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A Simple Method for Measuring Functional Residual Capacity by N_2 Washout in Small Animals and Newborn Infants

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ABSTRACT. An open circuit N_2 washout technique is described for the determination of functional residual capacity in infants. Either 100% O_2 or any oxygen/helium mixture can be used as the washing gas. The subject breathes the washing gas through a T-tube and the washed out nitrogen is mixed with this gas in a mixing chamber, placed into the exhalation part of the circuit. The N_2 concentration of the mixed gas is analyzed continuously, and the concentration signal is electronically integrated over time. Calibration of the system is accomplished by injecting known amounts of nitrogen or room air into the circuit. The gas flow through the system must remain constant and is adjusted to approximate peak inspiratory flow of the infant. *In vitro* testing of the system showed that the technique gives reproducible values (coefficient of variance <1.0%) and that the integrated signal output has a close linear correlation with the amount of N_2 washed out ($r = 0.99$). *In vivo* measurements in 10 cats confirmed the accuracy and reproducibility of the method when compared with N_2 collection. The technical advantages of the system are simplicity of components, absence of valves, easy calibration, low dead space, and no need to collect or measure expired gases. For the infant this means no added resistance during washout and no risk of hypoxia, hyperoxia, or hypercapnea. In the presence of pulmonary disease and poor gas mixing the washout period can be prolonged as needed. There is no lower limit of weight or size for functional residual capacity measurements in small infants or animals. (*Pediatr Res* 19: 1165–1169, 1985)

Abbreviation

FRC, functional residual capacity

Measurement of lung volume is an important part of pulmonary function testing in the newborn and older infant. Relating lung compliance and pulmonary conductance to FRC enables valid comparison of pulmonary function between infants of different weights and ages. Serial determinations of FRC are useful to evaluate lung growth.

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Lung volume can be determined in the newborn and infant by whole body plethysmography, by inert gas dilution, or by nitrogen washout. The techniques employed are the same used in the adult, but the equipment has to be scaled appropriately. This often causes technical problems, involves complex and expensive equipment, and the method may still not be applicable to very small perterm infants with pulmonary disease.

Krauss and Auld (1) and later Ronchetti *et al.* (2) described simple helium dilution and N_2 equilibration techniques where infants rebreathe for 30–60 s from a bag filled with 12–14% helium or 100% oxygen, respectively. The risks of these methods for the newborn are hypercapnea, hypoxia, or hyperoxia. Other limitations of these methods are that the rebreathing time necessary for equilibration between the gas concentrations in bag and lungs may be longer than 30–60 s in infants with abnormal distribution of ventilation or in infants who breathe with a small tidal volume in relation to the dead space of the mask and valve. During rebreathing the rate of O_2 uptake and CO_2 elimination changes, leading to a reduction in gas volume in the system, increase in helium or nitrogen concentration, and further uncertainty about the time when equilibration between lung and bag is reached (2). Because of these limitations this technique can only provide a crude measure of lung volume in small infants.

The plethysmographic method to measure thoracic gas volume described by Dubois *et al.* (3) has also been modified for the use in newborn infants (4, 5). The method requires highly sensitive transducers, and the results are susceptible to changes in box temperature and to small leaks. Errors secondary to this are so frequent that it has been recommended to display pressure/volume and pressure/flow loops on a xy oscilloscope in order to detect these potential problems (5). Because of the complexity of the equipment required this method cannot be performed at the bedside.

Richardson *et al.* (6) described a four breath N_2 washout method for the determination of FRC. It is based on the assumption of a one-space lung model and requires the simultaneous measurement of flow rate and N_2 concentration of each expired breath at the airway. The exhaled N_2 volume was determined by integrating the tidal N_2 concentration with respect to the exhaled flow rate. This method gave good results in infants with short time constant, but may underestimate FRC in patients with a longer time constant. The analysis of the results is time consuming and lacks accuracy if calculations are done by hand because of the phase shift between the recording of volume and N_2 concentration signals.

