CO_2 Monitoring with Experimental Animals

One of the most useful means of assessing the respiratory health of experimental animals is *capnography*, or the monitoring of expired carbon dioxide. In its most general form, capnography refers to the continuous measurement of the CO_2 fraction of expired air (see figure). This is routinely used for human patient monitoring during surgical procedures, artificial ventilation, general anesthesia, and other situations where respiratory status must be continuously evaluated. As will be seen, capnography in humans and large experimental animals is much easier to perform than in small animals. Recent improvements in the required instrumentation (the *capnograph*) allow this important measurement to be easily performed on small animals as well.

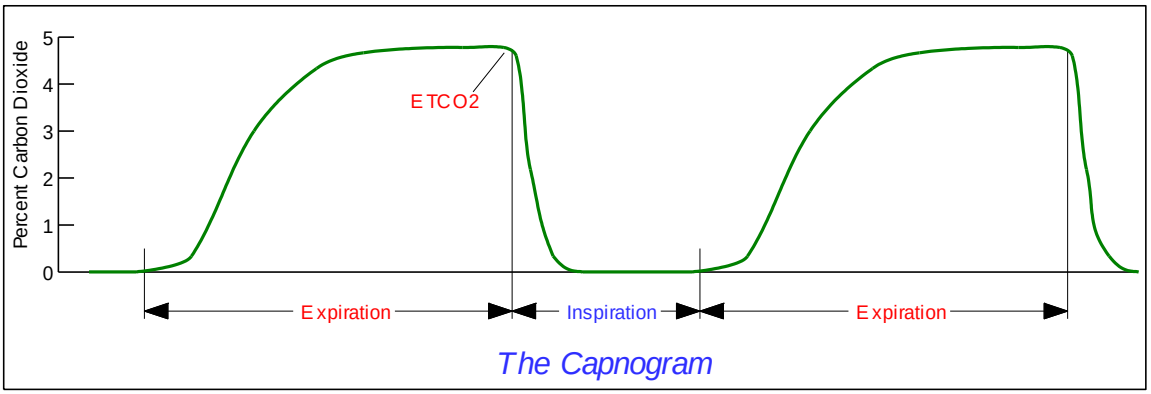
Background and measurement principles

The term capnography is often used synonymously with end-tidal CO_2 measurement (or $ETCO_2$). $ETCO_2$ measurement is actually a subset of capnography, and refers to the peak level of CO_2 observed during a breath. This measure is important since it theoretically captures the CO_2 concentration of respired air in communication with the lung alveoli. This fraction of the expired air is equilibrated by diffusion with the $PaCO_2$ of the arterial blood. It is this fact that makes respiratory CO_2 measurement so useful: $ETCO_2$ provides a non-invasive means for estimating $PaCO_2$ without recourse to frequent blood gas analysis.

Although $PaCO_2$ and $ETCO_2$ will vary proportionately during normal ventilation of the lungs, the values are not identical. $ETCO_2$ is typically a few mmHg less than $PaCO_2$ due to imperfect alveolar ventilation and perfusion; i.e., some alveoli are not completely "washed out" during ventilation, and some may receive inadequate perfusion due to anatomical or local blood flow variations. In addition, pulmonary emboli or other conditions will upset the ventilation-perfusion balance. For normal experimental animals, however, $ETCO_2$ measurement suffices as a good estimate of arterial CO_2 concentration, and thus of respiratory status. For most small mammalian species, an $ETCO_2$ should fall in the range of 3.5 – 5.5% (27 – 41mmHg).

Capnography is almost universally performed today using infrared optical gas analysis. This relies on the convenient fact that CO_2 absorbs infrared light energy in the 4.2 – 4.4μm wavelength band. A beam of infrared light is passed through the gas sample and onto a sensitive photodetector. With higher concentration of CO_2, more infrared energy is absorbed by the sample gas, and less energy falls on the photocell. The photocell output is thus inversely related to the CO_2 concentration. The instrument then linearizes the photocell signal, and presents the result as a digital readout, an analog voltage for external recording, or some other useful means. Early CO_2 measurement apparatus used dual or split beams of light, one beam passing through a zero-CO_2 reference cell, and the other passing through the gas sample. The two measurements were compared, and the difference corresponded to the CO_2 concentration in the gas sample. Two beams were necessary because the detectors were also sensitive to temperature and other environmental factors. The reference measurement served to correct for these changes in the instrument. Most modern capnographs use a single beam technique with a heated and tightly temperature-controlled measurement cell, thus eliminating the background effects of varying temperature. The best commercially available infrared analyzers have a response time of less than 100mS, making them suitable for capnography in even rapidly breathing animals. Many capnographs also make use of the CO_2 waveform to compute respiratory rate.

