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The bias flow nitrogen washout technique for measuring the functional residual capacity in infants

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The bias flow nitrogen washout technique for measuring the functional residual capacity in infants. M.G. Morris, P. Gustafsson, R. Tepper, M. Gappa, J. Stocks, on behalf of the ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. ©ERS Journals Ltd 2001.
ABSTRACT: The functional residual capacity (FRC) is the most commonly measured static lung volume in infants. It is important for interpreting volume-dependent pulmonary mechanics, *e.g.* airway resistance, and defining normal lung growth. The bias flow nitrogen washout technique is widely used for measuring FRC because the dead space and circuit resistance are low, making it suitable for small or sick infants. Moreover, data acquisition and calculation are easily programmed for a personal computer.

The aim of this paper is to provide recommendations pertaining to equipment requirements, study procedures and reporting of data for functional residual capacity measurements. While measuring the functional residual capacity is regarded as physiologically and clinically important, the accuracy of the measurement is undoubtedly equally important. Hence, the paper also emphasizes factors influencing the accuracy of functional residual capacity measurements independent of equipment requirements. These recommendations represent the "State of the Art" in 2000.
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The present paper represents the consensus of investigators from the American Thoracic Society/European Respiratory Society Task Force on Standards for Infant Lung Function tests, who have considerable experience in the measurement of lung volume in infants. The recommendations contained within deal with equipment requirements, study procedures and reporting of data for measurements of the functional residual capacity (FRC) by nitrogen (N_2) washout. It is anticipated that as knowledge progresses these guidelines will be updated regularly. Furthermore, the acceptance and application of these recommendations will be of particular value when attempting to compare data between centres, develop or use reference data, or participate in multicentre trials which use FRC as an outcome measure.

FRC has been the only static lung volume routinely measured in infants [1, 2]. FRC is important for interpreting volume-dependent pulmonary mechanics such as airway resistance or forced expiratory flows, and for defining normal lung growth. Longitudinal measurements of FRC are useful in infants with suspected impairment of alveolar growth such as in premature infants or pulmonary hypoplasia. Atelectasis, decreased lung or increased chest compliance also decreases FRC. Increased FRC commonly occurs in relation to gas trapping associated with airway obstruction. Measuring FRC has been clinically useful in the evaluation of infants with lung diseases, such as bronchiolitis, bronchopulmonary dysplasia, cystic fibrosis, and respiratory distress syndrome, as well as the evaluation of treatment efficacy [1–3].

FRC can be measured by body plethysmography (FRC_{pleth}) or by multiple breath inert gas (x) washout/dilution technique (FRC_{gasx}). The latter includes the closed circuit (*e.g.* closed circuit helium dilution) and the open washout systems. The latter in turn can be divided into bias flow and breath-by-breath washout systems. Different inert gas tracers can be used such as N_2, helium (He), argon (Ar), and sulphur hexafluoride ($SF6$). The gases can be measured with various devices such as a helium catharometer, N_2 emission spectrophotometer (known as the N_2 analyser), mass spectrometer, main stream or side stream infrared (JR) detector. Breath-by-breath systems provide information on gas distribution and ventilatory efficiency that are not provided by the bias flow washout systems. N_2 washout employs 100% oxygen (O_2), but room air can be used when other inert gas tracers are washed out. N_2 washout can also be performed using 21% O_2/79% Ar mixture when using a mass spectrometer. The bias flow N_2 washout technique has been the most commonly used approach. It is relatively simple and inexpensive to set up in the laboratory [1–14].

Definitions and terminology

The technique referred to in the present paper is for measuring the FRC in spontaneously breathing infants by the bias flow N_2 washout technique. The FRC is the volume of air contained in the lung and airways at end-tidal expiration [1–3]. The FRC measured is referred to as FRC_{N_2} to differentiate it from measurements made with other tracer gases or plethysmography. For measurements of FRC_{N_2} in ventilated infants the reader is referred to recent publications [6, 10, 15–19].

Equipment

See previous publications in this series [20, 21] for further details and justification of the recommendations presented.

The nitrogen washout circuit

Further details of the theoretical background and basic methodology have been described by TEPPER *et al.* [3].

