

# Bioengineering/Physiology 6000 Lab Exercise

## Control of Respiration

Rob MacLeod and Brian Birchler

April 26, 2012

### Contents

<b>1 Purpose and Background</b>	<b>1</b>
1.1 Purpose . . . . .	1
1.2 Background . . . . .	2
1.2.1 Ventilation volumes . . . . .	2
<b>2 Lab Procedure</b>	<b>3</b>
2.1 Organization . . . . .	3
2.2 Spirometry . . . . .	4
2.2.1 Spirometer circuit . . . . .	4
2.2.2 Lab steps . . . . .	5
2.2.3 Forced expiratory volume . . . . .	6
2.2.4 Comparison with theoretical values . . . . .	6
2.3 Respiration rates and depth . . . . .	6
2.4 Breath holding . . . . .	7
2.5 Physical models of ventilation . . . . .	7
2.5.1 Alveolar filling . . . . .	8
2.5.2 Lung model . . . . .	8
2.6 Re-breathing and control of respiration . . . . .	8
2.6.1 Re-breathing room air . . . . .	9
2.6.2 Re-breathing oxygen-rich air . . . . .	10
2.6.3 Data analysis . . . . .	11
<b>3 Acknowledgments</b>	<b>11</b>
<b>4 Lab report</b>	<b>11</b>

## 1 Purpose and Background

### 1.1 Purpose

The goal of this lab is to illustrate some basic mechanisms of respiration and especially the control of respiration. One of the main questions is to determine which stimuli influence respiration rate

and which among them is the most powerful—why do we breath!?

## 1.2 Background

The regular class notes and the text book provide the basic background we need for this lab. We include here some points that are especially important in this lab.