Anesthetic gasses can complicate the measurement, since nitrous oxide (N_2O) also strongly absorbs infrared energy at wavelengths close to, but slightly longer than CO_2. This "interference effect" is usually compensated for in the instrument, and some analyzers have an additional N_2O measurement output as a bonus. Most other commonly used gaseous anesthetics have a negligible effect on CO_2 measurement.



Practical capnography using experimental animals

There are two main techniques for obtaining a respiratory gas sample for CO_2 measurement: *mainstream* and *sidestream*. Mainstream monitoring places the CO_2 measurement cell directly in the tracheal tube connection. This has the advantages of fast response and lack of non-respiratory gasses being mixed with the sample. This is widely used for clinical monitoring of humans, but because of the relatively large internal volume of the sample cell, it is impractical for use with small animals.

Sidestream monitoring uses a gas sample drawn from an airway connection at the tracheal tube, and carried to a remotely located measurement cell for analysis. This has the advantages of not adding dead space to the breathing circuit, and can be used with intubated or non-intubated animals (e.g., using a face mask). The principal disadvantage is slower response time due to the gas transport time to the measurement cell. Additionally, provision must be made to prevent water or secretions from being drawn into the cell, which would cause measurement errors.

Because of the limitations of mainstream monitoring, sidestream monitoring is almost invariably used with small animals. For animals of several hundred grams or larger, a respiratory gas sample can be continuously withdrawn and analyzed, providing a good $ETCO_2$ measurement. Practical problems arise with smaller animals, however, since the sample cannot be withdrawn at a higher flow rate than the animal's natural expiratory flow rate. Exceeding this flow would result in mixing non-respired air into the analysis sample and an inaccurate measurement. This sample flow limitation has long been a deterrent to CO_2 measurement in very small animals, such as mice, since a suitably small sample flow would result in an unacceptably sluggish analyzer response.

An effective but technically challenging workaround to the small sample flow problem is the *carrier flow analyzer*. This technique uses a relatively large flow of carrier gas coming out of the analyzer, down a tubing to the sample site, and then actively drawn back into the analyzer.

The net sample flow drawn from the animal is thus equal to the sample drawn into the analyzer minus the carrier flow. This has the strong advantages of very small flows drawn from the animal's airway, and rapid response time. It can be seen that the CO_2 concentration flowing through the sample cell is but a small fraction of the true expired CO_2: the sample is diluted by the ratio of the net sample flow to the carrier flow (typically 5:1 – 10:1), and the measurement must be artificially multiplied to recover the real concentration. For this technique to work adequately, the CO_2 measurement cell must have great stability and accuracy at the low end of its measurement range, since that is where it will be operating. Further, the carrier – sample flow ratio must be tightly controlled for the multiplication factor to be accurate. Fortunately, advances in sensor and flow control technology have made such analyzers practical.

Why use CO_2 monitoring during experimental procedures?

There are compelling reasons to use capnography with experimental animals. Foremost among these is that $ETCO_2$ monitoring provides a continuous check on adequate ventilation when working with either mechanically ventilated or spontaneously breathing anesthetized animals.

With non-assisted anesthetized animals, depth of anesthesia can be estimated by $ETCO_2$ and respiratory rate, since many anesthetics are respiratory depressants. Both these measurements are provided by the capnograph instrument. With assisted ventilation, capnography provides a convenient check in setting ventilation parameters. Hypoventilation will result in $ETCO_2$ being above the normal levels, and hyperventilation will cause abnormally low $ETCO_2$ values. Both of these states are clearly undesirable, and should be avoided whenever possible. Besides the desirability of maintaining a normal physiological status during procedures, abnormal CO_2 levels can lead to unintended experimental consequences; e.g., with nerve recording where changes in acid-base balance can directly impact firing thresholds or frequency.

A few of the benefits of routine CO_2 measurement are:

- Verification of proper endotracheal intubation (avoiding esophageal intubation, as often happens with very small animals)
- Aids in setting appropriate ventilator parameters to avoid hypo- or hyper-ventilation (assisted animals)
- Assessing ventilator and/or anesthesia circuit configuration or problems (e.g., rebreathing may be occurring if CO_2 waveform does not return to baseline)
- Gauging anesthesia levels (spontaneously breathing animals)
- Reduces the requirements for blood gas analysis
- Indirect effects: e.g., hypo- or hyperthermia will result in lower or higher $ETCO_2$

To summarize, capnography can provide considerable practical information about your experimental animal setup. This information verifies (and can document) that the animal is in a healthy respiratory physiological state, and thus leads to confidence that the experimental

protocols will lead to usable and reproducible results. Capnographic instruments are reliable and easy to use, and are now available for animals of any size.

References:

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