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ERS/ATS CONSENSUS STATEMENT

Consensus statement for inert gas washout measurement using multiple- and single-breath tests

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ABSTRACT: Inert gas washout tests, performed using the single- or multiple-breath washout technique, were first described over 60 years ago. As measures of ventilation distribution inhomogeneity, they offer complementary information to standard lung function tests, such as spirometry, as well as improved feasibility across wider age ranges and improved sensitivity in the detection of early lung damage. These benefits have led to a resurgence of interest in these techniques from manufacturers, clinicians and researchers, yet detailed guidelines for washout equipment specifications, test performance and analysis are lacking. This manuscript provides recommendations about these aspects, applicable to both the paediatric and adult testing environment, whilst outlining the important principles that are essential for the reader to understand. These recommendations are evidence based, where possible, but in many places represent expert opinion from a working group with a large collective experience in the techniques discussed.

Finally, the important issues that remain unanswered are highlighted. By addressing these important issues and directing future research, the hope is to facilitate the incorporation of these promising tests into routine clinical practice.

KEYWORDS: Adult, lung function, monitoring, paediatric, validation

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INTRODUCTION

The architecture of the airway tree promotes even distribution and optimal mixing of inhaled gas with resident gas. Multiple-breath and single-breath inert gas washout tests (MBW and SBW, respectively) assess the efficiency of ventilation distribution [1, 2]: in principle, efficiency of inert marker gas clearance from the lungs, or gas mixing within the time frame of a single breath, respectively. Suitable inert gases must be safe to inhale at the concentrations used, not participate in gas exchange, and not dissolve significantly in blood or other tissues. Options include both endogenous (nitrogen: N_2, and argon) and exogenous gases (sulfur hexafluoride: SF_6, helium: He, and methane). Marked ventilation distribution abnormalities occur in obstructive lung disease [3, 4] despite normal ventilatory capacity as measured by spirometry [5–10]. Washout tests may provide insight into mechanisms behind abnormal ventilation distribution and localisation of pathology. MBW is particularly attractive as it uses either relaxed tidal breathing (mostly in paediatric settings) or a fixed tidal volume (usually 1 L in adults) without need for maximal effort, thereby offering feasibility in all age groups [5, 7, 9, 11–14], driving recent strong paediatric interest. Despite this and unique insights into disease onset, widespread clinical use has yet to be achieved and further work that is required is limited by a lack of carefully validated robust commercial washout systems.

Washout recording systems determine inspired and expired inert gas volumes, by continuously measuring inert gas concentrations synchronised with respiratory flow. The overall aims of this standardisation document are to promote and facilitate use of open-circuit washout systems (*i.e.* minimal rebreathing of expired air), and achieve quality assured results, comparable between laboratories, using validated systems suitable across age groups and disease conditions. This paper is directed to manufacturers, researchers, clinicians and respiratory technicians. Recommendations are made for testing infants, children and adults, reflecting broad clinical and research interest. Application in different age groups may require age-specific modifications, assumptions and limitations.

