Online Supplement

E1. Glossary of terms

BTPS	Body temperature, pressure and saturated. The condition under which lung volumes, respired volumes and flows are reported. Ideally BTPS correction should be dynamic, reflecting the fluctuations of temperature and humidity through the breathing cycle, and variations with flow. Changes in hardware may alter the BTPS correction required, prompting reassessment. The equation to convert ATPS to BTPS is: $V_{BTPS} = V_{ATPS}$ [310° / (273° + T_A)][(P_1 - P_{H2O}) / (P_1 - 47)] P_1, ambient barometric pressure (mmHg). T_A, ambient temperature (in centigrade, °C). P_{H2O}, ambient partial pressure of water. Body Temperature is assumed to be 37°C, hence standard temperature is 310°K (i.e. 273°K + 37°C) with a P_{H2O} of 47 mmHg.
C	Concentration of gas.
CDI	Convection-Dependent inhomogeneity.
C_{et}	End-tidal gas concentration. A representative measure of alveolar concentration of a particular gas at the end of expiration. Ideally, free of phase IV contribution,
CEV	Cumulative expired volume, defined as the sum of the expiratory tidal volumes measured up to the point of interest.
CEV_{alv}	This is the cumulative alveolar expired volume, and represents the cumulative expired volume directly participating in gas exchange. CEV_{alv} contribution for each breath is calculated by subtracting airway and external dead space from the expired volume of the breath.
Closing capacity (CC)	The volume of gas remaining in the lungs when phase IV starts. Closing capacity = closing volume + residual volume.
Closing volume (CV)	The volume expired from the start of the phase IV to the end of the expiration.
Convection	Net volume change driven by differences in pressure. “Bulk flow”.
DCDI	Diffusion-Convection-interaction-Dependent inhomogeneity.
Diffusion	Gas molecule movement driven by concentration gradients.
Diffusion convection front	Quasi-stationary gas front arising in the zone where convective and diffusive gas mixing mechanisms are of similar importance for gas molecule movement, separating alveolar and bronchial gas concentrations.
EELV	End expiratory lung volume
Expirogram	Plot of gas concentration vs. expiratory volume for given breath.
FRC	Functional residual capacity, a representative measure of end expiratory lung volume after a relaxed expiration.

FRC_{gas}	FRC measured as the lung volume communicating with the atmosphere.
FRC_{Pleth}	FRC measured as the intra-thoracic gas volume using whole body plethysmography, representing all compressible intra-thoracic gas.
gs	Gas sampling point
IGW	Inert gas washout.
Inert marker gas	A non toxic gas that does not participate in gas exchange or other metabolic processes that has a relatively low solubility in blood and other tissue and that can be measured rapidly online.
LCI	Lung Clearance Index.
Mainstream	The main path of the respiratory flow.
MBW	Multiple breath inert gas washout test. An open circuit inert gas washout test involving either spontaneous or controlled tidal breathing, allowing determination of FRC and various indices of ventilation distribution non uniformity.
Open circuit washout system	System with minimal re-inspiration of exhaled gas.
Peclet number	Describes the ratio of convective (the product of gas velocity and airway diameter) and diffusive gas transport in the airway. The diffusion-convection front arises between a Pe number of 1 and 0.1. Peclet number = ud/D Where, u is gas velocity, d is airway diameter and D is gas diffusivity.
Phase I	Phase of expiration when no alveolar gas has yet appeared (i.e. no increase of inert gas concentration after previous inspiration), also referred to as absolute dead space volume.
Phase II	Portion of expirogram with a rapid increase of inert marker gas concentration representing the average of the sequential arrivals of diffusion fronts from the lung periphery. Termed the bronchial phase of the expirogram.
Phase III	Phase of expirogram representing alveolar gas concentration. Termed the alveolar phase of the expirogram.
Phase IV	Late phase of expirogram occurring in the presence of airway closures. Onset of phase IV slope is marked by sudden increase in inert gas concentration occurring during the alveolar phase. Measured as intercept of phase III and phase IV slopes.
Relative humidity (RH)	Percentage of maximum water vapour pressure at a given temperature.
Response time (gas analyser)	By definition, the time required for a gas sensor's signal to raise from 10-90% of a step change in gas concentration.
Single breath washout (SBW)	An open circuit inert gas washout test involving a predefined controlled breath size, inspiratory and expiratory flow and pre- and post-inspiratory lung volumes. The SBW test is used to assess non-uniformity of ventilation distribution from the slope of the phase III, the closing volume, the closing capacity and/or the height of the phase IV.

Sequencing	Sequential filling and emptying among lung units due to differences in mechanical properties, i.e. Resistance (*R*) and/or Compliance (C), which determine time constant (τ; $\tau = R * C$). Sequencing is a prerequisite for phase III to arise in the conducting airway zone.
Sidestream	Sample taken from the mainstream.
Signal-to-Noise ratio	Measure of signal strength relative to background noise, and conventionally expressed as the mean/SD of a gas input of constant concentration over a period of 10 seconds.
S_{III}	Phase III slope. Mean rate of increase in gas concentration during phase III, measured by linear regression and expressed as percent per litre (%/L).
Sn_{III}	Phase III slope (S_{III}) normalised for the gas dilution occurring over the MBW. This can be performed by dividing S_{III} by one of three different reference inert marker gas concentrations. 1. Mean inert marker gas concentration over the entire expiration (**$Sn_{III,mb}$** = S_{III} / mean C_{gas} over breath). 2. Mean inert marker gas concentration over the interval used for S_{III} calculation. (**$Sn_{III,ms}$** = S_{III} / mean C_{gas} over slope interval). 3. End tidal gas concentration. (**$Sn_{III,et}$** = S_{III} / C_{et}). The choice of normalisation concentration must be clearly stated.
Specific ventilation	The change in volume of a lung unit with breathing as a proportion of its initial volume ($\Delta V / V$).
Synchronisation of gas to flow signal	Asynchrony between gas and respiratory flow signals created by transit time of gas within sampling tubing and analyser response characteristics. Transit time is the time from start of exposure to the first deflection of a step change in gas concentration. Response time is measured as the signal change from 10-90% of full signal deflection in response to a step change in gas concentration. Synchronisation time shift is defined as transit time plus from start of deflection to 50% of full deflection.
TO	Lung volume turnover. The cumulative expired volume (CEV, i.e. sum of the expired tidal volumes) expressed as the number of lung volume turnovers after a given breath during an MBW. The method of calculation must be clearly described (see below). Where pre-gas sampling point (gs) V_D dead space is defined, TO is referenced to the airway opening (TO_{ao}) $TO_{ao} = \frac{\text{CEV - number of breaths} \bullet (V_{D,pre\text{-}gs} + V_{D,post\text{-}gs})}{FRC_{ao}}$ Where pre-gs V_D is uncertain (e.g. infants, pre-schoolers), TO is referenced to the gas sampling point (TO_{gs}) $TO_{gs} = \frac{\text{CEV} - (\text{number of breaths} \bullet V_{D\ post\text{-}gs})}{FRC_{gs}}$