Concept. The open circuit N_2 washout method for assessment of FRC_{N_2} entails measuring the volume of nitrogen expired after end-tidal expiratory switching of the inspired gas from room air to 100% O_2. At a constant bias flow that exceeds the infant′s inspiratory peak flow during tidal breathing, the integrated mixed expired FN_2 (area under the curve of the N_2 concentration (F = fractional concentration) *versus* time (*t*)) is multiplied by the constant flow of O_2 (V'), to obtain the volume of expired N_2 (VN_2) [3, 5, 6]:

$$V_{N_2}(t) = V' \int_0^t FN_2(t)\,dt \qquad (1)$$

Therefore, the accuracy of the method depends on two conditions: 1) the background of O_2 flow remaining constant; and 2) the exhaled N_2 being well mixed with the bias flow of O_2 before the N_2 concentration is analysed [5].

A two-point calibration is performed with known air volumes. With the amount of N_2 washed out measured and the initial fractional alveolar N_2 concentration (FA_{i,N_2}) known (FA_{i,N_2}: room air = 0.79), then the lung volume at which the washout was initiated can be calculated:

$$\text{Lung volume (FRC)} = \frac{\text{Volume } N_2 \text{ washed out}}{(FA_{i,N_2} - 0.02)} \qquad (2)$$

The value of 0.02 is subtracted from FA_{i,N_2} because the washout is terminated at an end-tidal FN_2 of 0.02 at the airway opening. Since the N_2 is diluted within a mixing chamber (see later), the washout is actually continued until the nitrogen analyser reads an FN_2 of $\leqslant 0.0065$ in the chamber, which corresponds to the end-tidal FN_2 of 0.02. This dilution effect will depend upon the flow of gas through the N_2 mixing chamber and the size of the chamber. The end-tidal FN_2 cut-off of 0.02 reduces the overestimation of FRC, since previous tissue N_2 elimination studies during pure O_2 breathing in adults and animals suggested that FRC_{N_2} includes tissue-N_2 dissolved into the lung during prolonged washout [4, 22–24].

Corrections are made for the dead space of the mask and apparatus, the switching error above FRC and

body temperature, pressure and saturation (BTPS) (see later).

Equipment required

The equipment required is outlined in fig. 1 and includes a: clear face mask; three-way switching valve with two inlet/outlet ports and a mask port; T-piece; pneumotachometer (PNT); O_2 supply with a precision flowmeter ($0-15$ L·min^{-1}); N_2 analyser and mixing chamber; calibrating syringe; collapsible breathing bag and a T-connection (recommended; see later) [11, 12].

Face mask. The face mask and the estimation of the effective dead space have been described in detail elsewhere [20, 25].

Three-way switching valve. The three-way switching valve allows the infant to breathe either room air through the PNT or 100% O_2 through a T-piece. Computer-controlled electronic switching of the three-way valve *via* a computer keyboard stroke is preferred. The PNT is connected to one of the two-inlet/outlet ports of the three-way valve (fig. 1). The other inlet/outlet port is attached to the T-piece which carries the constant O_2 bias flow from the flowmeter, through the T-piece, tubing and N_2 mixing chamber. The infant breathes room air through the face mask, mask port of the three-way valve and PNT. When switched into pure O_2 during the washout, the infant no longer breathes through the PNT (fig. 1) [7, 12]. Connecting the PNT to the inlet/outlet port of the three-way valve is the preferred placement because this decreases the dead space and resistance during the washout. The PNT may be placed within the washout circuit by connecting it to the mask port, which is useful in preterm infants with unstable breathing patterns. However, variations in gas temperature, composition and viscosity need to be accounted for as well as the phase shift between the flow and N_2 concentration signals [3].