TABLE 1 Key recommendations from this standardisation document

Recommendations contained in this document are based on evidence where available. If no evidence exists, the recommendations are based on expert opinion, and will continue to evolve over time and be updated in future documents as further insight is gained.
SBW and MBW testing offer complimentary information, but the choice of test used may be age and disease dependent. Depending on the pathology under study, relationships between MBW-derived indices may help identify the type of structural changes.
A series of individual equipment component recommendations are provided in this document. It is, however, unlikely that all individual criteria outlined will be fulfilled by any one system, which is why overall system performance during validation and subsequent testing is the central aspect of importance.
FRC measurement validation is an essential step and should assess all the stages of the measurement including post-data acquisition processing procedures, such as BTPS correction. FRC measurement accuracy does not ensure accuracy of all other derived indices and biological control measurements and monitoring is essential.
Responsibility for commercial system validation and ongoing reliability of system performance should lie with the manufacturer. However, close vigilance by the end user is essential. Biological control measurement and monitoring during subsequent clinical and research testing is an essential component of this.
FRC and ventilation inhomogeneity indices must relate to the same geometric reference point in the airstream. FRC end-point for measurement during the washout should correspond to the end of test threshold used for ventilation inhomogeneity index analysis, *e.g.* LCI threshold.
The method of FRC determination, indices of ventilation distribution inhomogeneity calculation, and any corrections performed (*e.g.* V_T or VC) must be clearly described. Both corrected and uncorrected values should be reported to facilitate *a priori* analysis in the future.
Suitability of open-system inert gas washout equipment for use in different age ranges is determined by the overall contribution of characteristics such as equipment dead space and analyser dynamic properties.
The choice of inert gas used is dependent on many factors, but impacts on the results obtained. Normative values are inert gas specific. Comparison of multiple simultaneously measured inert gases may provide additional information about the location of underlying pathology. Correction for tissue N_2 diffusion into the lung is not currently recommended due to a lack of appropriate data to base corrections on.
A variety of factors may lead to differences in reported washout indices between centres and experimental conditions under which normative data are obtained should be clearly described.
Quality control during testing is critical and extends beyond equipment performance and software feedback to also include close observation by the operator of the subject's behaviour during testing and how this affects the data obtained. Adequate operator training and appreciation of all factors influencing test results is essential.
Breathing patterns during testing should be kept similar between subjects to facilitate comparison of results. In adults this is achieved by using strict breathing regimens where feasible and in younger children (aged $\leqslant$16 yrs) by distraction to encourage relaxed tidal breathing.
The end test threshold used for MBW tests will depend on the ventilation distribution index (or indices) being reported.
Formal FRC repeatability criteria for MBW indices should not be routinely applied, but FRC values within 10% should be viewed as encouraging. FRC values differing by more than 25% from the median of three test values should be excluded.

SBW: single-breath washout; MBW: multiple-breath washout; FRC: functional residual capacity; BTPS: body temperature, ambient pressure, saturated with water; LCI: lung clearance index; V_T; tidal volume; VC: vital capacity; N_2: nitrogen gas.

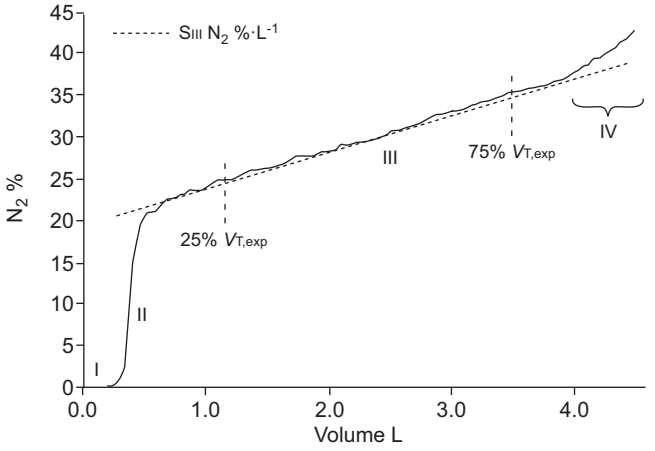

FIGURE 1. Example of a typical single-breath washout (SBW) trace. Nitrogen gas (N_2) expirogram showing calculation of phase III slope (S_{III}) in a vital capacity SBW test in a 60-yr-old smoker. S_{III} is calculated between 25% and 75% of the expired volume (S_{III} 4.4%·L^{-1}), to avoid the contribution of phase IV. The four phases of the expirogram are also demonstrated: phase I (absolute dead space), phase II (bronchial phase), phase III (alveolar phase) and phase IV (fast rising phase at end of expiration). Closing volume (CV) is the expired volume (L) from the start of the upward deflection where phase IV starts, to the end of the breath. If residual volume (RV) is known, closing capacity (CC) can be calculated: CC=CV+RV. $V_{T,exp}$: expired tidal volume.