### 1.2.1 Ventilation volumes

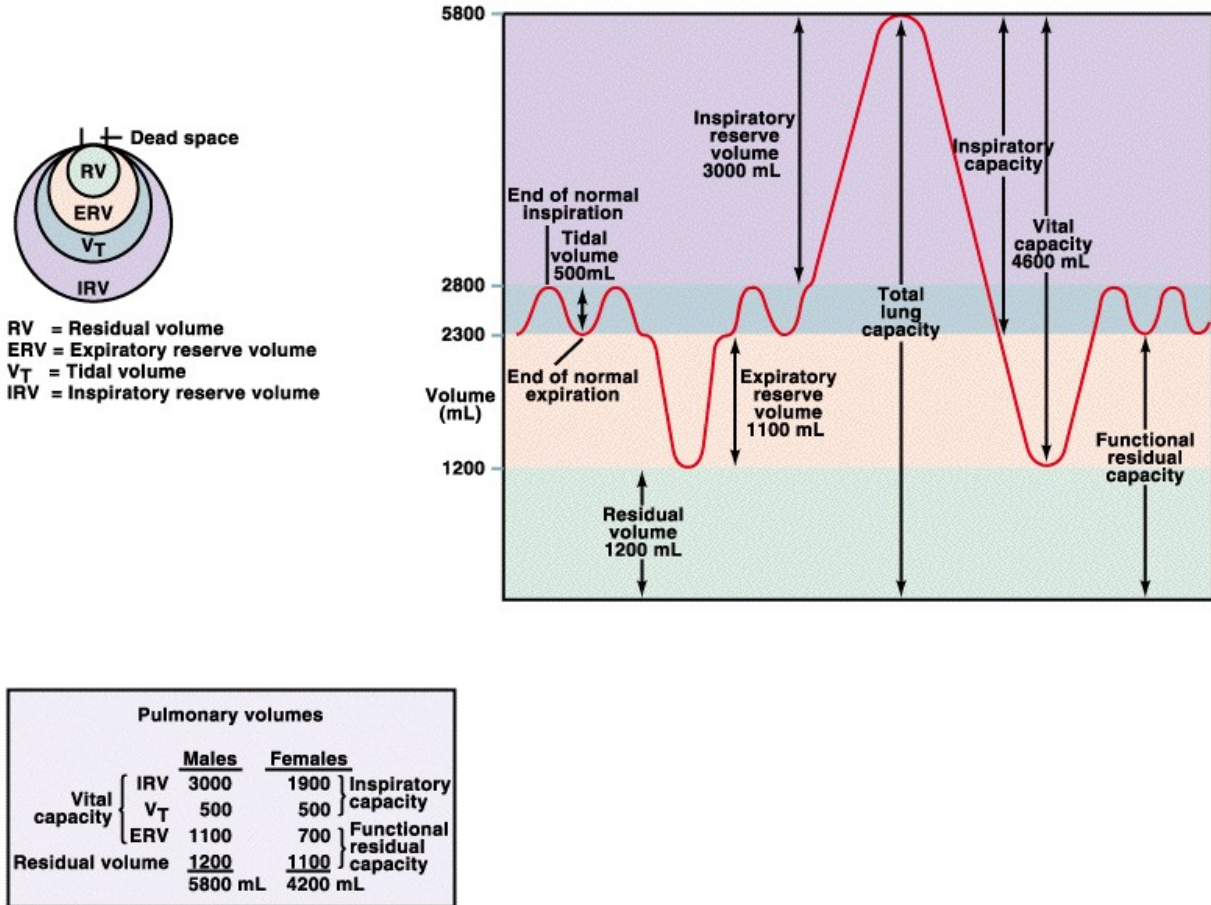


Figure 1: Ventilation volumes for humans

Figure 1 contains a diagram showing the relationship of the following respiratory volumes:

**Tidal Volume (TV):** the volume of gas inspired or expired during each respiratory cycle.

**Inspiratory Reserve Volume (IRV):** the maximal amount of gas that can be inspired from the end inspiratory position.

**Expiratory Reserve Volume (ERV):** the maximal amount of gas that can be expired from the end expiratory position.

**Residual Volume (RV):** the volume of gas remaining in the lungs at the end of maximal expiration.

**Total Lung Capacity (TLC):** the amount of gas contained in the lungs at the end of a maximal inspiration, *i.e.*, the total of all four volumes.

**Vital Capacity (VC):** the maximal volume of gas that can be expelled from the lungs by a forceful effort following a maximal inspiration *i.e.*, it is the sum of the Tidal Volume, Inspiratory Reserve Volume and Expiratory Reserve Volume.

**Inspiratory Capacity (IC):** the maximal volume of gas that can be inspired from the resting expiratory level. It is the sum of the Tidal Volume and Inspiratory Reserve Volume.

**Functional Residual Capacity (FRC):** the volume of gas remaining in the lungs at the resting expiratory level. The resting end-expiratory position is used here as a base line because it varies less than the end-inspiratory position, it is thus the sum of Expiratory Reserve Volume and Residual Volume.

**Forced Expiratory Volume in one second ( $FEV_1$ ):** the percentage of the Forced Vital Capacity which can be forcibly expired as rapidly as possible in one second after maximal inspiration.

What purpose would it have to measure  $FEV_1$ ? Compare this to the role of other ventilation volumes we measure in this lab.