V_D	Dead space volume. Constitutes the portion of the tidal volume which does not participate in gas exchange, and is composed of the external equipment related dead space ($V_{D,ext}$) and the volume of the conducting airways ($V_{D,aw}$). External dead space volume is partitioned into pre- and post-gs V_D. **1. Pre-gs dead space volume ($V_{D,pre-gs}$) :** V_D between the airway opening and the point at which inert gas is sampled. **2. Post-gs dead space volume ($V_{D,post-gs}$):** V_D between the gas sampling point and the bias flow. This can be calculated as the total amount of marker gas re-inspired, expressed as the corresponding volume of air. Equipment dead space can be estimated by water displacement, but may be considerably larger than actual functional (effective) dead space, measured using the Fowler method from the CO_2 expirogram [1, 2] or the MM signal [3]. Physical and physiological factors may also be influential: face shape and mask pressure, and time constants during the washout [4]. Pulmonary dead space [5], or non-equipment related airways dead space, can be partitioned into the following components. **1. Respiratory dead space volume ($V_{D,resp}$):** defined as the volume of airways and respiratory units that do not effectively take part in gas mixing, and as such constitutes a purely *respiratory* dead space. It consists of $V_{D,aw} + V_{D,alv}$. $V_{D,resp}$ can be estimated from the expirogram using the Bohr equation ($V_{D,Bohr}$; [6]). $V_{D,resp} = V_{D,Bohr} = V_{D,aw} + V_{D,alv}$. **2. Airways dead space volume ($V_{D,aw}$):** defined as the volume of the conducting airways, measured either using the Fowler [1] or Langley method [7], subsequently modified by Tang et al [4]. **3. Alveolar dead space volume ($V_{D,alv}$)**: $V_{D,alv} = V_{D,Bohr} - V_{D,aw}$. **4. Physiological dead space ($V_{D,phys}$):** defined as, arterial CO_2 concentration: i.e. (PaCO_2/Pambient) • V_T - expired volume of CO_2/FCO_2 to express as a volume of air.
Ventilation distribution inhomogeneity	Non-uniformity of gas distribution within the lung. Can be assessed using either global measures of ventilatory inefficiency (e.g. Lung clearance index, LCI, or moment ratios) or specific indices (e.g. S_{acin} and S_{cond}). The latter provide additional insight into the mechanisms and origin of the abnormality in distribution of ventilation.
V_T	Tidal Volume (i.e. breath size or volume). Defined as the breath size over a period of breathing. Unless specified V_T refers to expiratory tidal volume.
V_{TG}	Volume of Trapped Gas, conventionally measured as $FRC_{Pleth} - FRC_{gas}$. Alternatively this can be measured as the amount of inert marker gas, expressed as the corresponding volume of air,

	mobilised by 3-5 inspiratory capacity (IC) breaths performed at the end of the MBW test, in excess of the FRC as measured at the end of the MBW.

E2. Specific recommendations for components in inert gas washout systems

Section E2.6 outlines specific recommendations for individual components within a washout system. The combination of features not only determines overall accuracy but also suitability of systems for use in different age groups. Features of current systems and their suitability for different age ranges are discussed in section E2.7.

E2.1 Flow and volume measurement

A linear flow meter calibrated across the range of flows encountered during testing should be used, as described by Yeh *et al* [8-10]. High data sampling rates ensure accurate reconstruction of the flow signal and provide the resolution required for detecting changes in the breathing pattern [9]. A sampling rate of at least 100Hz has been recommended for measures of infant lung function [9, 11], where faster breathing rates encountered, and is adopted in these guidelines. Magnitude of aliasing error introduced with lower sampling rates is unclear for washout, but may decrease in older age groups with slower respiratory rates.

Variations in dynamic gas viscosity related to gas composition must be accounted for when using pneumotachographs [12, 13], which should be heated to above airway temperature to prevent condensation-induced errors in flow measurement. Washout system designs using a bag-in-box design avoid the need for this correction, but are too bulky for widespread clinical use [14, 15]. Alternative flow meters include

ultrasonic flow meters, mass flow sensors and turbines. Flows and integrated volumes should be reported at BTPS (body temperature, pressure, saturated with water). Volume drift during testing may occur due to physiological or methodological reasons (e.g. erroneous BTPS settings) or technical errors [8], which may be difficult to differentiate. Drift correction algorithms should be clearly described, as the effect of drift correction on washout results remains poorly defined.

When sidestream analysers sample from the middle of the airstream, proximal to the flow measurement point, gas drawn off by the analyser (sample flow) represents "lost gas" introducing marker gas and flow measurement error and affects accurate estimation of volumes. Error becomes additive in subsequent integrated volumes. Subjects with low tidal flows, e.g. infant and young children, are particularly susceptible to such errors. Sample flow should be corrected for and ideally minimised whilst meeting the requirements of a gas analyser for a fast effective rise time [16]. In sidestream systems sample flow has a direct effect on, and inverse relationship to, analyser response, both in terms of transit time of the gas bolus and the analyser rise time. Fixed correction factors used for high pressure systems with constant flow (e.g. respiratory mass spectrometer, RMS) may not be appropriate for high flow systems under lower pressure, where sample flow may vary with generated inspiratory and expiratory flow. Depending on its magnitude, loss of inspiratory flow, expiratory flow and volume of inert marker gas need to be accounted for. Magnitude of error introduced by sidestream sampling distal to the flow measurement point is likely to be lower but must still be assessed in any washout system based on this approach.

E2.2 Inert gas concentration measurement

Gas analysers using several different physical measurement principles are available. These generally measure partial pressure of gas, subsequently presented as a percentage of all gases contributing to atmospheric pressure, with or without the inclusion of water vapour pressure, and relative to a set span of values determined using dry calibration gases. During *in vivo* recordings, gas concentrations are measured during varying conditions (e.g. dry conditions during inspiration and approaching BTPS conditions during expiration). Changes in gas volume with temperature and humidity fluctuation affect all constituent gases equally but not their relative proportions. Ideally, analysers should measure and report gas concentrations under constant conditions throughout the washout (i.e. independent of variations in temperature and humidity), and preferentially report values under dry conditions for simplicity.

Using nafion tubing with sidestream gas analysers allows dry gas concentrations to be calculated, when taking ambient conditions into account [17]. Gas passing along nafion tubing (e.g. Perm-pure “drying” system, DuPont Corporation®) equilibrates to ambient conditions (water vapour pressure and temperature) but its use is not feasible with N_2 analysers (emission spectrophotometers) as these analysers operate under vacuum conditions. Constant dry conditions can be achieved with a RMS, when all constituent gases (excluding water vapour) are summed to 100% in each recording cycle. It is, however, generally not possible for analysers monitoring only one gas to report gas concentration under constant conditions. Detrimental effects of humidity and temperature occur, for example, in mainstream MM signals [18] and N_2 analysers (emission spectrophotometers) [19], which may cause significant non-linearity and

measurement errors unless accounted for. Subsequent correction is problematic due to difficulties measuring rapidly changing humidity and temperature profiles during respiratory cycles [20]. Accuracy of BTPS correction of flow presents a similar challenge.

Analyser signal-to-noise ratio (measured as the mean/SD of data measured over a time period such as 10 seconds) and signal resolution should be high enough to enable determination of stable end tidal inert gas concentrations within 1% relative to the inert gas concentration at the start (e.g. for 78% N_2 this is ±0.8%) and 5% relative to the inert gas concentration at the end of an MBW, typically $1/40^{th}$ of the starting concentration (e.g. for 2% N_2 this is ±0.1%). Linearity must be documented over the full measurement range encountered during clinical testing, and should include assessment of the impact of BTPS conditions. Analysers may be inherently nonlinear, and whist detailed non-linearity correction algorithms have been published, these are analyser specific [21]. Two point calibrations are recommended prior to each testing session. Linearity should be monitored using sufficient calibration gas points, determined by the shape of the calibration curve as directed by the manufacturer and based on the inherent properties of the analyser and its documented performance over time.

Long rise time, in relation to the frequency content of the physiological signal, related to respiratory rate, may lead to errors in determination of inspired and expired gas volumes [16, 22]. A sharp fall in inspiratory gas concentration occurs in early inspiration and inspiratory gas volumes are particularly susceptible to measurement error. Error is proportional to the size of re-inspired post-gs V_D. For a given minute

ventilation, relative magnitude of FRC error increases with smaller V_T and higher respiratory rates [4, 23]. A response time of less than 100ms is recommended and is felt to be appropriate for all age groups. This can be determined easily by the fast step reaction during inspiration, occurring during re-inspiration of post gs V_D (Section E5.2). Gas signal speeding algorithms have been developed [24] and validated for RMS [25] that may be beneficial for other analysers, particularly if multiple analysers with differing intrinsic rise times are used in a single washout system (e.g. O_2 and CO_2 analysers for indirect N_2 calculation, section E3.2).