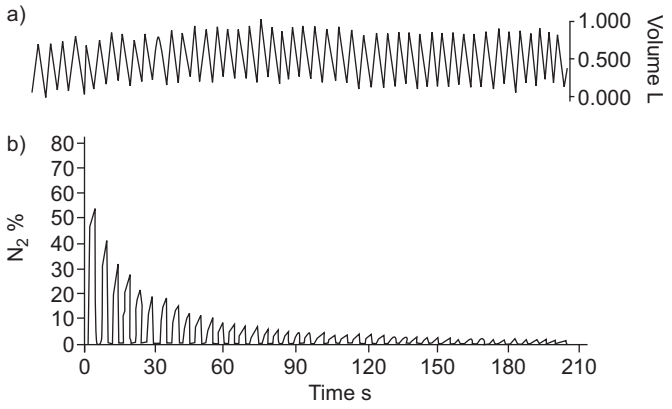

FIGURE 2. Example of a typical multiple-breath washout (MBW) trace. Time series display of a) volume and b) nitrogen gas (N_2) from an N_2 MBW test in a female aged 15 yrs with cystic fibrosis. Stable breathing and end-tidal inert gas concentration are seen prior to commencing the washout phase.

Specific aims of this document are to: 1) describe the principles and physiological concepts behind MBW and SBW tests; 2) outline equipment requirements, appropriate system quality control and validation; 3) describe available washout outcomes, factors influencing their calculation, and insights provided into underlying mechanisms of ventilation distribution inhomogeneity; 4) provide recommendations and test acceptability criteria in different age groups; and 5) highlight important future research.

Recommendations will continue to evolve as further insight is gained. Clinical utility has been summarised elsewhere [15–19]. Key recommendations are summarised in table 1.

MECHANISMS OF VENTILATION DISTRIBUTION INHOMOGENEITY

Ventilation distribution occurs by convection and diffusion [20]. Three principal mechanisms generate inhomogeneity [21]. 1) Convection-dependent inhomogeneity (CDI) in the conducting airway zone (*i.e.* airways proximal to terminal bronchioles) [22]. 2) Diffusion-limitation related inhomogeneity in pathologically enlarged acinar structures (rare). 3) Interaction between convection and diffusion in an intermediate zone at the level of the diffusion-convection front.

In adult healthy lungs, this quasi-stationary diffusion-convection front, which determines where these mechanisms can operate, is thought to arise around the acinar entrance [23]. Inhomogeneity of ventilation distribution is reflected in delayed MBW marker-gas clearance, raised SBW phase III slope (S_{III}), explained in figure 1, and magnitude and progression of MBW concentration normalised phase III slopes (Sn_{III}) through subsequent breaths (fig. 2); in the latter, S_{III} normalisation by expired alveolar inert gas concentration is required to compare progression.

CDI results from differences in specific ventilation between lung units sharing branch points in the conducting airway zone in combination with sequential filling and emptying among these units [24]. CDI contributes to increased S_{III} in SBW and generates a continuous rise in Sn_{III} through subsequent MBW breaths [25]. Diffusion convection-interaction-dependent inhomogeneity (DCDI), which occurs in the region of the acinar entrance, increases Sn_{III} if structural asymmetry is present at branch points (*e.g.* differences in cross-sectional area and/or subtended lung volumes). In normal adult lungs, DCDI is the major contributor to SBW S_{III} [24] and DCDI contribution to MBW Sn_{III} reaches its maximum at approximately five breaths [25].

SBW AND MBW TESTS

SBW and MBW assess ventilation distribution inhomogeneity at differing lung volumes. The most widely used is the N_2 SBW test [1], which involves a vital capacity (VC) manoeuvre performed at low constant flow (400–500 mL·s^{-1}): exhalation to residual volume (RV), inhalation of 100% oxygen gas (O_2) to total lung capacity (TLC), then washout during exhalation from TLC to RV [1, 26], where S_{III} is measured over the mid portion of the expirogram (fig. 1). For exogenous inert gas SBW, the inert gas is washed in during inhalation from RV to TLC, before washout during exhalation to RV. VC SBW S_{III} is influenced to a greater degree by gravitational and nongravitational inter-regional differences in gas distribution and airway closure during the inspiratory phase [27–29], compared to tidal breathing protocols. Actual peripheral airway contribution to VC SBW S_{III} is uncertain. Modification by initial wash-in from functional residual capacity (FRC) to either TLC or a volume above FRC (*e.g.* 1 L) [30], better reflects inhomogeneity present during near-tidal breathing and may be a more sensitive index of peripheral airway involvement [31].