## 2 Lab Procedure

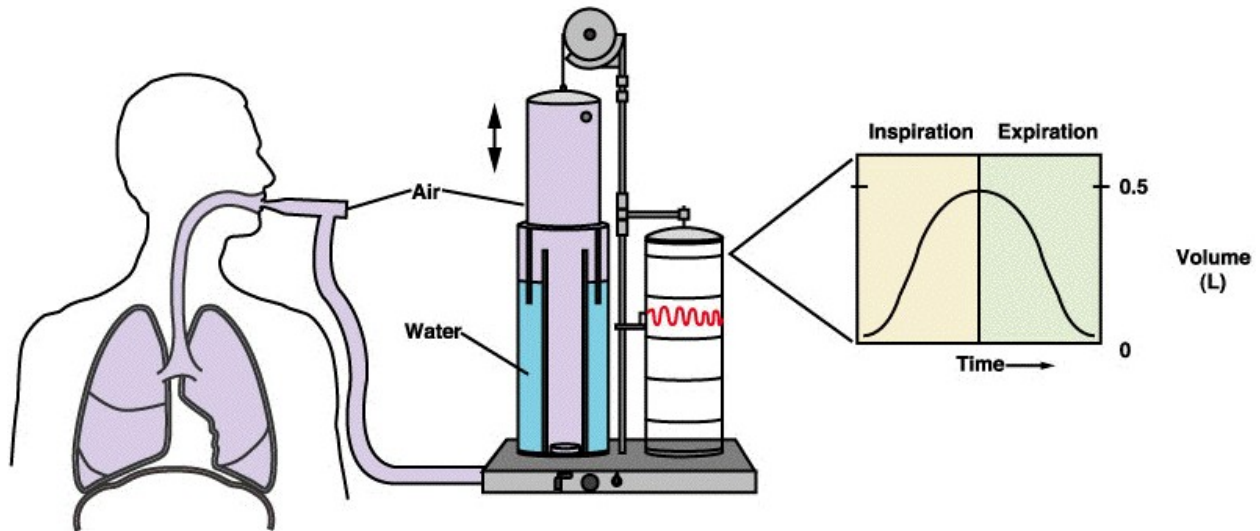


Figure 2: Principle of spirometry

### 2.1 Organization

We will have multiple stations around the lab and ask you to circulate through them all in small groups. We will need to form larger groups (6–8 people) for the re-breathing exercises but otherwise, pairs should be adequate for all the other exercises. There are 8 spirometer stations and one station with the physical model of the lung.

Each student should pick up a nose clip, mouth piece, and (if available) viral/bacterial filter. You can reuse your own equipment for each of the lab procedures. However, please don't use someone else's. Remember to use your nose clip for all applicable experiments (especially breath holding).

The order of performing the exercises is irrelevant.

## 2.2 Spirometry



Figure 3: Examples of modern spirometers.

The goal of the first part of this lab is to use a spirometer to determine the values in Figure 1 for each student. The schematic of the spirometer in Figure 2 illustrates the function of a “wet” spirometer, like the ones we have. In clinical use there are more modern versions of this device that uses a bellows to absorb the air and can write to a chart. Most current spirometers are electronic devices that measure air flow through a tube and compute volumes as needed. Figure 3 shows examples of the bellows type “gold standard” spirometry (left-hand panel) and the types of spirometers used in clinical practice (center and right-hand panels).

### 2.2.1 Spirometer circuit

Our spirometers use a simple yet effective method to sense a change in the volume contained within the spirometer. As the volume changes, a chain rotates a pulley and the angle of this pulley is sensed by a potentiometer mounted to the shaft of the pulley. To electrically sense the angle of the pulley and, hence, the volume of the spirometer's bellows, wire the potentiometer as illustrated below. **BUT FIRST DO THE FOLLOWING:** Set the Tenma Double Power Supply Outputs to Series Tracking. Use the two push buttons in the middle to do this (left button depressed and right button not). Also turn on the output (far left button, make sure green indicator light is on). For the older model, move single switch all the way to the left. Set the output to 7.5 volts (for the older model with Supply A's knob; for the newer model with the righthand or “Master” control knob). When in series tracking mode, both supplies are connected internally so that the output voltage ranges from the positive to the negative value adjusted on the display, *e.g.*,  $\pm 7.5$  V. This setting creates both a positive and negative voltage in reference to shared zero terminal. Access to the zero voltage is proved by connecting the positive of the lefthand supply with the these terminals. Also, the output voltage of both Channel 1 and 2 are controlled by Channel 1's knobs, with the result that both the positive and negative voltages having the same magnitude. Now you can wire the circuit as shown in Figure 4. Once you have the circuit set up, you may adjust the power supply voltage a little (make sure the voltage is between 7 and 8 V) to ensure the signal fits in the desired range on the software. If more change to the voltage is needed, adjust range on software.