Hanson and Shinozaki (7) and later Sjöqvist *et al.* (8) using a multiple breath N_2 washout method measured breath to breath ventilatory flow and nitrogen concentration and performed the calculation of FRC by computer. This method requires a computer and a specially written program taking into consideration the characteristics of the equipment used to measure flow and N_2 concentration.

In order to avoid some of the above mentioned limitations problems and expenses a simple open circuit nitrogen washout technique was developed to measure FRC in small infants.

METHODS

The design of the system used to measure FRC is shown in Figure 1. It consists of a gas mixer with a flow meter (MR-1 oxygen Controller, Veriflow Corp., Richmond, CA) connected to oxygen and helium supplies, which delivers a continuous and stable flow of 100% O_2 or any oxygen/helium mixture. The gas is warmed and humidified and delivered to the infant through a T-tube by mask, nosepiece, or endotracheal tube. From the exhalation part of the T-tube the gas passes through a mixing chamber (500 ml glass bottle). The N_2 concentration of the gas leaving the mixing chamber is analyzed continuously by a rapid N_2 analyzer or mass spectrometer (Med Spect II, Chemetron Medical Products, St. Louis, MO). After the sampling site the gas flows through additional tubing to prevent drawing room air into the sampling site during an unexpected sigh of the infant. The nitrogen concentration signal is integrated (Integrator Coupler, Gould Inc., Instruments Division, Cleveland, OH) and displayed on a recorder (Brush 260, Gould Inc.).

Calibration can be done by attaching a 50-ml syringe filled with room air to the T-tube. Moving the piston back and forth in small strokes will slowly wash out the gas from the syringe and add its N_2 content to the nitrogen free gas passing through the T-tube. The signal obtained from the integrator is proportional to the amount of N_2 added (Fig. 2).

Theory and assumptions. The accuracy of the method depends on two conditions: 1) The background flow delivered by the flow meter is constant. 2) The exhaled N_2 is well mixed with the gas flowing through the system before analyzing the N_2 concentration.

If these conditions are met, the amount of N_2 washed out is equal to the product of background flow, and the area under the curve of the N_2 concentration.

$$V_{N_2} = \dot{V} \int N_2(t)\, dt$$

The flow delivered by the flow meter has been measured to be constant over 2 h and varied less than 1%. The flow remained unchanged when the subjects breathed from the T-tube. This is because the system's resistance is low (1.2 cm H_2O/liter/s at a flow of 10 liter/min) and the pressure changes during breathing are minimal in relation to the high-pressure source.

Flow and integration of N_2 concentration are inversely related. An increase in flow by 10% will lead to a decrease of the integral by also 10% and therefore to an under estimation of N_2 volume by 10%, if this change in flow is not noticed.

The mixing chamber dampens breath to breath changes in N_2 concentration resulting in a typical washout curve with a fast rise and a gradual decrease in N_2 concentration over the entire duration of the washout (Fig. 2). The larger the chamber and the lower the flow the more pronounced is this dampening effect. In our experience a container with a volume 10 times the tidal volume of the subject tested, provided good mixing as demonstrated by the reproducibility of the N_2 measurements. Poor mixing resulted in an overestimation and a larger variability of the determinations.

The equation $V_{N_2} = \dot{V} \int N_2(t)\, dt$ assumes that $\dot{V}$ is constant. Although the flow from the flow meter is constant, the flow downstream from the subject changes with each breath. During inspiration, flow will be subtracted and during expiration added to the background flow. Since inspiratory and expiratory volumes are equal, the average flow over time will remain constant and the possible error resulting from these flow changes is balanced out: The amount of nitrogen passing by the sampling site is overestimated during inspiration (lower than baseline flow) and underestimated by equal magnitude during expiration (higher than baseline flow). For this reason the above equation is valid for the described conditions. For more explanations see addendum.