The operator should be able to choose one of two switching modes: 1) automatic, whereby the software programme monitors stability of the tidal volume (V_T) and expiatory time (t_E) so that when the user triggers the activation of the slide valve, it occurs at the end of the next expiration provided that V_T and t_E of that breath are within a set percentage (*e.g.* 10%) of the previous mean of $5-10$ breaths. This may be difficult to achieve in small babies with rapid or irregular breathing, and it may therefore, be necessary to allow user adjustment of the selected percentage. Nevertheless, this mode has the advantage of being simple to use and minimizing inter-observer variability. For a detailed discussion regarding the use of automatic breath identification, see BATES *et al.* [26]. 2) Manual, whereby the operator waits until a stable tidal breathing (end-expiratory level) pattern is observed in real time on the computer monitor and activates the slide valve as close as possible to end-expiration in a chosen breath [3, 7, 12]. This mode requires an experienced operator but is useful if the infant has irregular breathing.
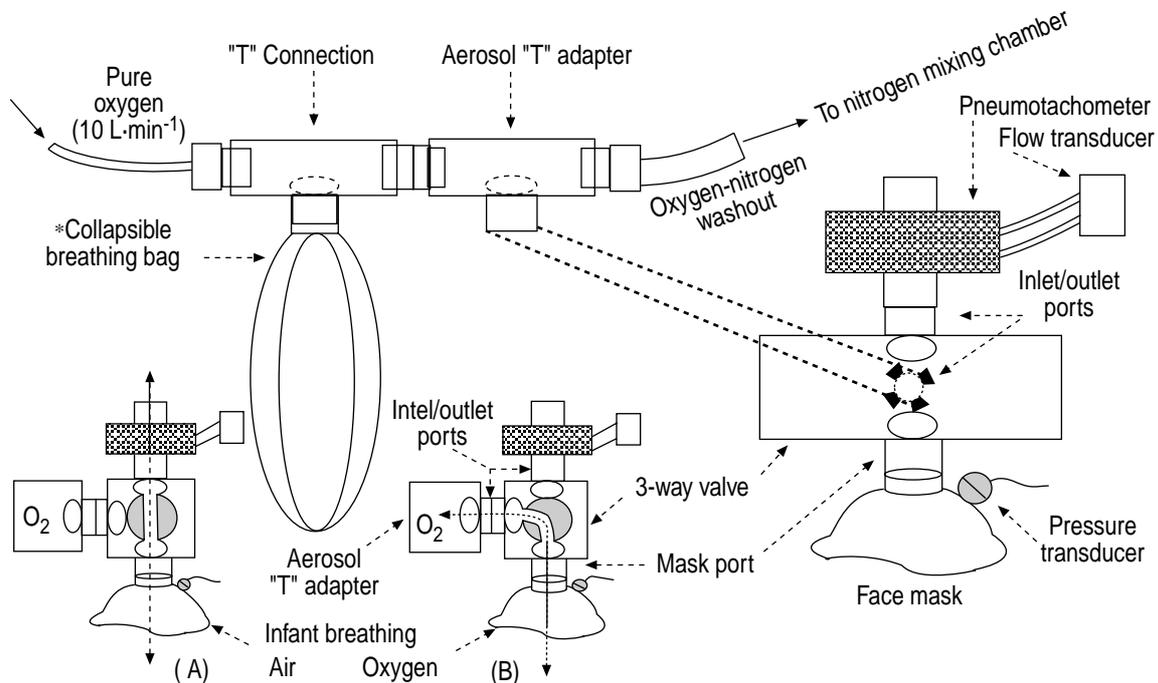

Fig. 1. – The nitrogen washout circuit. The long parallel dotted lines point to the connection site of the central port of the aerosol "T" adapter which carries the constant (10 L·min^{-1}) bias flow of oxygen into the inlet/outlet port of the slide valve. Side views of the circuit depict the path (arrows interconnected by a dotted line) of air (A) and, after activating the slide valve, of oxygen (B) in and out of the of the infant's airway. Note that after the slide valve switches the infant into oxygen, the infant no longer breathes through the pneumotachometer. *: Inclusion of a collapsible breathing bag is optional. (Adapted and modifed from Morris [12]).

Nitrogen analyser. N_2 concentration is usually measured using emission spectrophotometry in a low-pressure ionization chamber under conditions of constant flow, although a mass spectrometer can also be used. An adjustable needle valve mounted on the mixing chamber is connected to a vacuum pump in order to provide the optimum negative pressure and constant flow for the ionization of nitrogen. The analyser should have: a linear output with a range $0-100\%$ N_2; an accuracy of 1% full range; a resolution of 0.01%; a drift $<0.2\%$ $N_2 \cdot h^{-1}$ (stability close to zero N_2 concentration is particularly important); a range of gas sampling rate $9-50$ mL·min^{-1}; a recorder output of $0-10$ Volts Direct Current (VDC) full scale; a response time $(10-90\%$ full scale) of <100 ms.

The manufacturer should provide details regarding the necessary warming time for the equipment [11].