MBW assesses ventilation distribution inhomogeneity during tidal breathing from FRC, by examining inert gas clearance over a series of breaths. Exogenous gas washout requires an initial wash-in phase. MBW requires only passive cooperation and minimal coordination, but is more time consuming. It appears to be the most informative of these tests. In contrast to MBW, SBW S_{III} using a single inert gas does not separate CDI

and DCDI contributions, though some information about location of pathological processes may be gained by comparing simultaneous SBW SIII of inert gases with widely different molecular mass (as described in the section entitled Impact of inert gas choice). SBW may be sufficient for some patient groups: in patients for whom DCDI is thought to be the main mechanism, SBW initiated from FRC approximates the first tidal expiration of a MBW, which contains most of the DCDI information. Studies directly comparing SBW and MBW are rare or non-existent.

EQUIPMENT SPECIFICATIONS

Key components and principles exist when designing washout devices (fig. 3). Individual component recommendations are summarised in table 2 and section E2 in the online supplementary material. It is unlikely that all individual criteria outlined will be fulfilled by any one system, which is why overall system performance during validation and subsequent testing is the central aspect (table 3). Recommendations for online and offline washout software are summarised in tables 4 and 5.

Accurately measured flow and inert gas concentration must be meticulously synchronised. Asynchrony between flow and gas signals in real-time measurement is due to gas sample transit time from airstream to inert gas analyser and/or gas analyser response time. Inert gas concentration measurements should ideally occur across the mainstream to minimise the error introduced by streaming, and be synchronous with flow signal. Mainstream gas analysers generally have shorter rise times than sidestream analysers but may introduce additional equipment deadspace, which in turn may have detrimental effects on ventilation during testing. Short analyser rise times become increasingly important as breathing rate increases, such as in young infants. Overall contribution of characteristics such as these determines suitability for different age ranges, as illustrated by the detailed discussion of current published systems as shown in section E2.7 in the online supplementary material.

VALIDATION OF WASHOUT EQUIPMENT

Recommended washout equipment validation is FRC measurement accuracy: FRC values within 5% of known volume for at least 95% of values [32] across the range of lung volumes, V_T and respiratory rates encountered during subsequent clinical testing [34, 35]. Validation should assess all stages of measurement including post-data acquisition processing procedures, such as body temperature, ambient pressure, saturated with water (BTPS) correction. Recently, optimised lung model design [36] has incorporated simulated BTPS conditions for validation of both established and emerging MBW systems (fig. 4) [35] and is the recommended approach. Validation should be repeated if significant changes in hardware or