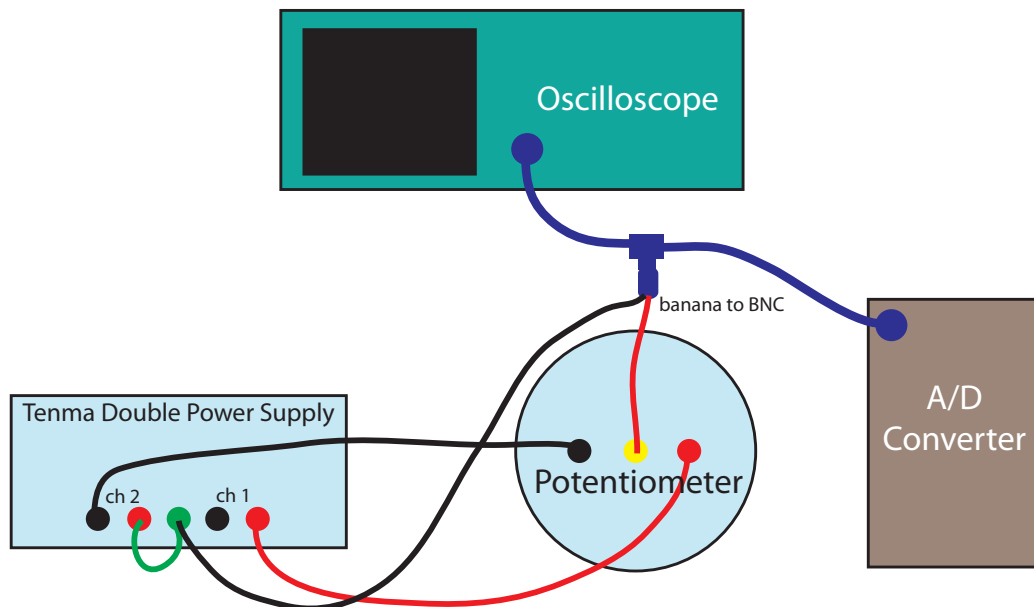


Figure 4: Circuit for spirometer sensing.

### 2.2.2 Lab steps



Figure 5: Calibration syringe for spirometry.

1. Move the spirometer up and down and adjust the oscilloscope settings and acquisition program until you get good tracings.
2. Using one of the large calibration syringes (see Figure 5), determine the scaling of the display of spirometer volume. **Make note of this conversion factor and apply it to all your data so that all values are expressed in liter (or milliliter) units. Also Be very careful not to drop or damage the calibration syringe! It is a precision instrument.**

3. Once the device is calibrated, have the subject sit comfortably near the spirometer, find a clean mouthpiece, and apply the nose clip. Make sure there are no leaks in the system and ensure that the subject always breathes only through the mouth.
4. **Gently** force the arm of the spirometer up and down in order to flush it and fill it with fresh room air. Before starting each experiment, lift the spirometer chain so that approximately 5 l is showing on the mechanical display (and about 0 V on the oscilloscope).
5. Start the acquisition running at a sample rate and duration that is enough for a minute of recording time.
6. Have the subject take a moderate inspiration, then take the spirometer mouthpiece in his or her mouth and breath normally into the tube.
7. Breath quietly for 5–6 breaths.
8. FEV<sub>1</sub>: At the end of these breaths, have the subject take one inspiration that is as deep as possible followed by one expiration that is as deep and as rapid as possible. The idea is to see how much air a subject can exhale in one second so exhalation must be **very forceful**.
9. Take the mouthpiece from the subject’s mouth and flush the spirometer again. Be careful not to let the subject breath too long into the spirometer as this will alter the composition of gas in the (closed) system and thus influence respiration rate.
10. Repeat the process three times for each person.
11. Repeat these steps until you have all the data you need in order to report values (mean  $\pm$  standard deviation) for TV, ERV, IRV, IC, VC, and FEV<sub>1</sub>.

### 2.2.3 Forced expiratory volume

We will also use a dedicated clinical system called AccuTrax to measure FEV<sub>1</sub>. Have the subject, with nose clip in place, breath normally with the device ready at hand. Then, as above with the spirometry measurement of FEV<sub>1</sub>, the subject should take a large inspiration, put the device to his/her mouth and blow out as hard and fast as possible; be sure to encourage the subject to really exhale forcefully. Repeat this three times and the device will then report the best result.

### 2.2.4 Comparison with theoretical values

Go to the following website [http://www.medicine.mcgill.ca/physio/vlab/resp/calcul\\_n.htm](http://www.medicine.mcgill.ca/physio/vlab/resp/calcul_n.htm) and navigate to the “Volume Calculator” page, under Respiration and then Practice. Use the applet to get theoretical values for the lung volumes and capacities and compare them to the values you obtained from the above exercises.

If you are interested, see the web site <http://www.medicine.mcgill.ca/physio/vlab/resp/vlabmenuresp.htm> for a virtual respiratory lab.

## 2.3 Respiration rates and depth

The following two tests require a measure of your respiration rate. For any such measurement, the subject should breathe normally for a while and have a groupmate time and count the number of breaths you take in one minute. The subject should breath normally and try consciously not to count his/her own breaths but rather think about something else. **It may seem easier to count**

**the number of breaths over  $x$  seconds ( $x < 60$ ) and then extrapolate to a minute, but you should not do this—can you explain why?**

Please apply the pulse oxymeter to the subject and record heart rate and saturation levels at each stage of the experiment, during control, after mild exercise, and during dead space experiment.

