

## Application Note 268 Metabolic Analysis Systems and Propane-based Verification

*This application note examines a conventional equipment setup for metabolic measurements. The setup is exposed to a propane-based, combustion verification source that emulates subject's steady-state consumption of oxygen and production of carbon dioxide. A propane-based, combustion source is a very reliable and accurate metabolic gas generator that can be used to verify the performance of a metabolic measurement system.*

### Metabolic Analysis System Setup

For this application note, the three input signals have been assigned to MP150 data collection channels:

- CO<sub>2</sub>e** Carbon dioxide concentration via CO2100C module, set to a gain of 1% CO<sub>2</sub> per volt
- O<sub>2</sub>e** Oxygen concentration via O2100C module, set to a gain of 10% O<sub>2</sub> per volt
- Fi** Inspired flow from TSD107B transducer via DA100C amplifier, set to a gain of 1,000

Figure 1 illustrates the typical connections when performing metabolic analysis.

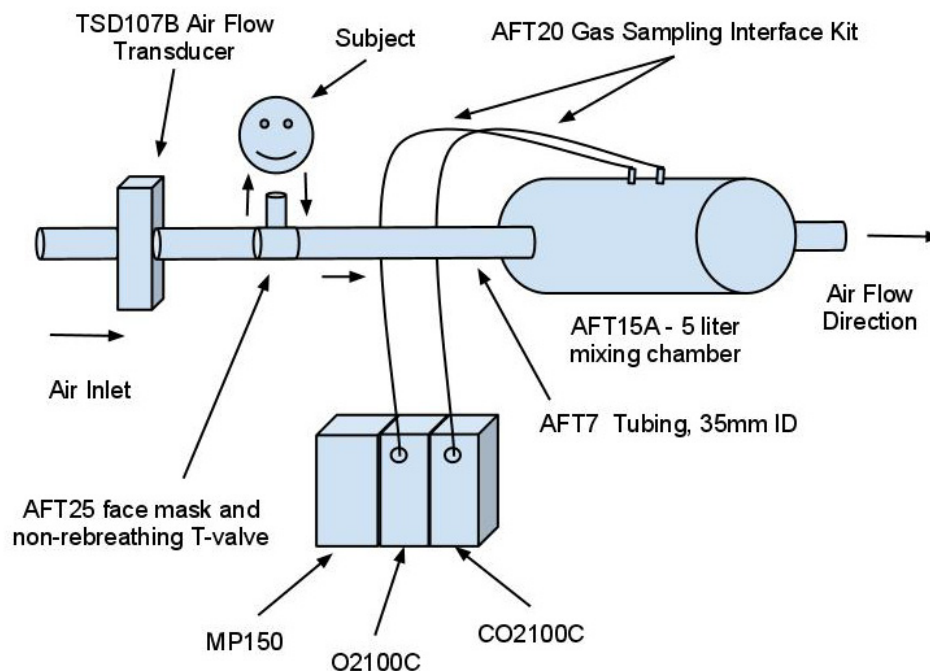


Figure 1 Typical connections when performing metabolic analysis

The subject breathes through the non-rebreathing “T-valve” facemask/mouthpiece (AFT25) which attaches directly to the TSD107B air flow transducer on the inspiration side and the AFT15A mixing chamber on the output side.

When the subject inspires, air is drawn through the TSD107B, as shown by the arrows, and then passes through the AFT25 non-rebreathing “T-valve” into the subject’s lungs. The TSD107B is placed on the inspiration side to eliminate any effects associated with expired air humidity and associated condensation.

When the subject expires, air is directed out to the 5 liter mixing chamber (AFT15A). The CO2100C and O2100C gas concentration sampling modules connect to the mixing chamber via separate AFT20 gas sampling interface kits. The gas sampling interface kits act to filter and dehumidify (via Nafion® tubing) 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 CO<sub>2</sub> and O<sub>2</sub> concentrations to vary in accordance to 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 liter (as during resting metabolic rate measurements), 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 MP150 system along with the CO2100C and O2100C modules. Accordingly, there are three channels of analog data that are acquired to properly calculate metabolic Oxygen Consumption (VO<sub>2</sub>) and the Respiratory Exchange Ratio (RER).

*AcqKnowledge* is setup to record on three analog channels and multiple calculation channels. Data is acquired to computer memory, or hard disk, with an acquisition time of at least several minutes.