E2.3 Synchronisation of flow and gas signals

Gas signals must be synchronised precisely with flow prior to data presentation and analysis, and should occur after any applied volume or gas analyser drift correction (if performed). Respiratory flow is assumed to be the same throughout the recording system at a given point in time, while the profile of gas concentration is generally different. In cases where gas concentration is assessed at a different location to the actual gas sampling point (e.g. attempts to measure dead space volume at lips), synchronisation may become respiratory flow dependent (with greater separation occurring at lower flows), and may differ between inspiration and expiration depending on equipment layout [3]. This may also occur if two or more gases are sampled at different points in the recording system. Washout systems should be assessed for this phenomenon and ideally designed to avoid the need for this complicated type of correction.

The time shift required for synchronisation should be assessed using a step response for each gas of interest with a simultaneous measurement of flow. Various methods

exist to synchronisation data, each with advantages and disadvantages (section E5.1). The optimal method and thresholds for synchronisation accuracy are unclear, but both published [26] and unpublished data (personal communication C. Buess, ndd Medical Technologies Switzerland) suggests within 10ms. Viscosity-dependent synchronisation is necessary for N_2 MBW performed using RMS and 100% O_2 for washout [27, 28], due to variations in dynamic viscosity of gas samples (O_2 is markedly more viscous than air) on transit times along the narrow RMS capillary tubing. This may lead to errors of up to 17% in FRC and 70% in V_D measurement [27]. Proposed dynamic algorithms for synchronisation have not yet been applied to physiological data. For this reason N_2 MBW using RMS, whilst possible, should not be undertaken without due consideration of these issues. When using an USFM, both MM and flow signals can be measured simultaneously in the mainstream without any signal delay.

E2.4 Minimisation of equipment deadspace volume (V_D)

Equipment-related V_D may exert detrimental effects for both the subject and subsequent analysis and should be minimized to less than 2 mL/kg [9, 29], or 70 mL for adults, and accounted for. Increased V_D/V_T may increase respiratory rate and/or V_T [30, 31], and alter ventilation distribution inhomogeneity, and is particularly critical for infants. In infants equipment $V_D < 1$ mL/kg is ideal [9]. CEV is assumed to increase by the same amount per breath as that externally added to the equipment V_D, and is accounted for accordingly. Pre-gs V_D may be minimised using silicone putty within facemasks in infants and preschoolers, taking care not to obstruct the airway opening. Nasal masks have been used in neonates and are feasible due to the preferential nasal breathing during this period [32], but whilst FRC measurements

may be very similar to those collected using facemasks, measures of ventilation distribution inhomogeneity may differ. Disposable inserts may avoid the need for bacterial filters, which may introduce prohibitively large additional V_D in younger subjects [33]. Post-gs V_D should be minimised to, firstly, avoid rebreathing impact of large post-gs V_D, and, secondly, to minimize effects of any error in measured re-inspired inert gas volumes on subsequent indices. Measures to reduce V_D should not increase equipment resistance [9], which may alter breathing pattern, FRC, as well as respiratory mechanics, reflected by altered pressure-volume curves.

E2.5 Delivery of washin and washout gas

Gas can be delivered using a variety of options: using a bias flow (by-pass or flow-past) system; using a passive two-way valve system and delivery of gas from a pre-filled bag; or using a demand valve and gas delivered from a pressurised gas cylinder. Two-way valves and demand valves increase resistance to flow and may alter breathing pattern, but conserve gas. Bias flow systems have minimal effect on breathing pattern, minimise post-gs V_D and resistance, but at the cost of greater gas use. Bias flow must be flushed during expiration before commencing the washout phase, and chosen bias flow should eliminate re-inspiration of expired gas beyond post-gs V_D, by ensuring bias flow exceeds the subject's peak inspiratory flow. A period of initial inspiratory flow monitoring to determine optimal bias flow may help conserve gas.

E2.6 Recommendations for inert gas washout system characteristics

Component	Recommendation	Comments
Flow and Volume measurement		
Flow measurement	Instantaneous flow accuracy within 5% across the range of flows encountered during clinical testing and volume accuracy within 3% using a precision calibration syringe.	Based on previous infant lung function guidelines [8, 9, 11]. Accuracy at low flows is increasingly important as age decreases. Methodology to achieve linearity of pneumotachometers has been reported [10].
Sample flow	Ideally, all sidestream washout systems should correct for sample flow. If correction is not performed or achievable, sample flow should be minimised: proposed acceptable thresholds are <20mL/min for paediatric apparatus and <40mL/min for adult apparatus where gas sample point is proximal to flow meter.	Currently, thresholds for acceptable sample flow are not evidence based. Excessive sample flow may also have detrimental effect on breath detection. Thresholds depend on site of sampling i.e. proximal or distal to flow measurement point. The effects of distal sampling on flow measurement should still be assessed in any IGW system. Dynamic sample flow corrections may be required for some systems.
Volume drift	Accurate correction of volume drift is problematic due to difficulty separating technical and physiological components to observed drift. When an excessive volume drift appears, beyond the range usually observed, attempts to identify physiological and/or technical causes (e.g. leaks) should be made as part of the routine quality control.	Recommendations from previous published infant lung function guidelines have suggested that volume drift of <1mL/s should be corrected automatically [9]. This may not be readily extrapolated to washout systems, as the magnitude of error introduced into subsequent washout outcomes of volume drift is unclear. If volume drift correction is performed it should be clearly stated and described.
Inert Gas Analyser		
Gas analyser accuracy	Demonstrated linearity within 1% relative of full	Based on consensus view, not evidence based criteria.

	scale (e.g. 0-80% is +/- 0.8% at 80% N_2) to ensure appropriate assessment of starting concentration, and within 5% relative of any lower value (e.g. 0.25% at 5% N_2) down to 1/40th of the starting concentration. Initial assessment should be in both dry and humid conditions. Monitor gas analyser linearity annually using at least three reference points of gas concentration. Analyser signal-to-noise ratio should be high enough to facilitate this.	The impact of non-linearity on measurement accuracy is well established. The upper end accuracy criterion corresponds to a signal-to-noise ratio of >100. Frequency of monitoring based on consensus view but is also influenced by the stability of the gas analyser and ideally should be determined by the manufacturer. Non-linearity correction algorithms may require more detailed ongoing assessment depending on the pattern of non-linearity. Algorithms should be clearly described [21], and appropriate for humid conditions.
Gas analyser rise time	Analyser rise time should be short enough to adequately characterise the respiratory cycle (frequency content of physiological signals) of the smallest subject being tested. A rise time of <100 ms is recommended across all age groups. In principle, the shorter the rise time, the better. This recommendation does not apply to and would be insufficient for measurement during high frequency ventilation.	Based on consensus view, not evidence based criteria. Whilst the response required for accurate estimation of alveolar concentration are likely to be age dependent, correction for reinspired gas (and any index based on steep transition phases of the expirogram) require fast responding analysers in all age groups.
Other characteristics		
Data sampling frequency	Data sampling should ideally be ≥100 Hz for both flow and inert gas concentration measurement and at greater than twice the fundamental frequency of the faster physiological signal of flow and gas concentration (Shannon sampling theorem) [9].	Based on consensus view, not evidence based criteria. Error introduced by slower sampling rates is likely to be age dependent, and decrease as respiratory rates fall. Sampling frequency should ideally be identical for both flow

		and inert gas concentration.
Synchronisation of flow and inert gas concentration signals	Alignment accuracy within 10 ms or one sample (whichever is greater). Should ideally be measured at the start of each testing session, and accuracy of alignment should be possible to review, re-measure and adjust off-line as necessary.	Optimal method for acceptable synchronisation accuracy are currently unclear. Thresholds for synchronisation accuracy are based on both published and unpublished data. Thresholds are dependent on the breathing pattern: error is directly proportional to respiratory rate.
Equipment related dead space	Total equipment dead space for young children should be <2 mL/kg [9, 29], and ideally <1 mL/kg in infants. Recommendations should be adhered to in older subjects, until further evidence is available. An upper limit of 70 mL should be adhered to for adults including hygiene filters if used.	Based on consensus view, not evidence based criteria. Exact thresholds may differ across age and disease groups. Infants and preschoolers are most susceptible. Large deadspace affects gas mixing indices, breathing pattern, comfort and feasibility. Methods of dead space volume measurement should be clearly described (e.g. by water displacement).
Equipment related resistance	Should be minimised for both inspiration and expiration to avoid effects on breathing pattern and FRC during test.	Significant increases in resistance increase expiratory time constant and potentially influence end-expiratory level (i.e. FRC).