1. The subject should sit quietly and take a measurement of baseline respiration rate. Repeat three times and take the average. This is the control measurement.

2. Then have the subject walk **briskly** around the MEB hallways for a few minutes.

As quickly as possible after the subject returns, repeat the measurement by counting the number of breaths in one minute and note the result. **Observe and comment on any other changes in the breathing, heart rate, or oxygen saturation that you observe.**

3. Some percentage of the respiratory volume contains dead space, air that moves back and forth but does not reach the alveoli and hence does not play an active role in ventilation. Measure another control under quiet conditions, *i.e.*, after recovery from the exercise; have the subject breathe normally and count the rate. Now have the subject breathe through the length of tube provided (for quicker results, begin with an exhalation instead of an inspiration). After a minute or two of rebreathing, again count the rate while the subject is still rebreathing. **Observe and note any qualitative changes in breathing (*e.g.*, depth of inspiration), heart rate, or oxygen saturation. When complete, ask the subject for a report of his or her sensations.**

## 2.4 Breath holding

The idea of this exercise is to measure the ability of a subject to hold his or her breath under different conditions. Here again, please apply the pulse oxymeter and record heart rate and saturation during each intervention.

1. At Rest: Have the subject breathe normally and then when ready, take a large inhalation and then hold the breath for as long as possible. Start timing at the end of the inspiration.
2. After Hyperventilating: Allow the subject to take a few slightly deeper than normal breaths; try not to over-do this as the exertion of too forceful breathing will counteract the effect of hyperventilation. After the ventilation, have the subject take a full inspiration and hold his or her breath for as long as possible.
3. After exercise: Have the subject walk briskly around the building and then as soon as he/she returns, have the subject take a large inspiration and then hold his or her breath for as long as possible.

**Note any changes in breath hold duration, heart rate, and oxygen saturation and explain the mechanism for these changes.**

## 2.5 Physical models of ventilation

The goal of this exercise is to use physical models to illustrate some features of ventilation.

### 2.5.1 Alveolar filling

We talked about the need for surfactants to reduce surface tension in the alveoli and this model demonstrates the reason for this need.

1. Take one of the alveolar models, which consists of 2 balloons connected to opposite arms of a T-connector, with a section of rubber tubing in the third arm.
2. Place the rubber tube in your mouth and pinch the neck of one balloon to inflate the other balloon to be about 2/3 full.
3. Now pinch the neck of the inflated balloon and inflate the second balloon with somewhat less air than the first balloon.
4. With your other hand, fold over and pinch the rubber tube and you should have one hand pinching the neck of the large balloon and one hand pinching the filler tube.
5. Now release the hand holding the neck of the balloon and watch what happens.
6. Repeat for different filling volumes of both balloons.

**Explain the findings on the basis of surface tension and then explain the consequence of this mechanism if it were to occur in the alveoli of the lungs. What happens instead in the lungs?**

### 2.5.2 Lung model

This model replicates the essential physics of ventilation in a very simplified form.

1. With the lung model sealed, pull down the membrane at the bottom of the model (the diaphragm) and observe the response of the lungs (the balloons).
2. Open the seal at the top of the model and repeat.

**Explain the mechanics of what you saw and describe how it relates to the pneumothorax we discussed in class**

## 2.6 Re-breathing and control of respiration

This experiment seeks to evaluate the roles of  $\text{CO}_2$  and  $\text{O}_2$  in the control of respiration. Be sure to perform this experiment only with the assistance of the instructor and gather in groups of 6–8 students. You can participate in this experiment any time during the lab period, *i.e.*, there is no need to wait until the end.

The procedure consists of two repetitions of the same basic experiment, each under slightly different conditions. In this pair of experiments the subject lies on the table and breaths into a spirometer with a nose clip applied. The spirometer is a closed system so in this configuration the subject breaths the same air in and out, with the result the oxygen concentration will go down and carbon dioxide concentration will rise over time. In one experiment, “hypoxia”, the subject will experience progressively reduced oxygen concentrations but no elevation in  $\text{CO}_2$ . The the second experiment, the subject will receive high concentration  $\text{O}_2$  and gradually elevated levels of  $\text{CO}_2$ .