Once the setup is completed, with all the plumbing fixed and secure, gas concentration calibration can proceed. If airflow plumbing, gas sampling line plumbing or gas measurement module pump speed are changed, it's generally necessary to repeat the gas measurement module calibrations.

### Gas Concentration Calibration

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

- 1) Carbon Dioxide concentration (calibrate in range of .04% to 4% CO<sub>2</sub>)
- 2) Oxygen concentration (calibrate in range of 20.93% to 16% O<sub>2</sub>)
- 3) Inspired Flow (calibrate within the range of 0 to 13 liters/sec)

To calibrate the gas concentration sensors, the mixing chamber is flooded with the appropriate gas. First flood the chamber with fresh (ambient) air by attaching the calibration syringe to the subject mouthpiece and cycling fresh air into the mixing chamber. Monitor the gas concentration changes using *AcqKnowledge*. When the levels appear stabilized with ambient air, obtain the first calibration point (CO<sub>2</sub> at 0.04% and O<sub>2</sub> at 20.93%). Then flood the chamber with the desired gas mixture (recommended - BIOPAC's GASCAL and GASREG: 4% CO<sub>2</sub>, 16% O<sub>2</sub> and 80% N<sub>2</sub>). When the levels appear stabilized, obtain the second calibration point. In this fashion, the CO200C module will be calibrated between 0.04% to 4% carbon dioxide and the O2100C module will be calibrated between 20.93% and 16% oxygen.

The airflow transducer -pneumotach- (TSD107B) has a factory scale factor of 90uV per liter/second. This scale factor implies that when 1 liter/second of air is moving through the TSD107B, then it will output a signal of 90uV. The TSD107B is attached directly to the DA100C, set to a gain of 1000. This amplification results in a 90mV signal under the same airflow conditions.

Calibration is estimated using the following procedure:

- 1) Record the first calibration value (value X) with no airflow through TSD107B
- 2) In the scaling dialog window, X volts will map to 0 liters/second
- 3) Calculate the inverse of the factory scale factor –  $1/0.090 = 11.111$  (liters/sec per volt)
- 4) Add 11.111 to X to obtain value Y
- 5) In the scaling dialog window, Y volts will map to 1 liter/second

The second point calibration is estimated by adding the inverse of the factory scale factor to the offset recorded by the first calibration point.

The TSD107B can be precisely calibrated by using a calibration syringe to inject a specific volume of air through the airflow transducer. While recording the airflow signal in *AcqKnowledge*, note that the signal will rise and then fall in accordance to the syringe movement. The "area" pop-up measurement in *AcqKnowledge* is used to perform an area calculation on the airflow signal as shown in Figure 2.

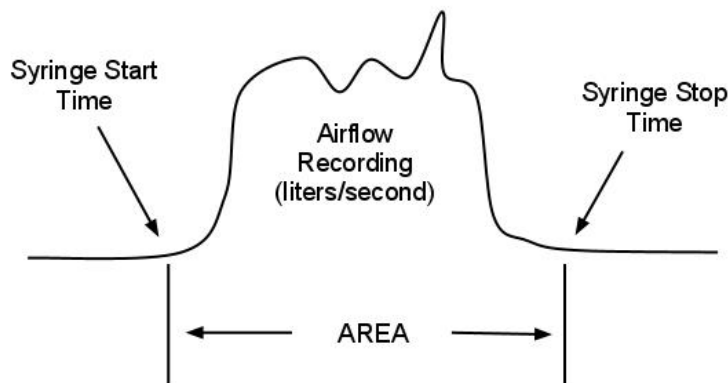


Figure 2 Airflow signal area calculation

Assuming a 2-liter calibration syringe (BIOPAC AFT26) is used, the measured area should be 2 liters. If the measured area is something different, then the scale factor of 11.111 liters/second (used in scaling dialog window) should be adjusted upwards or downwards in direct proportion until the area measurement returns 2 liters.