It is anticipated that these requirements will need to be amended for use with newer generations of equipment or alternative ways of measuring FRC$_{N_2}$, such as those based on CO_2 and O_2 subtraction techniques, which have not yet been validated or sufficiently tried in infant testing. Time characteristics of these systems may have to be assessed using Fast Fourier Transform (FFT) analysis of the N_2 signal using a rapid N_2 analyser.

Nitrogen mixing chamber. The mixing chamber should contain at least three serial channels with baffles to recirculate and mix the gas prior to exiting the chamber. A long (~ 2.0 m), low resistance tubing is attached to the outlet port of the mixing chamber to prevent ambient air from diffusing back into the mixing chamber. A chamber volume of about 500 mL would be adequate, even in toddlers, particularly if a collapsible breathing bag is incorporated in the circuit (see later) [11, 12].

Collapsible breathing bag. A collapsible breathing bag (0.5 L) can be incorporated into the washout circuit *via* a second T-connection. It should be placed between the infant and the O_2 source but closer to the former. This has been reported to enhance the reproducibility of measurements by acting as a buffer reservoir. This minimizes flow swings within the N_2 mixing chamber during the breathing cycle as well as the retrograde movement of mixed O_2 and N_2 gas after it passes beyond the N_2 sampling needle port. In addition, the bag can be used to monitor the infant's breathing pattern both during the washout and when determining the "end of test" (see later) [11, 12].

Bias flow of oxygen. The bias flow of O_2 should be standardized to facilitate comparison of washout times between laboratories: 10 L·min^{-1} and 5 L·min^{-1} are suitable for infants weighing $\geqslant 5.0$ kg, respectively [3, 12]. Nevertheless, the volume of the washout circuit may influence the circuit time constant.

Flowmeter. This has been described elsewhere [20, 26, 27].

Calibration syringe. The exact dead space of the calibration syringe should be known. The combined volume of the syringe once connected to the mask port is generally less than the sum of their individual volumes as determined by water replacement. If both have been made by the same manufacturer, the latter can provide these data, but further confirmation by the investigator using water displacement with the equipment assembled as for use *in vivo* is recommended [11].

Measurements in infants who require an oxygen supplement. In infants who require an O_2 supplement, FRC can be measured in one of two ways: calibration is performed with gas volumes of the same FN_2 as the infant is breathing, and FRC is calculated as described earlier; calibration is performed with room air, with the calculated volume subsequently being multiplied by a correction factor that accounts for the difference in FN_2 between calibration and alveolar gas [3].

The bias flow N_2 washout technique becomes inaccurate when the fractional concentration of inspired oxygen is >0.7 [3].

Helium/O_2 (80/20%) has been used in premature infants to prevent exposure to high O_2 concentration [28] but may not be used interchangeably [29]. In addition, the effect of a helium/oxygen mixture on gas mixing, equilibration time and lung volume have yet to be evaluated [3]. While pure O_2 has been suggested to decrease tidal breathing significantly [30, 31], there are no data indicating that any potentially harmful effects could result from such short exposures to pure O_2 as during FRC measurement.

Data acquisition. Data acquisition requirements are dealt with elsewhere in this series [21, 26, 27, 32]. Particular points of relevance to lung volume measurements are: 1) a sampling rate of 100 Hz is normally adequate for the acquisition of FRC$_{N_2}$ data. However, 200 Hz may be required for rapidly breathing infants or the washout volume/time measurements (see later). 2) Prior to data acquisition, the operator should be prompted to enter whether a subject is being tested or an *in vitro* assessment performed, so that any body temperature, pressure and saturation (BTPS) corrections can be switched on or off, respectively. The type of measurement should be indicated in the report [11, 12]. 3) Prior to the FRC measurements, tidal flow and hence volume should be corrected to BTPS conditions, assuming that inspired air is at ambient temperature, pressure and saturation conditions (ATPS) and expired air at BTPS conditions [26]. 4) Volume drift, any drift of the tidal volume signal prior to switching the infant into O_2 during FRC measurement should be minimized. 5) A representative end expiratory level must be established (see later). 6) In the absence of any injected air into the O_2 circuit, there should be no drift in the baseline of the integrated nitrogen signal (INS), that is the INS should read zero (arbitrary units), after the three-way valve is activated. When testing patients with airway obstruction, the period during which there is no drift must persist for at least $90-120$ seconds [11, 12]. 7) End of test should be operator-controlled rather than automatic. End of test is defined by a FN_2 decreasing to 0.0065 within the mixing chamber and N_2 analyser, in the presence of regular breathing. Before ending the washout, the operator must ensure that this low N_2 concentration has been recorded