The tasks required for both experiments are as follows:



**Timer:** keep track of time and initiate data recording every 30 seconds minute and announce time for others.

**Computer operator:** run the acquisition system on the computer, taking a measurement at each minute.

**Gas level recorder:** record manually the gas concentration values and the pulse oxymeter values at each time indicated by the Timer. (Often requires more than one observer.)

**Pulse oxymeter recorder:** record heart rate and O<sub>2</sub> saturation values and ensure stable operation of the pulse oxymeter throughout the measurements.

**Subject monitor:** keep an eye on the subject at all times and ensure there are no air leaks or that the subject is not in any distress. Operate the spirometer and insert and remove the mouthpiece as needed.

**Observers:** the rest of the group should observe and keep notes of the subject's response. Every minute, count manually the respiratory rate and compare this with the output of the gas analyzer.

### 2.6.1 Re-breathing room air

In this experiment, the subject starts with room air and re-breaths it through a circuit that contains a CO<sub>2</sub> scrubber (*i.e.*, a filter that removes all CO<sub>2</sub> from the air that passes through it).

The specific steps are as follows:

1. Have the subject lie comfortably on his/her back with the head closest to the spirometer.
2. Flush out the spirometer by moving the bell gently and slowly up and down 5–10 times.
3. Set up the computer to record continuously for 10 minutes.
4. Make sure the gas monitor is running normally and reset the trend display; apply the pulse oxymeter to the subject's finger and make sure it is displaying properly (saturation at east 95% and heart rate stable).
5. With the bell about two-thirds full, apply nose clips and place the mouthpiece in the subject's mouth and have him/her breath normally.
6. Start the computer acquisition.
7. At the start of the experiment and again at each 30-second interval, measure and record from the gas analyzer and pulse oxymeter the following:
  - (a) inspired and expired O<sub>2</sub> concentration,
  - (b) inspired and expired CO<sub>2</sub> concentration,
  - (c) oxygen saturation,
  - (d) heart rate, and
  - (e) respiratory rate.
8. **If any of the following occurs, remove the mouthpiece and end the experiment:**
  - inspired O<sub>2</sub> concentration drops below 10%.

- inspired CO<sub>2</sub> partial pressure climbs above 5 mm Hg (or 0.8% if barometric pressure is 640 mm Hg) (this means that the filter is not working properly).
  - Oxygen saturation drops below 70%.
  - Subject shows any sign of distress or indicates that he or she wishes to stop.
9. As soon as you remove the mouthpiece, ask the subject for his or her impressions and subjective feelings with regard to respiration. Make note of these comments.

### 2.6.2 Re-breathing oxygen-rich air

This experiment runs exactly as the previous one, but with one important change. In this case, we remove the CO<sub>2</sub> filter from the circuit but begin by filling the spirometer with oxygen. Otherwise, the steps are just as in the previous section with the exception of the stopping criteria, which become a rise in CO<sub>2</sub> partial pressure above 40 mm Hg.

The specific steps are as follows:

1. Have the subject lie comfortably on his/her back with the head closest to the spirometer.
2. Flush out the spirometer by moving the bell gently and slowly up and down 5–10 times.
3. **Load up the spirometer with oxygen from the provided cylinder.**
4. Set up the computer to record continuously for 10 minutes.
5. Make sure the gas monitor is running normally and reset the trend display; apply the pulse oxymeter to the subject's finger and make sure it is displaying properly (saturation at least 95% and heart rate stable).
6. With the bell about two-thirds full, apply nose clips and place the mouthpiece in the subject's mouth and have him/her breathe normally.
7. Start the data acquisition.
8. At the start of the experiment and again at each 30-second interval, measure and record the
  - (a) inspired and expired O<sub>2</sub> concentration,
  - (b) inspired and expired CO<sub>2</sub> concentration,
  - (c) oxygen saturation,
  - (d) heart rate, and
  - (e) respiratory rate.
9. **If any of the following occurs, remove the mouthpiece and end the experiment:**
  - expired (end tidal) CO<sub>2</sub> partial pressure climbs above 60 mm Hg (or 9.4% if barometric pressure is 640 mm Hg)
  - inspired O<sub>2</sub> concentration drops below 20%.
  - oxygen saturation drops below 70%.
  - subject shows any sign of distress or indicates that he or she wishes to stop.
10. As soon as you remove the mouthpiece, ask the subject for his or her impressions and subjective feelings with regard to respiration. Make note of these comments.