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

- Ta** Ambient temperature
- Pb** Ambient barometric pressure in mmHg
- PH<sub>2</sub>O** Pressure of water vapor at ambient temperature in mmHg

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

**VO<sub>2</sub> and RER Measurement Setup Analysis**

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

$$N_{2e} = 100 - [O_{2e} + CO_{2e}]$$

Since the TSD107B is placed in the "Flow In" line, the flow of inspired air can be determined over a running 60-second interval, by integrating the flow signal using the expression (60 seconds equals 6000 samples at a 100Hz sampling rate):

$$V_i = \text{Integrate } (F_i) \text{ over 60 second period}$$

Convert gas at ATP to STPD:

$$V_{is} = V_i * (273 / (273 + T_a)) * ((P_b - P_{H_2O}) / 760)$$

- Where T<sub>a</sub> is ambient temperature (e.g. nominally 24 deg C)
- P<sub>b</sub> is ambient barometric pressure (e.g. nominally 745 mmHg)
- P<sub>H<sub>2</sub>O</sub> is ambient pressure of water vapor (e.g. nominally 22.4 mmHg)

Calculate the expired volume from the inspired volume by using the Haldane transformation (Nitrogen factor):

$$V_{es} = (V_{is} * 79.03) / N_{2e}$$

- Where the value 79.03 is the percent nitrogen in ambient air

The real-time oxygen consumption volume can be determined by using the expression:

$$VO_2 = (1/100)*[(Vis*20.93) - (Ves*O_{2e})]$$

Where the value 20.93 is the percent oxygen in ambient air

The real-time carbon dioxide production volume can be determined by using the expression:

$$VCO_2 = (1/100)*[(Ves* CO_{2e}) - (Vis*.04)]$$

Where the value .04 is the percent carbon dioxide in ambient air

Finally, RER can be calculated by:

$$RER = VCO_2 / VO_2$$

**Definitions**

**CO<sub>2e</sub>** Carbon Dioxide fractional concentration in expired air

**O<sub>2e</sub>** Oxygen fractional concentration in expired air

**Fi** Inspired air flow (ATP)

**N<sub>2e</sub>** Nitrogen fractional concentration in expired air

**Vi** Inspired air volume (ATP)

**Vis** Inspired air volume (STPD)

**Ves** Expired air volume (STPD)

**VO<sub>2</sub>** Volume of oxygen consumed (STPD)

**VCO<sub>2</sub>** Volume of carbon dioxide produced (STPD)

**RER** Respiratory Exchange Ratio

**Note** All volume-related units are expressed in liters per minute, because the integration interval is over a 60 second period.

Figure 3 illustrates typical values obtained when performing gas concentration and flow measurements from a subject. The CO2100 module was set to a gain of 1% CO<sub>2</sub> per volt, the O2100 module was set to a gain of 10% O<sub>2</sub> per volt and the DA100C amplifier (connected to TSD107B) was set to a gain of 1000.

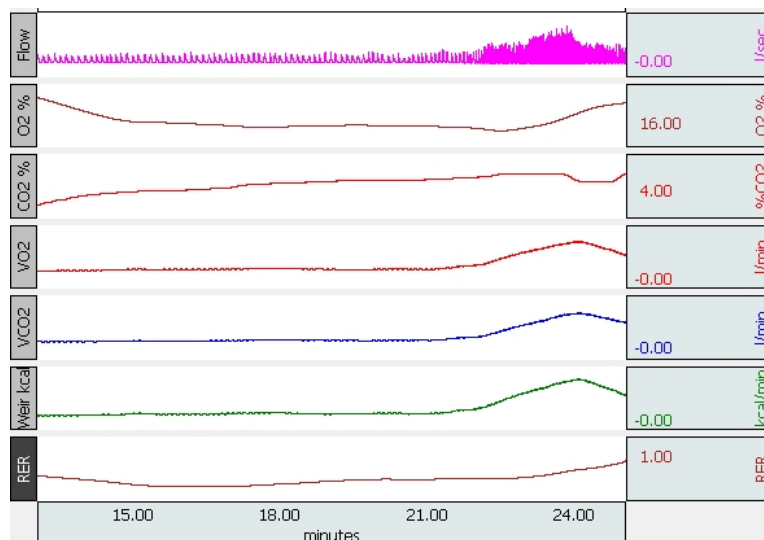


Figure 3 Typical gas concentration and flow measurements

The preceding graph illustrates the complete real-time Oxygen Consumption and Respiratory Exchange Ratio measurement taken from a subject riding a stationary bicycle. Both raw and calculated waveforms are displayed.