during regular breathing. This can be confirmed by watching the movements of a collapsible breathing bag provided this has been incorporated in the washout circuit (see earlier) [11]. In the absence of such movements the test should continue, since the low N$_2$ concentration may simply be due to a brief apnoea. The infant's head may need to be repositioned if there is any suggestion that such an apnoea could be due to a temporary upper airway obstruction rather than periodic breathing [11]. 8) For calculation of FRC$_{N_2}$ see later. Measured FRC must be converted to BTPS conditions.

Calibration of the nitrogen analyser. Equipment calibration has a significant influence on the calculated results and should be performed with utmost care and according to the manufacturer's recommendations. It is essential that: adequate equipment warming times be used according to the manufacturer's recommendation; calibration is performed with the same equipment configuration as during measurements; the calibration tools are checked periodically; qualified personnel, who understand both the procedure and data acquisition, perform the calibration; manual calibration is performed intermittently to check automatic calibration procedures.

After stabilization of the INS baseline, calibration with known low (LV) and a high (HV) room air volumes, below and above the infant's expected FRC, is performed. For a computerized system, integration of the mixed FN$_2$ signal (INS) (arbitrary units) begins when the mixed FN$_2$ value is >0.006. Washout is complete when FN$_2$ falls <0.0065 in the mixing chamber [3].

The slope and intercept of the calibration line are calculated as follows [3]:

$$\text{Slope} = \frac{(\text{HV} - \text{LV})}{(\text{INS(HV)} - \text{INS(LV)})} \quad (3)$$

$$\text{Intercept} = -(\text{INS(HV)} \times \text{Slope}) + \text{HV} \quad (4)$$

$$\text{FRC} = (\text{INS} \times \text{Slope}) + \text{Intercept} \quad (5)$$

With any BTPS correction switched off, calibration should be confirmed by using the same syringe volume on two consecutive occasions; the calculated washout volume should be within 1% of the known volume. After infant testing has been completed, calibration should again be confirmed with a known volume after installing a clean N$_2$ circuit to avoid contamination of the calibrating syringe. This is important to check that there has been no drift of the N$_2$ analyser during the testing period. The use of calibration volumes equivalent to the infant's measured FRC is recommended, as is the use of similar VT/FRC ratios and respiratory rates to those of the infant [7, 9, 11, 12].

Monitor display. See also F$_{REY}$ *et al.* [10].

Tidal breathing prior to FRC assessment. During data collection and/or replay, time-based displays of flow and volume are required to observe breathing pattern and facilitate activation of the slide valve at end-expiration.

During measurement of FRC$_{N_2}$. Once the slide valve is activated, a time-based display of the following are required: the N$_2$ concentration (FN$_2$) curve over time (s); the initial N$_2$ concentration at the time of switching the subject into O$_2$ (should read 0.00); the changes in the N$_2$ concentration (displayed to two decimal points) during washout; the integrated N$_2$ signal (arbitrary units).

After the washout is completed the operator needs to be able to: reject a curve before storage due to technical patterns, together with the ability to exclude (but not delete) selected trials during the analysis process; read off respective values of flow and volume during the tidal breathing recording prior to washout by, for example, moving a cursor through such data; examine/print any cumulative calculations/plots of volume by washout as a function of time, *e.g.* from time zero up to a certain point in time or over any specified time period.

In summary, it is essential that both graphics and tabulation be of sufficiently high standard to allow the user to decide when a washout is complete. Furthermore, printouts of washout curves should be of sufficient resolution for later quality assurance. It is essential that such curves are saved for later inspection.

Measurement protocol

Further practical details of how to apply this technique and interpret results have been described previously. The task force has published a book that collates much of the relevant information and discusses background issues that may influence the measurement [3].