### 2.6.3 Data analysis

By recording the spirometer data during the re-breathing experiment, there is a large amount of rich data to analyze. In the interests of time, during the lab, just gather the discrete measurements of inspired and expired gas concentrations, heart rate, and oxygen saturation, *i.e.*, the values available from the pulse oxymeter and gas analyzer. Transfer this information to a spreadsheet or wait for the data to appear on the class web site, together with the spirometer signals.

The spirometry time signals provide raw data to validate respiratory rates and to extract tidal volumes. Because you are looking at a combined effect, it is better to use minute volume for your analyses, *i.e.*, the total volume of the tidal volume of all the breaths taken over a single minute.

Keep in mind that the Datex gas analyzer actively draws an average of 200 mL/min of gas for sampling, resulting in a steady decline in the spirometer volume. Another source of decline is the fact that O<sub>2</sub> consumption exceeds slightly CO<sub>2</sub> production, the ratio of CO<sub>2</sub> production to O<sub>2</sub> consumption is known as the *respiratory ratio*  $R_Q$ , a number that varies with exercise and metabolic activity but at rest is in the range of 0.7–0.8. This O<sub>2</sub> consumption typically exceeds CO<sub>2</sub> production and there is a net loss in the gas volume in the closed system of the lungs and spirometry.

Should you need to convert between percent and mm Hg, you need to know the barometric pressure. On a normal day, the barometric pressure is 640 mm Hg in Salt Lake City. You can use the following website to determine exactly what it was during the lab period:

[www.usairnet.com/weather/maps/current/utah/barometric-pressure](http://www.usairnet.com/weather/maps/current/utah/barometric-pressure)

The values in this map are in hPa (hecto Pascals) and have to be converted to mm Hg, *i.e.*, multiplied by  $760/1013.25 = 0.75$ .

## 3 Lab report

For the lab report, follow the usual format of:

1. Brief introduction and background to describe the purpose of the lab and perhaps a short description of control mechanisms for respiration.
2. Methods section that describes only particular features of the methods you used or variations from the description in this manual.
3. Results section that includes the following:
  - (a) The data necessary to answer or respond to all the questions in the description. Pay special attention to the **passages in bold that ask you to observe and describe** what you saw and measured.
  - (b) For the rebreathing experiments, from tables of all the relevant variables you measured, create plots as functions of time, as we discussed in class (see notes for this lab on the class web site).
  - (c) For the rebreathing experiments, also plot the data from the spirometry recordings, calibrated to units of volume, as functions of time, and integrate these into the plots described above so it is possible to scan all the relevant measurements for each of the two scenarios. Again, see the lab notes for examples.

- (d) Compute minute ventilation values from the spirometry time signals and create a graph of these values, again as a function of time and at the same time scale as the other variables.
4. Discussion section that explains the results you recorded. Describe the relevant measurements for each exercise and any additional qualitative findings you made. Some specific topics to address include:
- The answers to all the questions posed (especially those typeset in **bold font**) throughout this lab description.
  - Any differences between what you measured and what you expected to find based on the textbook or theoretical values.
  - How did the measurements of  $FEV_1$  with the two devices compare and how could you explain any differences?
  - Formulate an interpretation of the results from the rebreathing experiment. Did the two protocols produce different results and what do those results suggest about the control mechanisms for respiration?
  - Summarize your impressions about the role of  $CO_2$  and  $O_2$  in controlling respiration and back the points up with data from the experiments. Note any limitations in either the measurements or the way you processed and analyzed them, acknowledging that experiments are never perfect and measurements never as pure as we might wish.

## 4 Acknowledgments

Besides the usual excellent help from Paul Dryden, for this lab we wish to thank Joe Orr from the Department of Anesthesiology and Korr Medical Devices and Dwayne Westenskow from the biomedical lab at the Department of Anesthesiology for providing all the specialized measurement instrumentation. A series of great TA's have created some of the physical models of the lung, none of which has been able to withstand the test of time (just in case you are interested in a challenging problem)