Note that the  $VO_2$ ,  $VCO_2$ , Weir Calculation and RER measurements (bottom four 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 as a function of time.

This measurement setup will provide a reading that, at any time, indicates the oxygen consumed, the carbon dioxide produced and the respective respiratory exchange ratio in the last 60 seconds. Accordingly, the peak  $VO_2$  reading – at 24 minutes - of 4.7 liters of oxygen consumed is indicative of the amount consumed by the subject in the previous 60 seconds. The RER at the same point in time is determined to be 0.91 and the Weir calculation indicates a peak value of 23.1 kcal/min.

### Gas Concentration Sensor Response Time

A very important issue associated with metabolic systems' calibration is the issue of gas concentration sensor response time. Because the RER is calculated as  $VCO_2/VO_2$ , the response times of the carbon dioxide and oxygen sensors must match very closely.

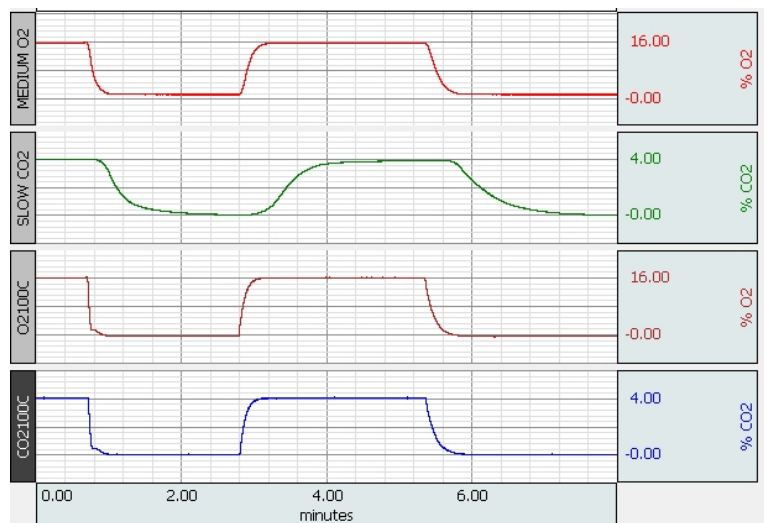


Figure 4 Gas concentration sensor response times

As an example, consider the gas sensor response times indicated in Figure 4. In this rise-time and fall-time test, nitrogen is used to flood the chamber to bring both the  $CO_2$  and  $O_2$  concentrations to 0%. After 2-3 minutes, the chamber is flooded with a gas mixture containing 4%  $CO_2$ , 16%  $O_2$  and 80%  $N_2$  (BIOPAC GASCAL).

The first two waveforms are representative of gas sensors that have very different response times. If these sensors are used in a metabolic system, they will only provide accurate RER measurements about 100 seconds after a quick change in gas concentrations. For these sensors, because the  $CO_2$  sensor responds much slower than the  $O_2$  sensor, the RER will be underestimated for quickly rising  $VCO_2$  (and falling  $VO_2$ ) and overestimated for quickly falling  $VCO_2$  (and rising  $VO_2$ ).

However, with steady-state conditions, as with resting and basal metabolic rate measurements, RERs will be reported correctly – even on gas systems that have very different response rates between the oxygen and carbon dioxide sensors. This is because the rate of change in gas concentration is very slow, so both sensors can track equivalently.

The bottom two waveforms are representative of gas sensors that have very similar (and fast) response times. These sensors (CO2100C and O2100C) will accurately report RER under a very wide range changing gas concentrations.

In the case of the CO2100C and O2100C, both measurement units are very high speed (under 500 msec response time), so RERs are reported very accurately – even under rapidly changing gas concentration conditions. Generally used, the outputs from the CO100C and O2100C are low pass filtered (1 Hz LPF) in AcqKnowledge to create smoothly varying waveforms.

**Propane-based Verification of Metabolic System**

Figure 5 illustrates the setup to perform a propane-based combustion verification of the previously referenced metabolic analysis system.