Preparing the infant for measurements

The following are necessary when preparing the infant for measurements: full resuscitation equipment, including suction, should be available at the site of infant lung function testing; two individuals (other than parents) should be present during testing, one of whom has the prime responsibility for the infant's well being. The infant must never be left unattended; the infant must be monitored continuously using at least a pulse oximeter; the hospital-specific protocol for sedation must be adhered to; measurements should be generally obtained with the sleeping infant laying supine. If other postures are used, these should be clearly indicated; the neck and/or shoulders should be supported in the midline in slight extension and position stabilized by using a neck-roll or head ring; the face mask should be transparent. It should cover the mouth and nose and be placed with minimal pressure. An airtight seal can be maintained with a thin ring of silicone putty; measurements should be restricted to periods of regular quiet breathing. It is particularly important to avoid switching the infant into O$_2$ during rapid eye movement sleep when FRC may be very unstable [33]; if a squeeze jacket

had been applied to perform forced expiatory manoeuvrers, it must be unfastened before measuring FRC.

Preparation for collecting data for functional residual capacity

The following is necessary when preparing for data collecting of the functional residual capacity: connecting the PNT-three way valve assembly to the face mask and recording at least 30 s of tidal breathing prior to FRC measurement; once a stable tidal breathing is observed on the monitor, switching the infant into O_2 as close to end-expiration as possible; depending on the bias flow of O_2 used and the length of the washout tube carrying the mixed O_2/N_2 gas from the infant to the N_2 mixing chamber, the $FRCN_2$ washout curve usually rises above the baseline within one [11, 12] or more seconds. It reaches an initial sharp peak followed by a stepwise decrease until the washout is complete. When the nitrogen analyser reads an FN_2 of 0.0065 within the N_2 mixing chamber at the end of the washout period, the three-way valve is activated switching the infant back into room air; the waiting period in between tests should be at least twice the washout time. After a washout has been completed and the infant is switched to room air, giving the infant a few sigh breaths *via* a Y-adapter carrying a bias flow $(12-15 \text{ L·min}^{-1})$ of air and connected to the PNT can speed the restoration of FAi,N_2 [11].

Potential sources of error when measuring $FRCN_2$

In addition to equipment related errors, factors that may adversely affect the accuracy of measurements [3, 7, 9, 11, 12, 25] include: insufficient equipment warming times; inadequate equilibration of the calibrating syringe with room air N_2 after it had been used for calibration; mask or circuit leaks; drift of the tidal volume signal and changes in the end-expiratory level; baseline drifting of the N_2 washout curve; PNT calibration errors and effect of heating; errors in estimating mask/apparatus dead space; retrograde movement of mixed O_2 and N_2 gas after it has passed beyond the N_2 sampling needle port; switching errors above FRC; errors in BTPS correction; infant's apnoea during a washout; inadequate interval between washouts; malfunction of the gas sampling needle or the N_2 analyser; incorrectly connecting the tube carrying the washout mixed O_2/N_2 gas (fig. 1) to the outlet instead of the inlet port of the N_2 mixing chamber.

Calculations

Tidal breathing parameters. The tidal breathing parameters that need calculating include (see also BATES *et al.* [26, 32]): End expiratory level (EEL); tidal volume; respiratory rate.

The end expiratory level (*i.e.* FRC) should be established over at least 5 breaths prior to switching the infant into O_2, after correcting for any volume drift. For consistency, it is suggested that the EEL is calculated as the mean of all selected end expiratory points after drift correction, and that the selected level

is clearly displayed on a time based trace for verification of accuracy by the operator. Some user flexibility may be required in those cases where EEL is particularly variable.

Calculation of $FRCN_2$. As described earlier, the integrated mixed expired FN_2 *versus* time (t) is multiplied by the constant flow of O_2 (V') to obtain the volume of expired N_2 (VN_2):

$$VN_2(t) = V' \int_0^t FN_2(t)\, dt \qquad (6)$$

The total volume ($Vtot$) measured by the washout, which is at ATPS, is calculated (see earlier):

$$Vtot = VN_2 \div (FAi,N_2 - 0.02) \qquad (7)$$

The volume of apparatus dead space (Vds,app (mL)), including the face mask (see earlier), (assumed to be at ATPS) is subtracted from $Vtot$ to obtain the effective washout volume ($Veff$):

$$Vtot - Vds,app = Veff \qquad (8)$$

The $Veff$ is converted to BTPS ($Veff\text{-}BTPS$) [24].

The switching errors above FRC should be corrected for by subtracting any volume above EEL (previously converted to BTPS conditions) from the $VEFF\text{-}BTPS$ to obtain $FRCN_2$ [7, 12]:

$$FRCN_2 = Veff\text{-}BTPS - V_{>FRC\text{-}BTPS} \qquad (9)$$

Switching errors should be negligible if automatic switching into O_2 has been employed and the infant has regular breathing (see earlier).