The amount of nitrogen washed out can be calculated from the background flow and the integrated nitrogen concentration as follows. 1) Flow is measured to be f ml/min. 2) The integrated nitrogen concentration of a calibration gas mixture with p% nitrogen is determined over 1 min and results in c chart division. 3) The N_2 washout is performed and yields n chart divisions.

$$\text{Total } N_2 \text{ washed out: } V_{N_2} = \frac{f \cdot p \cdot n}{c}$$

It is easier to calibrate the system by washing out a known amount of room air into the N_2 free gas flow because then background flow and the value for the integration of a known N_2 concentration do not need to be measured. 1) Washout of v ml of air results in the integrated N_2 concentration of c chart divisions. 2) The N_2 washout in the patient yields n chart division.

$$\text{Total } N_2 \text{ washed out: } V_{N_2} = \frac{0.78\ v \cdot n}{c}$$

The background flow through the system is adjusted to the infant's peak inspiratory flow. This prevents rebreathing, and the N_2 concentration in the mixing chamber rises to 5–10% in all patients independent of their size.

The N_2 analyzer must have a linear output within this range, but the signal to noise ratio is not important because the integration of the noise is zero.

The accuracy of this technique was evaluated in four ways.

1) *Reproducibility of integrated N_2 signal.* The calibration procedure with the 50-ml syringe was performed 15 times on three different occasions, on each occasion with a different

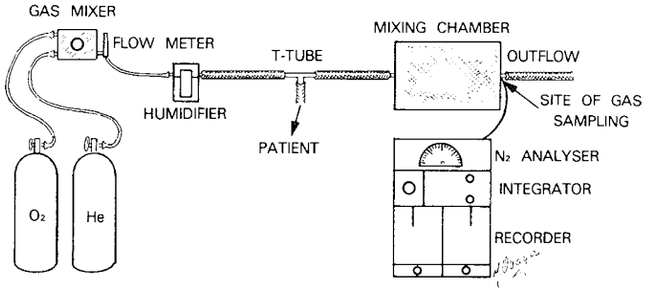

Fig. 1. The system used to measure FRC by nitrogen washout.

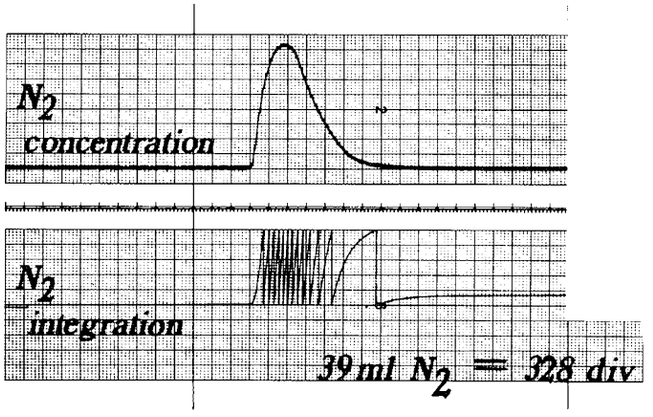

Fig. 2. Example of calibration procedure: 50 ml of air are washed into the circuit. Integration of N_2 concentration gives 328 chart divisions. Thirty-nine milliliters of N_2 (78% N_2 in air) correspond to 328 divisions. Calibraton factors: 39/328 = 0.119 ml N_2/div.

background flow (2.6, 6.0, 8.8 liter/min). The signal of the integrated N_2 concentration was recorded and the values were analyzed statistically determining mean ± SD and the coefficient of variation for each background flow.

2) *Linearity of N_2 signal.* The calibraton procedure was done with different volumes of 50, 100, 150, and 200 ml of air. Washouts were repeated five times with each volume and lasted about 20 s. Linear regression analysis was used to analyze the correlation between the volume of N_2 washed out and the signal of the integrated N_2 concentration.