E2.7 Characteristics of some published inert gas washout systems

The table below describes the characteristics of recognised published washout systems currently used. This is not intended to be an exhaustive list, but is shown to illustrate how differing characteristics affect suitability for different age ranges. It also illustrates that none of these systems fulfil *all* of the criteria outlined in section E2. However, overall acceptable system FRC validation, against the currently recommended lung model (Figure 4 main manuscript) has still been achievable despite this for a number of these systems to date [34, 35].

	RMS based [36]	N_2 analyser based [37, 38]	Photoacoustic analyser based [26]	Indirect inert gas concentration based [34, 35]	
Custom or Commercial	Custom	Custom	Custom (modified Innocor, Innovision)	Commercial (EasyOne Pro LAB™, ndd) MM based	Commercial (Exhalyser D, ECO Medics AG) O_2 and CO_2 analyser based
Flow and Volume measurement					
Flow meter	Pneumotachograph			Ultrasonic flow sensor	
Sample flow (mL/min)	20	5	120#	480#	200 for O_2 Mainstream CO_2
Inert Gas analyser characteristics					
Orientation of gas analyser	Sidestream gas analysis				Sidestream O_2 Mainstream CO_2
Inert gas	SF_6	N_2	SF_6	N_2	N_2
Response time (ms)	64	20	154	80	110 for O_2 55 for CO_2
Washin gas	4% SF_6, 4% He	Room air	0.2 % SF_6	Room air	Medical air
Washout gas	Room air	100% O_2	Room air	100% O_2	100% O_2
Sample rate (Hz)	33†	50	100	400	200
Sn_{III} analysis currently possible	Yes	Yes	Yes	No	Yes
Equipment-related dead space volume (mL)**					
Post gs V_D	Infant 5 Preschool 15	50	5	15	Preschool and above 16 Adult 27
Pre gs V_D	Facemask 7.5-12.5 Mouthpiece <5	Mouthpiece 5	Paediatric 36 mL Adult 46mL	20	30
Bacterial filter present and included	No	No	Yes	No***	Yes
Suitability for different age ranges					
Infants	Yes	No	No	No	No
Preschoolers		No		Yes	Yes
School aged children		Yes	Yes		
Adults					

*using indirect measurement method (see section E3.1). **by water displacement, ***disposable inert used for both dead space volume reduction and infection control. RMS, respiratory mass spectrometer. MM, molar mass. †measures all constituent gases in one cycle. #positioned distal to the flow measurement point.

E3. Indirect measurement of inert gas concentration

Indirect measurement of inert gas concentration is feasible but more complex, and contains additional inbuilt assumptions, which are important to appreciate when assessing devices. Simultaneous use of more than one type of gas analyser requires precise synchronisation, given the differing analyser response profiles present, to ensure accurate inert gas concentration estimation. “Speeding” algorithms [25] may improve response times of slower analysers, and response profiles may also differ between inspiration and expiration. The principles of two indirect methods used in commercial devices are outlined below, but may or may not reflect exact methods currently used by manufacturers, which should be clearly described in supporting documentation for indirect washout systems.

E3.1 Molar mass measurement using an ultrasonic flow meter (USFM) based system

USFM infers inert gas concentration by measuring relative change in MM of sample gas through the breathing cycle. Methodology has been described in detail elsewhere [39], and is briefly summarised here. Simultaneous measurements of flow and gas density are feasible. Flow is determined from difference in transit times for ultrasonic pulses emitted between two transducers across a fixed distance [40], and based on the principle that sound travelling through a streaming medium is accelerated or slowed by movement of the medium. MM can be derived from transit times as it is directly proportional to density of the medium:

$$\varphi = (MM*p)/(R*T)$$

where, φ is gas density, MM is molar mass, p is pressure, R is the gas constant, and T is temperature.

This temperature dependence means that precise temperature along the sound transmission path must be known. Measurement of rapidly changing temperature profiles within the breathing cycle is difficult, especially given the conditions of cool dry inspired gas and warm humidified exhaled gas during testing. As a result, for mainstream MM signals a fixed temperature is commonly used (measured within the USFM) and a subsequent algorithm applied to simulate estimated fluctuations occurring within-breath, based on known temperatures of inspired and expired gas and the USFM dead space volume. Accuracy of this algorithm has been optimized for infants [18], but has not been achieved to date for older subjects.

Sidestream USFM can avoid these fluctuating temperature and humidity conditions if the gas sample is first passed through nafion tubing, creating a relatively constant environment for MM measurement [17]. Additional corrections to MM change can be used if other gases are measured, such as CO_2 (e.g. using an infrared analyser). Sidestream MM prototypes have been validated for SF_6 measurement [41], and more recently N_2 measurement [34]. In the latter, a further assumption is that respiratory exchange ratio (RER) remains constant through the washout: O_2 is calculated from measured CO_2, using the constant RER, and residual change in MM is assumed to be due to change in N_2. Use of a sidestream USFM (for gas concentration) in parallel to a mainstream USFM (for flow measurement) also allows detailed synchronisation of the two signals. This synchronisation may become flow-dependent if concentrations are sampled at different location to flow. The small change in MM occurring within latter breaths of the washout may be below the resolution of the MM signal. Sn_{III} analysis has not been reported from MM based washouts.

E3.2 Indirect N_2 measurement based on simultaneous O_2 and CO_2 analysis

It is possible to measure N_2 concentration indirectly by simultaneous O_2 and CO_2 measurement, based on Dalton's law of partial pressures.

$C_{alv} = C_{N2} + C_{O2} + C_{CO2} + C_{H2O} + C_{Ar}$

where C is the concentration of each gas, and C_{alv} equals 1.

Concentration of argon (C_{Ar} = 0.93%) is treated as a fixed proportion of N_2 concentration during washout ($C_{Ar} = C_{N2}$*0.93/78.81). Variations in temperature and humidity are minimised passing sampled gas through nafion tubing prior to arrival at the gas analysers, and applying a correction for ambient temperature and humidity, measured at the time of testing. These steps allow the equation to be simplified to:

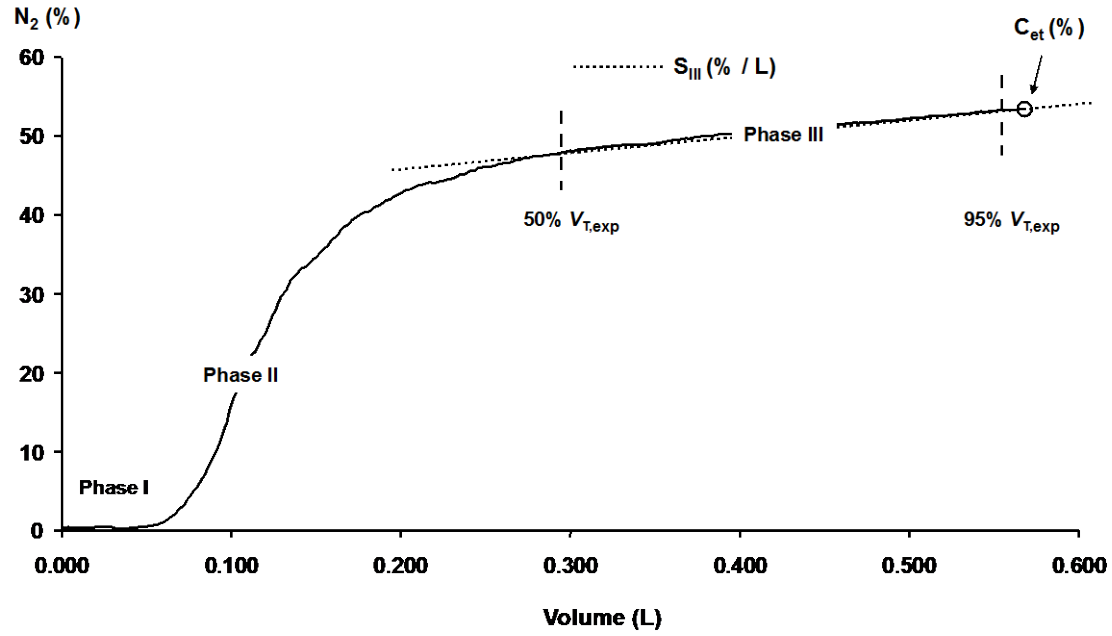
$C_{N2} = 100 - C_{O2} - C_{CO2} – (C_{N2} \bullet 0.93/78.81)$