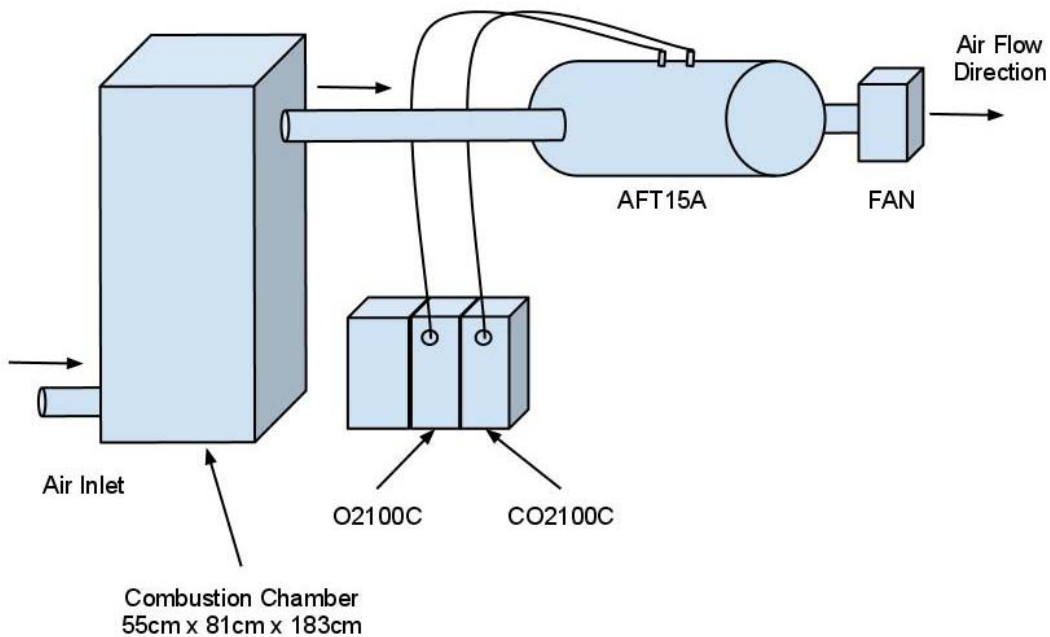


Figure 5 Typical connections when performing propane-based combustion verification of metabolic analysis system

In this situation, a propane-based combustion chamber replaces the subject. Also, a fan is added in-line to the AFT15A output. The fan draws air from the combustion chamber and then to the AFT15A where the resulting gas concentrations are sampled by the CO100C and O2100C.

The combustion chamber has dimensions of 55cm x 81cm x 183cm, providing an internal volume of 815,265 cm<sup>3</sup> or approximately 815 liters. Inside the chamber is a burning propane torch. The propane torch is set to a moderate flame size and the resulting flame is well-oxygenated. The fan draws air through the AFT15A and then from the top of the combustion chamber. Fresh air enters through the bottom of the combustion chamber to continue to source oxygen to the propane torch.

Inside the combustion chamber, the propane torch sits on a sensitive scale that measures weight with a resolution of 0.1grams. As the propane burns, it produces 11.92 kcal per gram of propane used. The scale determines the amount of propane consumed by the torch over some period of time.

As the propane burns it has an effective RER of 0.60. This means that the volume of carbon dioxide produced by the torch is 60% of the volume of oxygen consumed by the torch. For industrial propane, the effective RER is in the region of 0.58 to 0.60. For instrument grade propane, the RER is 0.60.

The Weir equation can also be evaluated with the combustion-based calibration test. The Weir equation is:

$$\text{kcal} = (3.9 \cdot \text{VO}_2) + (1.1 \cdot \text{VCO}_2) - \text{all dimensions on a per minute basis}$$

VO<sub>2</sub> and VCO<sub>2</sub> are the volume of oxygen consumed and the volume of carbon dioxide produced, respective, in units of liters/minute.

The graph in Figure 6 demonstrates proper metabolic system behavior, when subjected to propane-based verification. Once stabilized, after approximately 60 seconds,  $VO_2$  is 0.297 liters/min,  $VCO_2$  is 0.180 liters/min and the Weir calculation is 1.36 kcal/min and RER is approximately 0.6.