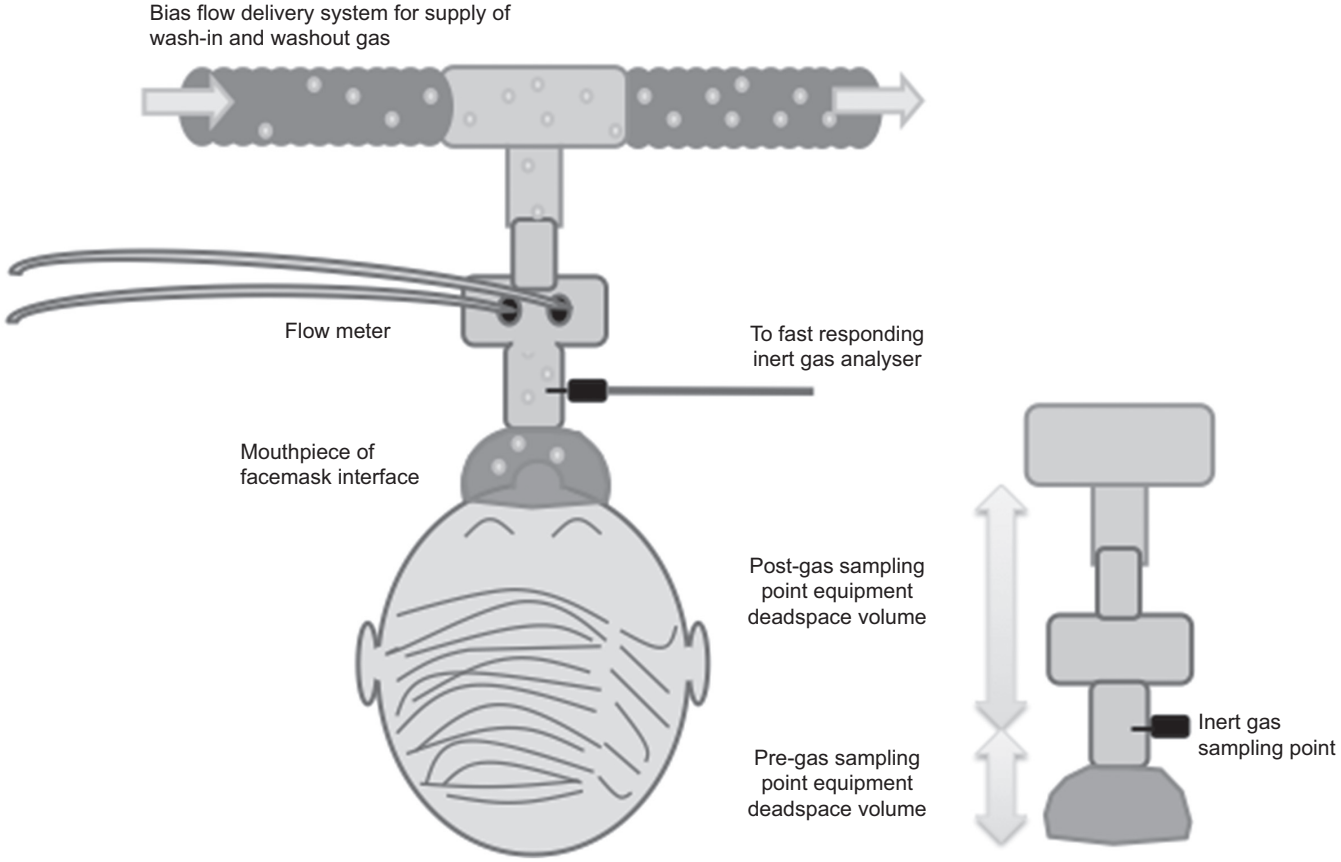

FIGURE 3. Schematic illustration of a generic inert gas washout system. The figure illustrates a generic washout system. Hardware required for washout is relatively simple: a flow meter, a fast responding inert gas analyser, a gas delivery system and a patient interface. The equipment-related deadspace volume (V_D) can be divided into pre- and post-gas sampling points. Post-gas sampling point V_D effectively introduces a small rebreathing chamber. Pre-gas sampling point V_D is an extension of anatomical V_D.

TABLE 2 Summary of component recommendations for inert gas washout system characteristics

Component	Recommendation
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.
Sample flow	Ideally, all side-stream washout systems should correct for sample flow. If not performed or achievable, sample flow should be minimised: <20 mL·min^{1} for paediatric and <40 mL·min^{-1} for adult apparatus where gas sample point is proximal to flow metre.
Volume drift	Accurate correction of volume drift is problematic due to difficulty separating technical and physiological components to observed drift. When an excessive drift, beyond the range usually observed, appears, attempts to identify physiological and/or technical causes (*e.g.* leaks) should be made as part of the routine quality control.
Gas analyser accuracy	Linearity within 1% relative of full scale (*e.g.* 0–80% is $\pm$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 1/40 of the starting concentration. Initial assessment should incorporate both dry and humid conditions. Monitor gas analyser accuracy, stability and linearity annually using at least three reference points of gas concentration.
Gas analyser rise time	A 10–90% analyser rise time of <100 ms is recommended across all age groups.
Data sampling frequency	Data sampling should ideally be $\geqslant 100$ Hz for both flow and inert gas concentration measurement.
Synchronisation of flow and gas signals	Alignment accuracy within 10 ms or one sample (whichever is longer).
Equipment-related dead space	Total equipment deadspace for young children should be <2 mL·kg^{-1} bodyweight, and ideally <1 mL·kg^{-1} 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.
Equipment-related resistance	Should be minimised for both inspiration and expiration to avoid effects on breathing pattern and FRC during test.