3) *Collection of N_2.* To assure that no N_2 was lost from the system, and to compare the integration method with the conventionally used N_2 washout technique a large 10-liter rubber bag was attached to the end of the circuit and gas was collected during the washout procedure. The amount of nitrogen in the bag was determined from the volume measured with a calibrated syringe and the N_2 concentration of the collected gas. Again linear regression analysis was employed to describe the correlation between the amount of N_2 measured by the integration method and the N_2 collected in the bag. Paired t test was used to determine the probability of both values being different.

4) *In vivo comparison with other methods.* Finally the method was tested in 10 cats (weight 1.9–3.6 kg) intubated with a cuffed endotracheal tube (size 3.5 Fr) after being narcotized with ketamine (25 mg/kg intramuscular). The flow through the system was 1.5–2.0 l/min of 100% O_2. After calibration of the system the endotracheal tube was connected to the circuit at the end of expiration by fitting the endotracheal tube adapter into the open part of the T-tube. No valves were necessary for this switch over from room air to breathing the N_2 free gas. The cats were allowed to breathe into the system for 2 min. This time was sufficient for a complete pulmonary washout. End tidal N_2 was consistently around 6 mm Hg after 1 min and close to 3 mm Hg after 2 min of washout (0.5% N_2). Each N_2 washout measurement was repeated five to seven times. After measurements were completed the calibration of the system was rechecked to assure reliability of the measurements.

End tidal N_2 was measured before the washout on several occasions and consistently found to be 5–10 mm Hg higher than the nitrogen tension in room air. An alveolar nitrogen concentration of 79% was therefore used to calculate FRC according to the equation:

$$FRC = \frac{V_{N_2}}{F_A N_{2,a} - F_A N_{2,p}}$$

$$V_{N_2} = \text{total volume of washed out } N_2$$

$$F_A N_{2,a} = \text{fraction of alveolar } N_2 \text{ before washout}$$

$$F_A N_{2,p} = \text{fraction of alveolar } N_2 \text{ at end of washout}$$

No correction was made for N_2 washed out from blood and tissues. All measurements of FRC were corrected for body temperature and 100% water vapor saturation (BTPS).

Again, the measurements were compared with the amount of nitrogen washed out and collected in a bag as described above. Because the method described herein is a variation of the classical N_2 washout method the correlation between both should be close. The technique was also compared to the rebreathing methods described by Kraus and Auld (1) and Ronchetti *et al.* (2). For this determination a 500-ml anesthesia bag was filled with 200 ml of a mixture of 10% helium and 90% O_2. Rebreathing was started and terminated at the end of a respiratory cycle and was continued for 2 min. Each animal had seven to ten rebreathing determinations of FRC. Helium, nitrogen, and CO_2 concentrations were measured in the bag before and at the end of the rebreathing time using a mass spectrometer. Calculation of FRC is based on the assumption that equilibration of helium or nitrogen concentration between lungs and bag has occurred at the end of the rebreathing period. Calculation was done taking into consideration the reduction in bag volume present after 2 min of rebreathing due to decreasing respiratory quotient, and correcting the measurements to BTPS.

RESULTS

The reproducibility of the system's output tested at different flows was very good. Adding 39 ml N_2 to the system with low background flow resulted in an average signal from the integrator of 835 ± 4.3 chart divisions with a coefficient of variation of 0.51%. Using the same amount of N_2 at medium background flow produced an average of 366 ± 1.9 divisions with a coefficient of variation of 0.52%, and at high background flow an average of 250 ± 1.6 divisions with a variation of 0.64% were obtained. The observed decrease in signal with increasing flow illustrates the inverse relation between flow and integral of N_2 concentration. Important is that at each flow the integrated N_2 signal was highly reproducible, as reflected by the small coefficients of variation. This means that the method can be used with different background flows as long as the flow remains constant after calibration has been completed.

