significant information regarding the changes of these variables over time.

This particular setup will provide a reading that, at any point in time, indicates the oxygen consumed, the carbon dioxide produced and the respective respiratory exchange ratio in the last 30 seconds. Accordingly, the VO$_2$ reading of 391.67 ml of oxygen consumed is indicative of the amount consumed by the subject in the last 30 seconds. The RER at the same point in time is determined to be 0.7768.