The table is expanded with further explanation in section E2.6 in the online supplementary data. N_2: nitrogen gas; FRC: functional residual capacity.

software algorithms occur [39]. All MBW ventilation inhomogeneity indices depend on accurate FRC determination, but FRC validation alone may not be sufficient to ensure accuracy of derived ventilation distribution indices. During subsequent clinical or research testing, biological controls should monitor measurement stability (*e.g.* three to four healthy staff members performing MBW in triplicate, monthly). Marked variation beyond normal observed pattern should prompt further careful evaluation of device performance and procedures.

A variety of factors may generate differences in reported indices between centres (table 6), and until standardisation is achieved, normative data is at best tentative and likely to be inert gas, equipment and software specific. Experimental conditions under which normative data are obtained should be clearly described in manuscripts.

SUITABILITY OF CURRENT WASHOUT SYSTEMS ACROSS AGE GROUPS

The only current system applicable across all age groups is custom built and based on the respiratory mass spectrometer (RMS). RMS is the current gold standard gas analyser offering simultaneous measurement of multiple gases in constant conditions, full linearity, low sample flow and short response time [39]. This custom washout system exists in several centres [5, 7, 40–42], but may be too expensive and impractical for widespread use.

In MBW using N_2, inhalation of 100% O_2 may alter breathing patterns in infants [43] and subsequent MBW outcomes, but impact on breathing pattern beyond infancy is considered minimal. As an alternative to emission spectrophotometer N_2 analyser systems (requiring vacuum pumps), indirect N_2 measurement systems have been proposed based on simultaneous O_2 and CO_2 measurement [35] or changes in molar mass (MM) [34] (see section E3 in the online supplementary material). Potential for additive errors with indirect measurement places even greater emphasis on adequate quality control.

MM based measurement of SF_6 or He are also feasible [44–46]. Mainstream MM SF_6 washout has been validated in infants [46, 47]; however, lack of validated correction algorithms for detrimental temperature and humidity fluctuations limit utility beyond infancy [48]. Sidestream MM washout incorporating Nafion® tubing to stabilise temperature and humidity [49] has been validated for older age groups [38, 50], but current equipment deadspace volume (V_D) precludes use in infancy.

Modified photoacoustic analyser based systems have been validated for use in adults and school age children [9, 51], but are not currently commercially available. Feasibility into younger ages will depend on minimisation of longer analyser response times. Detrimental impact of high sample flow used in these systems on measured flows may be reduced by gas sensor placement distal to flow measurement, but requires careful evaluation. Sampling bias flow gas during low expiratory flows must also be avoided. The commercially available photoacoustic analyser based closed circuit system is not discussed in this manuscript [52].

OUTCOMES

Functional residual capacity

FRC measured by MBW (FRC_{gas}) represents the volume of lung gas, at end expiration (assessed at the breath immediately preceding washout), in direct communication with the airway opening, excluding gas trapped in lung regions not ventilated by

TABLE 3 Overall recommendations for washout systems

Instantaneous flow within 5%, and V_T and CEV measurement accuracy within 3%.

Quality of gas signals allowing determination of FRC, end-tidal gas concentration and S_{III} down to 1/40 of the starting inert-gas concentration with sufficient accuracy and resolution (see below).

FRC measurement accuracy within 5% of the true FRC value (for 95% of values),using a realistic lung model incorporating BTPS conditions across the intended volume range and breathing pattern of the system. Commercial systems manufacturers should perform this validation both prior to sale and whenever significant hardware or software modifications are made to existing devices. Re-evaluation should be performed as necessary if marked variation occurs beyond the normal observed pattern for biological controls during clinical research use.

The static and dynamic properties of the gas analyser (accuracy, response time and signal-to-noise ratio) should ensure a linear and accurate gas signal. End-tidal inert-gas concentrations should be within 1% relative to inert-gas concentrations at the start and 5% relative to inert-gas concentrations at the end of the washout (*i.e.* at 1/40 of the starting concentration).