Linearity of the response while washing out 50, 100, 150, and 200 ml of room air into the stream of nitrogen free gas was excellent. Results are given in Table 1. The signal from the integrator increased in close proportion to the amunt of N_2 washed out. The correlation coefficient for the 20 pairs of values was 0.999.

The amount of N_2 collected in the bag was almost identical to the amount of N_2 added to the system (Table 1) ($p = 0.5948$). This indicates that no nitrogen was lost from the system.

The amount of nitrogen determined by the integration method was very closely related to the amount of N_2 collected in the bag (Table 1) ($r = 0.997$). The integration method gave results nearly identical to those obtained by the conventional N_2 washout technique ($p = 0.5327$). The coefficient of variation was significantly smaller for the integration method.

The method was evaluated *in vivo*. The individual FRC values obtained in the 10 cats by the four different methods are given in Table 2. The values obtained by N_2 integration in 10 cats varied between 20–45 ml/kg with a mean of 30.2 ± 8.6 ml/kg. The values in each animal were reproducible with a coefficient of variation smaller than 10% (mean coefficient of variation 7.1%).

FRC values obtained by N_2 collection were very similar ($p = 0.5166$) and closely correlated to the ones obtained by N_2 integration ($r = 0.95$) (Fig. 3). The intercept of the regression line with the y-axis was close to 0 (y intercept = 4.3) and its slope close to 1.0 (0.94). The mean coefficient of variation for the N_2 collection method was 9.2%.

The He dilution method gave also reproducible results, with a mean coefficient of variation of 10.9%. The results, however,

Table 1. *Correlation between N_2 added to the system, the signal of integrated N_2 concentration and the amount of N_2 measured by integration and by collection in a bag (mean ± SD)*

N_2 added to system (ml)	Signal of integrated N_2 concentration (chart divisions)	N_2 by integration (ml)	N_2 by collection (ml)
39	167.3 ± 4.4	39.3 ± 1.1	40.0 ± 1.7
78	327.6 ± 4.8	77.0 ± 1.1	78.2 ± 5.4
117	502.6 ± 6.4	118.1 ± 1.5	117.8 ± 5.3
156	663.2 ± 8.3	155.9 ± 2.0	151.8 ± 3.6
Coefficient of variation (%)	1.6	1.7	4.5

Table 2. *Comparison of measuring FRC by N_2 washout method and rebreathing method in 10 cats (means ± SD, FRC in ml)*

Wt of cat (g)	N_2 washout method		Rebreathing method	
	N_2 integration	N_2 collection	Helium dilution	N_2 equilibration
1930	75.6 ± 7.6	73.5 ± 6.2	88.0 ± 12.5	61.6 ± 9.5
2340	82.0 ± 4.8	87.2 ± 6.3	110.0 ± 7.8	81.1 ± 15.0
2900	113.0 ± 9.8	113.0 ± 11.9	116.0 ± 14.0	110.0 ± 24.0
2740	69.0 ± 6.1	72.6 ± 7.6	74.6 ± 9.3	56.0 ± 13.2
3180	68.7 ± 7.9	70.4 ± 10.6	78.7 ± 5.8	69.0 ± 20.5
2020	91.6 ± 4.9	87.7 ± 5.6	69.4 ± 7.4	59.2 ± 9.3
2400	77.8 ± 5.4	73.3 ± 4.2	88.0 ± 6.5	77.7 ± 9.9
3580	85.7 ± 4.5	83.1 ± 7.4	112.0 ± 18.0	102.0 ± 24.0
2600	52.0 ± 2.0			48.9 ± 3.7
2150	55.3 ± 2.9			60.0 ± 7.6
Coefficient of variation (%)	7.1	9.2	10.9	18.1
Correlation with N_2 integration method (r)		0.97	0.57	0.77