or $$C_{N2} = \frac{100 - C_{O2} - C_{CO2}}{(1+ (0.93/78.81))}$$

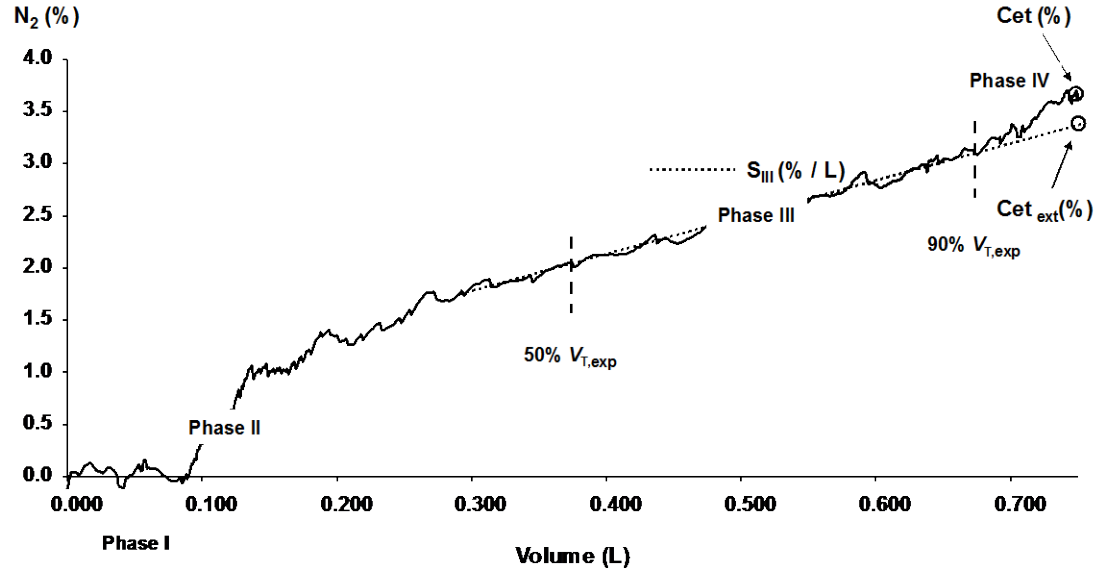
E4. Recommendations for expirogram based indices

E4.1 End tidal inert gas concentration

A.



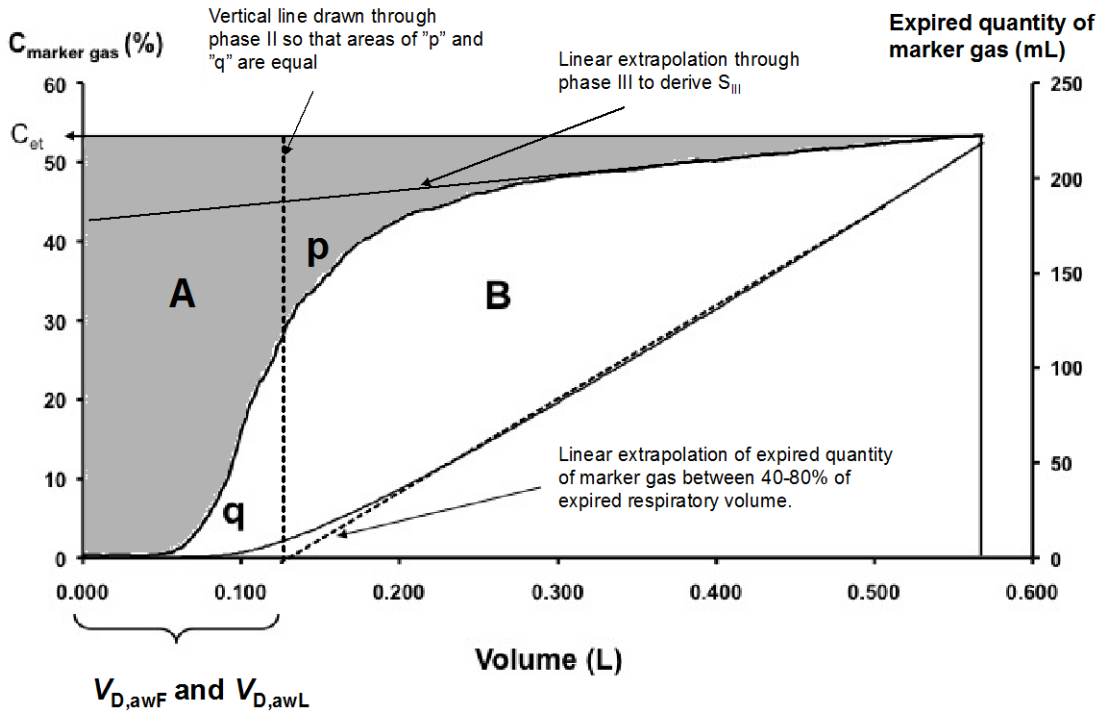
B.



The above expirograms from early breaths (breath number one, A) and later breaths (breath number 23, B) of a N_2 based MBW recording illustrate both the increasing noise at lower inert gas concentration and the potential effect of variation in C_{et} definition. Phase IV, present in this expirogram, elevates C_{et} away from the

extrapolated value from regression of the phase III slope ($C_{et,ext}$). Added noise within the gas signal at the low concentrations can be smoothed by averaging over a window of a number of samples (e.g. 5-10) or time period (e.g. 25-50ms), ending approximately 5 samples (or 25ms) before the end of the breath. Alternatively the average value between 95-98% of the expired volume can be used. Note that S_{III} upper interval limit has been changed in this expirogram from 95% to 90% of V_T due to the observed phase IV interference. The low frequency noise in the later expirogram are likely to represent cardiogenic oscillations.

E4.2 Dead space volume.



Respiratory dead space volume (Bohr deadspace, V_{DBohr}), expressed as a volume of exhaled marker gas, corresponds to area A (shaded area, i.e. calculated as C_{et} • expired V_T *minus* the area under the expirogram, unshaded area or B). A + B = C_{et} • V_T. A/(A+B) gives V_{DBohr}/V_T ratio. Airways dead space volume can be calculated, as volumes of air, using either the Fowler or Langley methods ($V_{D,awF}$ and $V_{D,awL}$, respectively). $V_{D,awF}$ is calculated by calculating the regression line of S_{III}, and then positioning a vertical line through phase II, such that the areas of triangles p and q are equal. $V_{D,awF}$ is the x-axis intercept of this vertical line [1]. $V_{D,awL}$ is calculated as the x-axis intercept of the linear regression line through the linear portion of expired marker gas volume vs. expired breath volume plot (typically 40-80% of the expired breath volume) [4, 7].