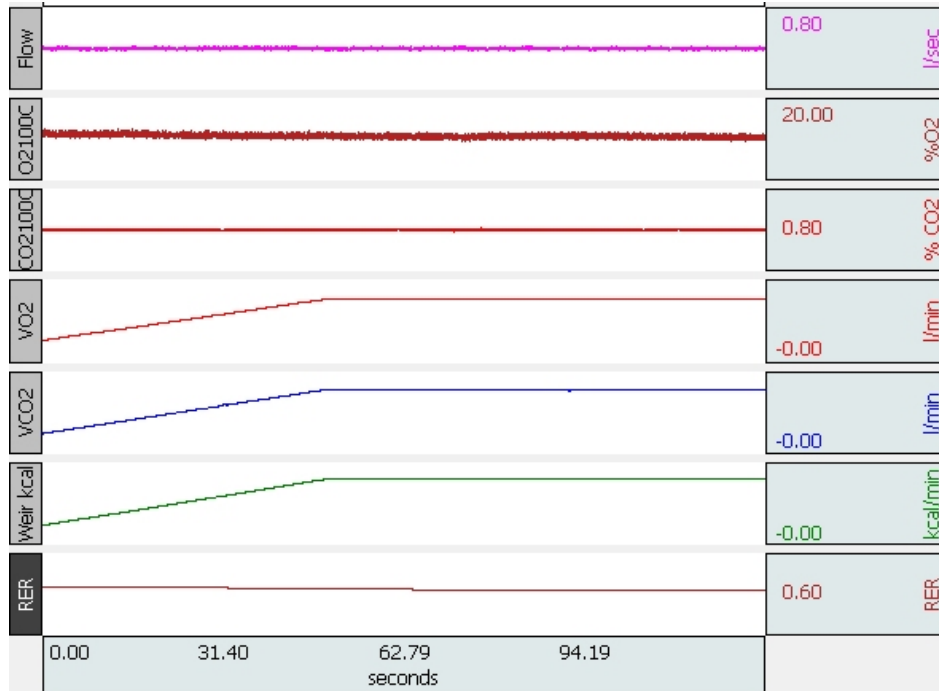


Figure 6 Proper metabolic system behavior, when subjected to propane-based verification

Artificial subject measurements are shown in Figure 7. In this case, the subject shown in Figure 1 is replaced with the propane-based combustion chamber. As illustrated in the graph, the RER is in the range of 0.58 +/- 0.01 throughout the duration of the test, even though the airflow is markedly changed over the course of the recording.  $VO_2$  maximum is 1.645 liters/min at 90 seconds into the recording, and at that time the Weir calculation is 7.46 kcal/min and RER is 0.575.

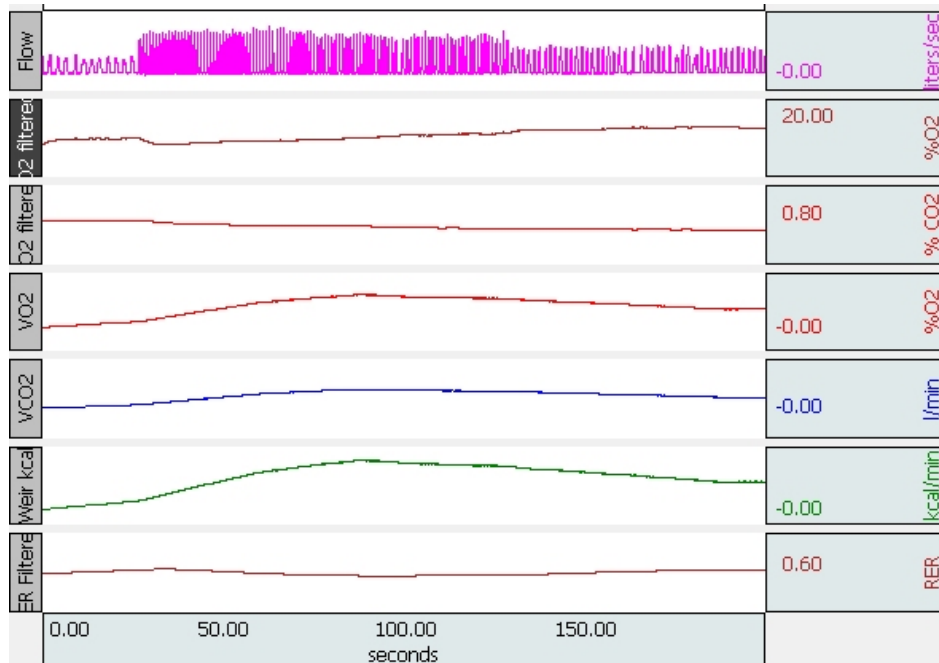


Figure 7 Artificial subject measurements