FRC should be calculated from at least two technically acceptable measurements that are within 10% or 10 mL of each other, whichever is the larger or, in the presence of increased variability, the mean of three technically satisfactory trials.

Quality control parameters. $FRCN_2$ is the only "outcome measure" that will be routinely reported from the nitrogen washout technique. Calibration and raw data of FRC should be saved in ASCII or similar format. Data that should be retrievable for validation purposes by the user, but not necessarily visible, include the uncalibrated analogue-to-digital (A/D) signal and all intermediate calculations leading to the final results. The parameters that should be displayed/available for each individual trial to assist in user or automated selection of the "best" data and to provide overall quality assurance, are listed below. Since many of these quality control features need to be summarized in publications describing $FRCN_2$ measurements in infants, it is essential that such information can be automatically saved and, if required, exported to a suitable spreadsheet.

The use of standard abbreviations as indicated would be of considerable benefit and is strongly recommended. The quality control and other parameters include: number of acceptable measurements (n); total number of measurements performed (n-tot); volume of apparatus dead space (Vds,app (mL)); type and size of mask *e.g.* Rendall Baker size; mean tidal volume prior to FRC measurement (VT (mL)); mean

respiratory rate prior to FRC measurement (RR (min^{-1})); minute ventilation (expired minute ventilation) prior to FRC measurement, calculated as $RR \times V_T$ ($V'E$; also referred to as MV); stability of EEL prior to switching the infant into O_2, expressed as % variability of end expiratory points over "n" (five) breaths prior to each switching of the infant into O_2 during FRC measurement (EEL%) [26, 27]; stability of EEL prior to each switching into O_2 during FRC measurement, expressed as the SD of the end expiratory points relative to the baseline over "n" (five) breaths prior to each manoeuvre (EEL-s, (mL)) [26, 27]; volume above EEL reflecting the switching error above FRC ($V>$FRC (mL)); FRC washout time (tFRCN_2 (s)); cumulative volume washed out at 50, 75, and 85% of the (total) washout time, FRC50N_2, FRC75N_2, FRC85N_2 (the potential usefulness of such parameters have yet to be explored).

Reporting

Data should be reported according to the following: for FRC (mL) the mean from three trials or a minimum of two if these are within 10% or 10 mL of each other (see earlier); the coefficient of variation (CV = $100 \times$ SD/mean) of 3–5 technically satisfactory FRC measurements should be available as a measure of the intra-subject variability; for quality control, a print out of the FRCN_2 nitrogen washout curve should also be provided; a print out of the time-based flow and volume traces at the time of switching into O_2 is helpful; ideally, data should not be presented as "per cent of predicted"; predicted values of FRCN_2 beyond the neonatal period should be expressed as the regression of FRC on crown-heel length along with the residual standard deviation (RSD). Observed values of FRCN_2 can then be reported as the number of RSD removed from the predicted mean [34]; FRCN_2 may be expressed per unit body weight during the first month of life as the regression of FRC *versus* weight is fairly linear and passes close to zero. FRCN_2 should never be expressed per unit body length, as neither of the conditions discussed earlier are met [34]; results can also be expressed in relation to the "normal range" according to the infant's age and sex; a reference equation from collated data of FRCHe [34] (table 1) that can be used until sufficient reference data is obtained for FRCN_2 is:

$$FRC_{He} = 0.0036 \cdot L^{2.531} \qquad (10)$$

where L is the crown-to-heel length in cm, RSD = 17.7% and 95% confidence interval (CI) = 71–141%.

See also TEPPER *et al.* [3]; STOCKS and QUANJER [34].

Table 1. – Representative values of functional residual capacity at arbitrary chosen values of length and body weight in infants from collated data [34]

Length cm	50	60	70	80	90
FRCHe mL	72	114	168	236	318
95% CI mL	51–102	81–161	119–237	166–333	226–448

FRCHe: Functional residual capacity by helium; CI: confidence interval.

Each individual laboratory should attempt to study at least some healthy infants to check whether available reference data are appropriate for their population. Regular checks should also be made to ensure that the equipment produces appropriate values during *in vitro* assessment over the full range of lung volumes likely to be encountered during studies.

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