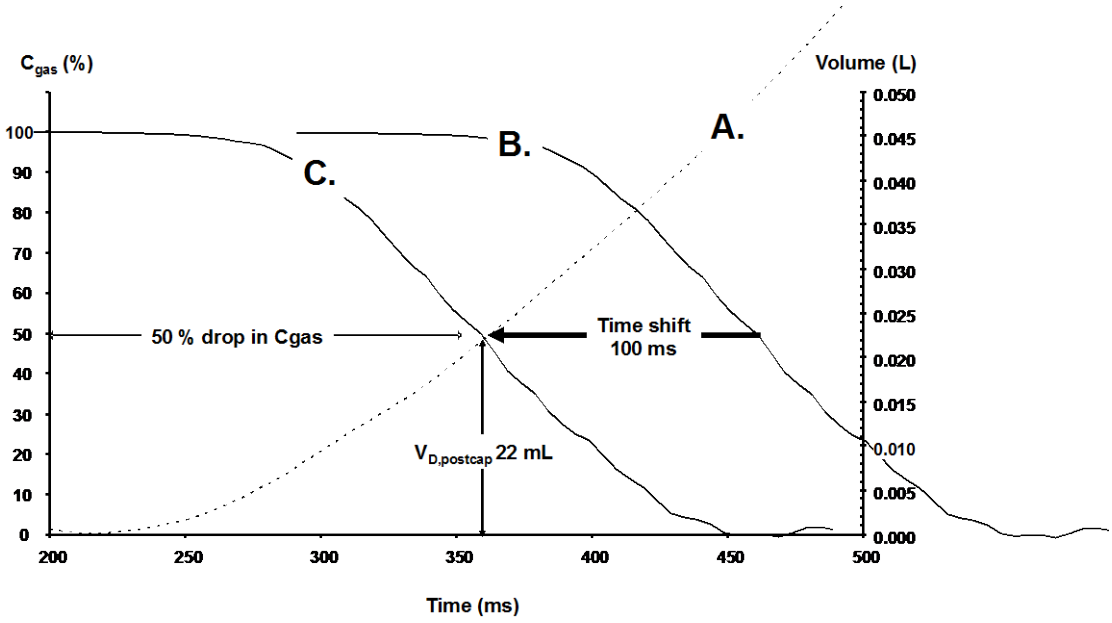
E5. Synchronisation of flow and inert gas concentration signals.

E5.1 Summary of currently available methods for synchronisation of inert gas and flow signals.

Synchronisation method	Advantages	Disadvantages
1. Brunner syringe method [27]	Very precise.	Difficult to perform prior to each test.
2. Based on re-inspiration of expired gas from the post-gs V_D [35]	Can be done prior to each test and is also feasible offline for each test. No additional equipment required.	Depends on the quality of the breathing pattern to allow accurate identification of the start and end of breaths Assumes that gas travels as a uniform front rather than in a cone (i.e. streaming).
3. Alignment of mainstream and sidestream molar mass signals (USFM)	Can be assessed breath by breath both on-line and off-line.	Patented approach Limited to a particular flow sensor.
4. Automated solenoid valve controlled step response [36]	Can be performed before each test. Validated against the Brunner syringe method for inspiratory step response.	Assessment is independent of the flow sensor.

E5.2 Synchronisation of gas and flow signals using re-inspired expiratory gas from the post-gas sampling point V_D at the start of inspiration.

Schematic illustration of synchronisation of inert gas signal to measured flow in a system with a V_D of 22 mL. Gas concentration and inspired volume are plotted on the left and right-sided y-axes, respectively.



where, A is the volume trace during inspiration, B is the gas signal before synchronisation, and C is the gas signal after synchronisation. The gas signal is time shifted such that $V_{D,post\text{-}gs}$ on the volume trace is reached at the same time as the 50% drop in inert gas concentration from starting inspiratory value. Time required for synchronisation in this washout system is 100 ms. In a modern recording system, these fittings can be done instantly by the computer over several breaths and a median value correction applied. The volume of re-inspired post-gs inert gas can be calculated by first plotting synchronised concentration of inert gas (y-axis) against inspired volume (x-axis) and then calculating the area under the curve.

E6. MBW ventilation distribution inhomogeneity indices

E6.1 Overview of published MBW indices

Historically the first assessments were based on a single time point, the alveolar N_2 concentration after seven minutes of 100% O_2 breathing [42]. Subsequently, indices based on the transition between two points on the inert gas washout curve were proposed.

Nitrogen clearance index (NCI) [43]	Number of lung volume turnovers (CEV/FRC) required to decrease C_{et} from 90% to 10% of its concentration at start of washout.
Lung clearance index (LCI) [44]	Number of lung volume turnovers (CEV/FRC) required to decrease C_{et} to 1/40th of its concentration at start of washout.
Becklake Index [45]	Number of lung volume turnovers to wash 90% of FRC free of Inert gas *divided by* 0.9.

Indices based on all points of the washout curve are less influenced by measurement error. These indices cannot differentiate between the effects of increases in V_D and gas mixing inefficiency.

Mean dilution number (MDN_1 and MDN_2) [46] or Moment ratios	Mean number of TO that a gas molecule remains in lung. Independent of changes in breathing pattern, except when V_D is significantly > 0 and when V_T/FRC decreases toward the end of the washout [47, 48] as occurs in infants during MBNW [49] Often referred to as moment ratios. M_1/M_0 (MDN_1), ratio between 1^{st} and 0^{th} moments of washout curve. M_2/M_0 (MDN_2), ratio between 2^{nd} and 0^{th} moments of washout curve (section E6.4).
Pulmonary clearance delay (PCD, %) [50]	Compares mean clearance time for gas molecule with ideal clearance value. Defined as 100 • (actual average time a tracer gas molecule remains in the lungs - the ideal time)/the ideal time.
PCD for moment ratio (%) [51]	Defined as 100 • (actual moment ratio - ideal moment ratio)/ideal moment ratio.

Curvilinearity [52] or "Slope Index" [14]	Based on the objective measurement of the degree to which the lung acts as separate compartments with differing time constants when emptying [53, 54]. Log values of C_{et} for each breath of the MBW are plotted against TO. Two compartments are defined by the slopes between the two compartments: either by TO value (e.g. TO 3-6, RS_2, and TO 0-3, RS_1 [52]) or by percentage of the TO value (10-50% TO, A, and 50-100% TO, B [14]). Then expressed as the ratio of distal potentially slower emptying compartment to the more proximal faster emptying compartment i.e. B/A or RS_2/RS_1.
Efficiency (%) (Eff) [55]	Ideal *versus* actual number of TO to reduce end-tidal inert gas concentration to 1% of original concentration (i.e. perfect mixing lung *versus* subject lung). Decreases as ventilation inhomogeneity increases.

Indices have been developed, based on alveolar ventilation, which are proposed to be independent of changes in dead space volume and breathing pattern.

Mixing ratio (MR) [47]	Ratio between observed and ideal number of breaths required to decrease C_{et} to $1/40^{th}$ of its concentration at start of washout. The ideal number of breaths should be calculated from the ratio between the logarithm for the C_{et} at end-washout and the logarithm for the FRC-to-(FRC + alveolar V_T) ratio. Alveolar V_T is calculated from average V_T during the MBW minus the predicted airway dead space (i.e. body wt (kg) x 2, mL) [56]. Ideal single uniformly ventilated space = MR of 1
Alveolar mean dilution number ($AMDN_1$ and $AMDN_2$) [48]	Similar to mean dilution number (moment ratios). However, TO refer to alveolar volume rather than FRC. Alternatively referred to as alveolar moment ratio.
Alveolar lung clearance index (ALCI)	Number of alveolar TO (CEV_{alv}/FRC) required to decrease C_{et} to $1/40^{th}$ of its concentration at start of washout.
Inspired gas distribution index (IDI) [57]	Ratio of theoretical and actual cumulative alveolar ventilation required to reduce alveolar inert gas concentration of FRC to given level. In ideal, single, uniformly ventilated space, ratio would be 1.0. Conceptually similar to mixing ratio.
Multiple-breath alveolar mixing inefficiency % (MBAMI) [58]	Defined as $100 \bullet (1\text{-}TO_{ideal}/TO_{actual})$ Where, TO_{ideal} is number of TO needed to wash 90% of FRC free of inert gas, and TO_{actual} is actual number of TO. TO is corrected for V_D (hence "alveolar")

Slope based indices measured during MBW.

Normalised phase III slope progression [59]	Progression of, or increase in, the S_{III}, normalised for gas dilution (Sn_{III}), through the breaths of the MBW. Provides information about relative contributions of the two main gas transport mechanisms within the lung, convection and molecular diffusion. Inhomogeneity of ventilation distribution can be partitioned into that arising from within the conducting airways, termed convection-dependent inhomogeneity (CDI), and that arising in the region of the entrance to the acinus, due to the interaction between convection and diffusion, termed convection-diffusion-dependent inhomogeneity (DCDI). These can be expressed as clinical indices, S_{cond} and S_{acin}, respectively [38]. The full DCDI contribution is equivalent to the y-axis intercept of the S_{cond} regression line [60].