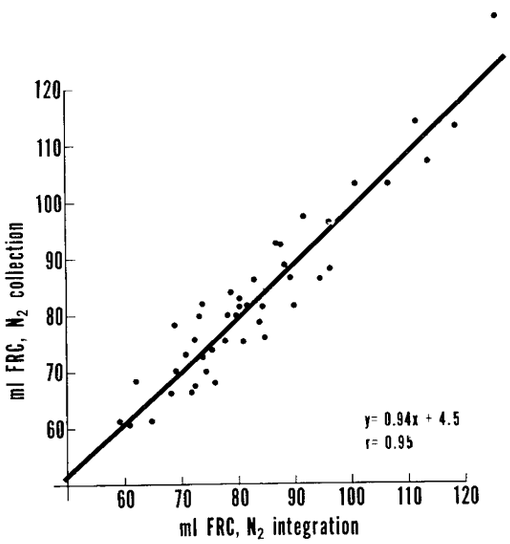

Fig. 3. Correlation between the measurement of FRC in eight cats by the N_2 integration and the N_2 collection methods.

were generally higher than the ones obtained by the N_2 integration and they did not correlate well with this method ($r = 0.57$). The intercept of the regression line with the y-axis was far from 0 (y intercept = 33), and the slope far from 1.0 (0.71).

The FRC values obtained with the N_2 equilibration technique showed larger variability than the ones obtained with the other methods (mean coefficient of variation 18.1%). The mean FRC values for each animal, however, were similar to the ones obtained by the integration method. Their correlation with the N_2 integration method was good ($r = 0.77$), the intercept of the regression line with the y-axis was close to 0 (y intercept = 4.8) and its slope close to 1.0 (0.88).

DISCUSSION

The rebreathing methods employed until now to measure FRC in infants are crude and less accurate than the more elaborated techniques with CO_2 absorption and stable spirometer volume used in adults. Calculation of FRC from these measurements in infants are unreliable because a decreasing bag volume will lead to increasing helium and N_2 concentrations interfering with equilibration of the inert gases between alveoli and bag (2). Furthermore, at the end of the rebreathing period the subjects are hyperventilating with increased respiratory rate and tidal volume making it difficult to terminate rebreathing at the end of expiration. The increased ventilatory drive may also change the FRC level during the test, thus changing the bag volume.

The method described herein to determine FRC in newborn and older infants is based on the open circuit nitrogen washout technique. It has several advantages as compared to other methods. The washout can be prolonged beyond 2 min in infants with lung disease and poor gas mixing. The washouts can be repeated without the need of emptying, refilling, and recalibrating the aparatus. Calibration is simple and fast.

The equipment used to assemble the circuit is simple, inexpensive, and available in most respiratory therapy departments while the integrator and recorder are common instruments in pulmonary laboratories. The only instrument specifically needed for the FRC measurement is a N_2 analyzer.

The advantages for the tested infants are also important. The added dead space is minimal, less than 1 ml if nasal prongs or endotracheal tube adaptors are used. Reproducible results can be obtained by inserting the nasal prongs into the nares at end expiration while tidal volume is monitored by impedance or pneumotachography. Leaks are avoided by using vaseline to improve the seal. A valve can also be used to switch from room air to the nitrogen-free gas.

The flow through the system can be adjusted according to the peak inspiratory flow of the subject tested. This prevents rebreathing avoiding the need for valves, thus reducing the resistance of the circuit. Also, by adjusting the flow through the system to the peak inspiratory flow of the infant the nitrogen concentration signal is kept in the same range for all patients. Because of this there is no lower limit for the size of infants to be tested and no reduction in the accuracy of the FRC determinations in very small subjects.

The inspired O_2 concentration does not change during the washout, as in most of the rebreathing methods, and can be adjusted to the oxygen needs of each infant by blending helium with oxygen. (If helium is used its possible effects on the function and calibration of the N_2 analyzer need to be considered.) Thus the potential complications associated with hyperoxia or hypoxia can be avoided. Because there is no rebreathing, hypercapnea and hyperventilation are also avoided.