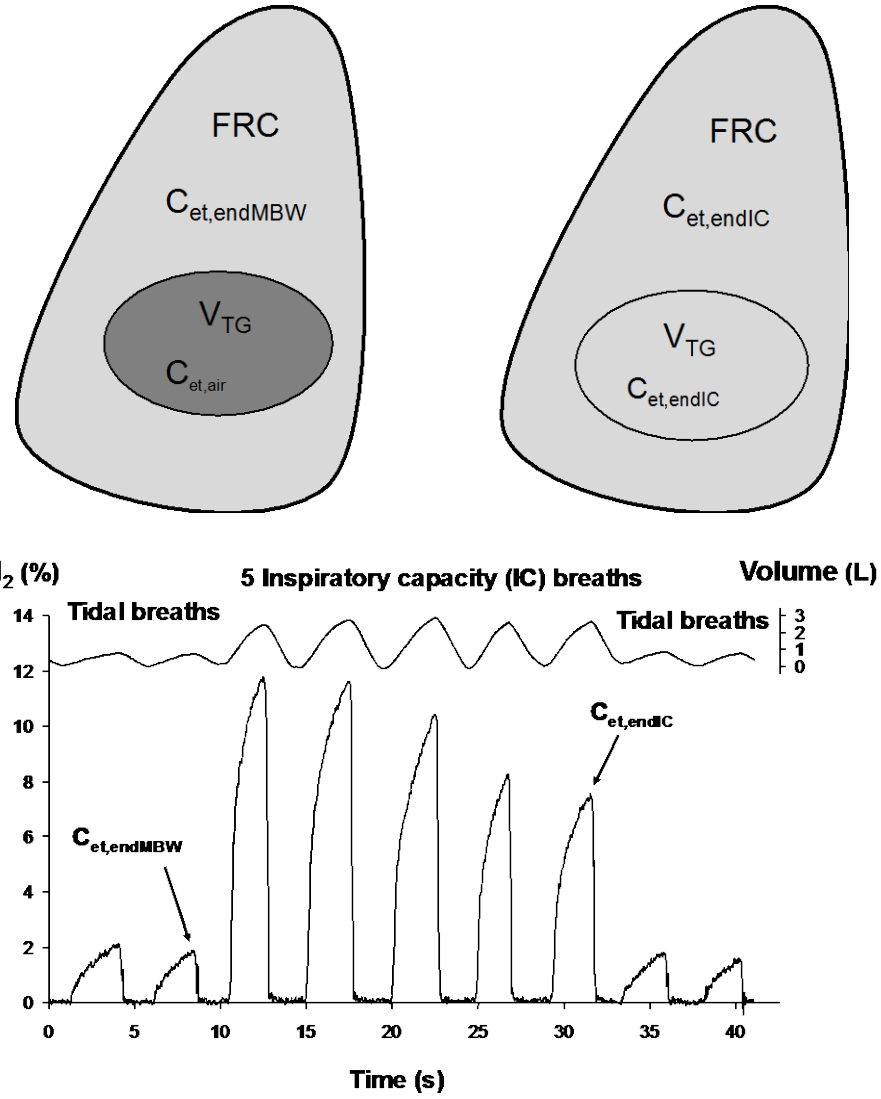
E6.2 FRC value used for calculation of ventilation distribution inhomogeneity indices.

FRC values chosen should correspond to ventilation inhomogeneity indices in two different ways: with respect to the geometric point of measurement in the airstream and with respect to time elapsed during the washout (i.e. the end-test threshold used for ventilation inhomogeneity index, e.g. LCI), as outlined in the main document. The geometric aspect is summarised in the table below. The CEV and FRC used for TO calculation must also be consistent.

Geometric point of reference for Ventilation Inhomogeneity assessment	**Corresponding FRC used**	**Correction of V_T required and V_T nomenclature**
1. Distal to post-gs dead space volume ($V_{D,\ post\text{-}gs}$)	FRC_{gs} *plus* $V_{D,\ post\text{-}gs}$	No correction required; full V_T
2. At the gas sampling point (gs)	FRC_{gs}	V_T *minus* $V_{D,\ post\text{-}gs}$, designated as $V_{T,gs}$
3. At the airway opening (i.e. lips)	FRC_{gs} *minus* $V_{D,\ pre\text{-}gs}$. Designated as FRC_{ao}	V_T *minus* both $V_{D,\ pre\text{-}gs}$ and $V_{D,\ post\text{-}gs}$. Designated as $V_{T,ao}$

E6.3 Calculation of trapped gas volume (V_{TG})

Trapped gas volume (V_{TG}) can be calculated by performing five inspiratory capacity breaths at the end of the MBW. MBNW V_{TG} is based on two assumptions: trapped gas has the same N_2 concentration as air, and that equilibration of N_2 concentration between the region of trapped gas and the remainder of the lung occurs after five IC breaths.



Where,

$C_{et,endMBW}$ = end-tidal concentration of N_2 at end of MBW, approximately 2.0%

$C_{et,air}$ = end-tidal concentration of N_2 during air breathing before start of MBW

$C_{et,endIC}$ = end-tidal concentration N_2 at last breath of the 5 IC breaths, assumed to be same in FRC and V_{TG}

$VolN_{2,\ IC5}$ = the volume of N_2 expired over the 5 IC breaths

Mass balance gives:

$$FRC \bullet C_{et,endMBW} + V_{TG} \bullet C_{et,air} = FRC \bullet C_{et,endIC} + V_{TG} \bullet C_{et,eIC} + VolN_{2,IC5}$$

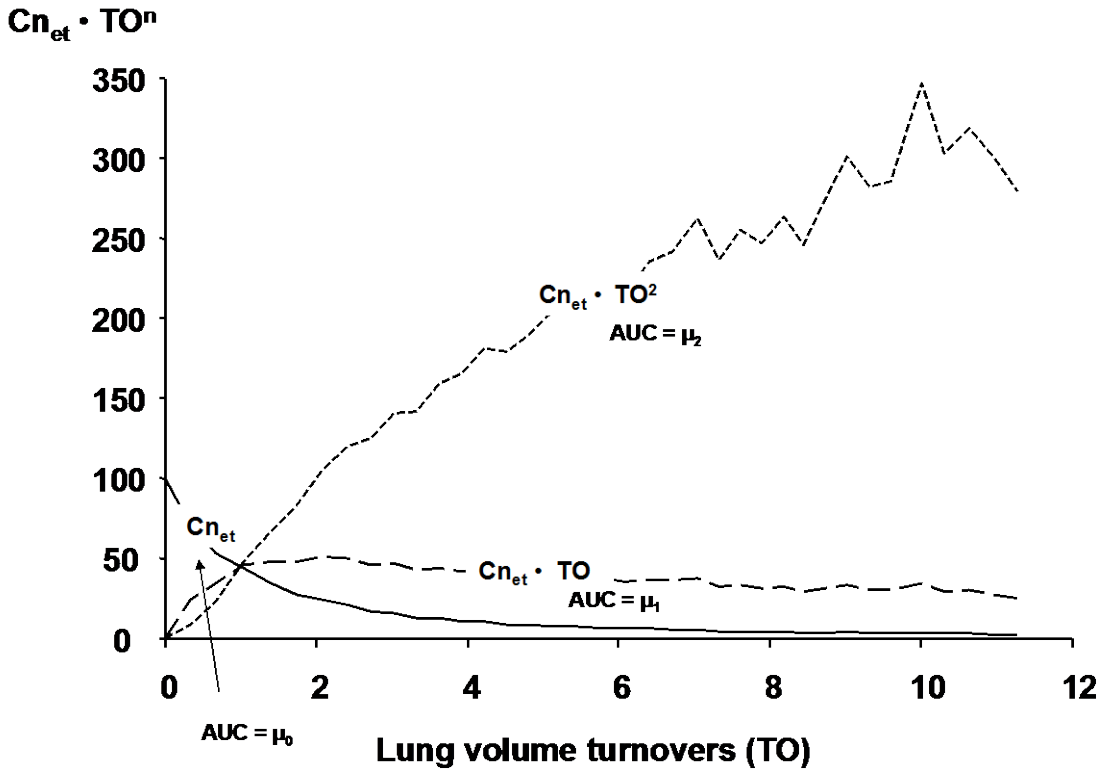
Rearranging gives:

$$V_{TG} \bullet C_{et,air} - V_{TG} \bullet C_{et,endIC} = FRC \bullet C_{et,endIC} - FRC \bullet C_{et,endMBW} + VolN_{2,IC5}$$