The accuracy of the integration method as reflected by high reproducibility and linearity was excellent. This indicates indirectly that baseline flow through the system remained constant as postulated.

The *in vivo* studies in cats confirmed the accuracy, reproducibility, and simplicity of the method. The mean FRC value of 30.2 ± 8.6 ml/kg and the range of individual values were very similar to FRC measurements in cats published previously (9, 10). The individual FRC values obtained by the integration method had a lower within subject coefficient of variation, and

the results were very similar and closely correlated to the values obtained by simultaneously measuring the N_2 collected in a bag attached to the end of the circuit. This similarity and close correlation supports the assumption that the potential errors secondary to changes in flow in the mixing chamber are indeed balancing each other and do not adversely effect the measurements.

In the initial description of the open circuit nitrogen washout technique correction was made for the amount of N_2 washed out from blood and tissues, which was estimated to be 220 ml in adults after breathing 100% O_2 for 7 min (12).

No values are available for newborn or older infants or for cats, but it can be expected that the amount of N_2/kg released from body tissues in cats or newborn infants is smaller than in adults. This is because the washout lasts only 2 min and during the 1st min release of N_2 from tissues is slow until alveolar N_2 tension has decreased significantly. For these reasons no estimate of N_2 washed out from tissues was used to correct the FRC measurements in cats in the present study.

In conclusion, a new method suitable to measure FRC in newborn or small animals is described. It is based on the nitrogen washout principle but instead of collecting the washed out gas in a spirometer, the N_2 concentration of this gas is measured continuously and integrated.

The method is easier to use and has fewer risks for the newborn than the rebreathing techniques. The results are highly reproducible and closely correlated to FRC measurements obtained by the conventional N_2 washout technique.

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ADDENDUM

The formula for the classic method of N_2 washout is as follows:

$$V_{N_2} = \int \dot{V}(N_2)\, dt \quad (1)$$

In this case, the flow signal and the N_2 concentration are sampled at regular intervals and their values are mutiplied together and then that produce is integrated for the total sampling time of the test.

In the case of the collection technique, all of the flow is collected into a bag and at the end of the test, assuming complete mixing of the N_2 in the gas mixture, a single reading of the N_2 concentration is made. The complete volume of the bag is then measured and the FRC is calculated from those data. Expressing the collection technique mathematically yields the following equation.

$$V_{N_2} = (N_2) \int \dot{V}\, dt \quad (2)$$

What this equation says is that it is assumed that the final value of the N_2 concentration is used as a constant N_2 concentration applied over the whole sampling period. This assumption can be made because only the end point is measured, and that can be applied as a constant over the whole test. In this case, the flow is integrated with respect to time and the final bag volume is the integral of the flow over that period of time.

In the present investigation, we are making the assumption that the flow is held constant over the sample time, and the N_2 concentration is then integrated and multiplied by the constant flow value to establish the correct value. The equation modeling this situation is as follows:

$$V_{N_2} = \dot{V} \int (N_2)\, dt \quad (3)$$

Since we are adding tidal volume flows to the constant bias flow through the system, we should expect some variability in this method. The test variability is minimal however and can be examined two different ways. The short-term variability can be high, giving low values during inspiration and high values during expiration. Since the results are integrated over the whole period of the test, the short-term variability is minimal, because the mean value of the tidal volume flow over a long time has to be zero unless there is a change in the level of FRC or there is a leak in the test apparatus. Therefore, the situation is essentially analogous to the collection method where there is short-term variability in the N_2 concentration, but final value is measured and assumed to have been constant over the whole sample period.

The second way to examine the variability is to compare experimentally the different methods on the same data sets. In the present investigation, we compared the methods of equations (2) and (3). Comparisons were made both *in vitro* and *in vivo* and are essentially identical. The data are examined in "Results" as well as in Table 1 and Table 2 and Figure 3. All our experimental results show methods of equations (2) and (3) to be identical, verifying our assumptions concerning constant flow.