Rearranging gives:

$$V_{TG} = \frac{VolN_{2,IC5} + FRC \bullet (C_{et,endIC} - C_{et,endMBW})}{C_{et,air} - C_{et,endIC}}$$

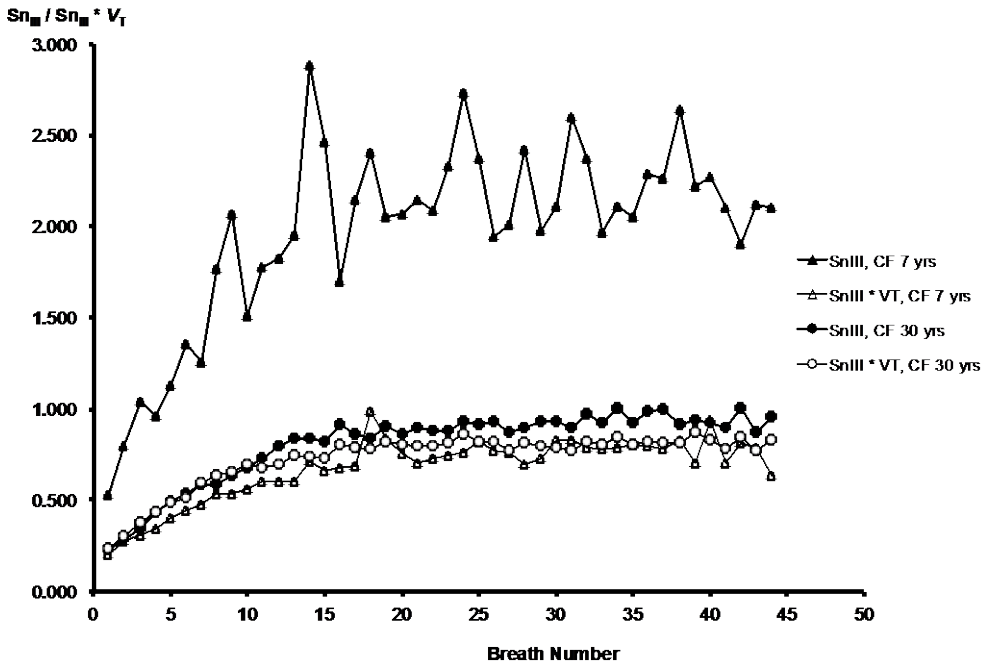
E6.4 Calculation of moment ratios



Moment analysis from a N_2 MBW of a 15 year old girl with CF. Initially, concentration normalised end-tidal concentration of inert marker gas (Cn_{et}) is plotted against TO. Moments 0, 1, and 2 (μ0, μ1, and μ2) are determined by calculating the area under the curves for Cn_{et}, Cn_{et} • TO, and Cn_{et} • TO^2, plotted against TO (x-axis), respectively. These are then expressed as moment ratios μ1/μ0 and μ2/μ0. The values obtained for this MBW are shown below, along with the values if truncation at either 6 or 8 TO is applied. Truncation is recommended to facilitate comparison between subjects [46].

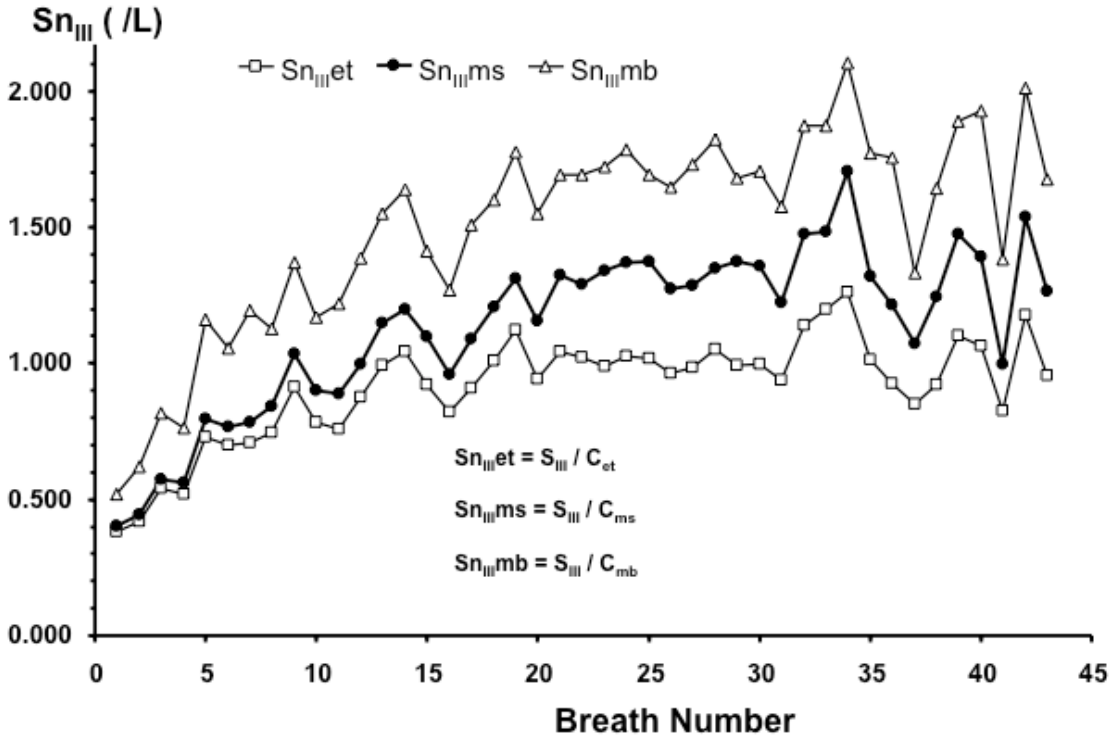
	μ0	μ1	μ2	μ1/μ0	μ2/μ0
Raw data	170	459	2625	2.70	15.44
Truncated to 6 TO	141	254	797	1.80	5.65
Truncated to 8 TO	156	326	1302	2.09	8.35

E6.5 Correction of Sn_{III} analysis for variations in lung size and breathing pattern



Sn_{III} progression over a MBW test from two different CF subjects. Breath number is used along the x-axis to allow a better visual comprehension of individual breath comparison, but should be plotted against TO for formal calculations. The adult subject breathes at V_T close to 1L and therefore the effect of V_T correction of Sn_{III} does not lead to a large change compared to uncorrected values. However, in the paediatric subject, there is a pronounced effect of V_T correction compared the uncorrected Sn_{III} values.

E6.6 Effect of choice of normalisation concentration on Sn_{III} progression through the MBW.



Both S_{cond} and S_{acin} are affected by the choice of reference gas concentration for S_{III} normalisation, and the reference concentration does differ currently in paediatric and adult literature [38, 61]. There are three different options for reference gas concentration when normalising phase III slope (S_{III}) to calculate Sn_{III} for each breath: the end tidal concentration (C_{et}), the mean concentration over the slope interval (C_{ms}), or the mean expired gas concentration (C_{mb}). However, these are not necessarily equivalent as they change in proportion to each other during the washout, affecting the absolute values and resultant progression of the Sn_{III} during the MBW [62]. Formal comparisons of the sensitivity and specificity of Sn_{III} indices calculated with each of these options are required before strict recommendations can be given. In the interim, authors should fully describe which reference gas concentration is used.

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