The manufacturers of commercial inert-gas washout systems should demonstrate these features prior to commercial release, with data being included within supporting documentation. V_T: tidal volume; CEV: cumulative expired volume; FRC: forced residual capacity; S_{III}: phase III slope; BTPS: body temperature, ambient pressure, saturated with water.

tidal breaths. FRC_{gas} is, therefore, often lower than plethysmographic FRC, especially in obstructive lung disease [53]. $FRC_{gas} = V_{IG}/C_{et,IG}$ (initial–final), where: V_{IG} is net volume of inert gas expired, and C_{et} is end-tidal concentration of inert gas. V_{IG} is the sum of the integral products of exhaled flow and gas concentration for each washout breath, corrected for re-inspired gas, contained within the V_D after the post-gas sampling point (fig. 3, see section E5.2 in online supplementary material).

TABLE 4 Recommendations for online washout software

Software to display flow, volume and respiratory rate monitoring are essential for both fixed breathing protocols (SBW and MBW in adults and older adolescents) and to monitor and stabilise tidal breathing in younger subjects

- Volume time series display of BTPS adjusted data should be of sufficient length and size to detect volume drift
  - Differentiating technical causes from physiological causes of volume drift may be difficult
  - Sudden step changes in volume may indicate leak

Graphical display of inert gas concentration traces both during the wash-in and washout phases

- To assess suitability of timing to start the washout phase
- To monitor for leaks (see table 5), this should include a clear display of the ''zero'' inert gas baseline concentration level, which may not be achieved in cases such as insufficient washout as supply or leak; if an automated correction of deviation from zero baseline is performed by the software, the magnitude of this deviation correction must be clearly visible to alert the user

Accurate breath detection of start and end of inspiration and expiration adhering to existing standards for identification of tidal breaths [32, 33]; these standards were developed for infants but are extendable for application in adults

- Distinguishing start and end of inspirations and expirations from minor fluctuations in flow during pauses and irregular breathing is usually accomplished using flow thresholds but a combination of flow and volume based criteria may be better

Accurate detection of end-tidal inert gas concentration

- Average over 5–10 samples (or 25–50 ms), ending five samples (or 25 ms) before the end of expiration (see section E4.1 in the online supplementary data)
- Alternatively average over 95–98% of the expired volume

If S_{nIII} progression is being measured then display the breath-by-breath inert gas expirogram to allow the user to ensure sufficient S_{III} is visible (⩾50% of the expired V_T)

To aid the user in determining when end-of-test thresholds are met, online analysis should display

- End-tidal inert gas concentration
- If S_{nIII} progression or moment ratios are being measured: FRC and lung turnover (CEV/FRC for each breath) as the washout proceeds

To limit the time required for testing, automated calculation of the following indices should occur at the end of each test

- FRC
- Breath-by-breath calculation and display of $V_{D,aw}$ (quality control for leak detection)
- Global ventilation distribution indices

Offline analysis and quality control can then be performed as required by the operator (as detailed in the section entitled Validation of washout equipment)

Warning messages should inform the operator when important quality control steps have not been fulfilled

SBW: single-breath washout; MBW: multiple-breath washout; BTPS: body temperature, ambient pressure, saturated with water; S_{nIII}: normalised phase III slope; V_T: tidal volume; CEV: cumulative expired volume; FRC: functional residual capacity; $V_{D,aw}$: deadspace volume of the conducting airways.

TABLE 5 Recommendations for offline washout software

Software transparency for
- All correction algorithms and factors applied to data (*e.g.* BTPS and temperature modelling)
- All algorithms used for subsequently calculated indices
- Method used to synchronise flow and inert marker gas concentration signals
- Normative data or upper limit of normal incorporated, including details of source and population characteristics (number of subjects, sex distribution, age range, ethnic group, *etc*.)

General recommendations
- Full availability of raw data, calibrated data and BTPS-converted data which should be saved and readily exportable in widely acceptable formats, *e.g.* ASCII (.txt) or .xls
- Ability to assess accuracy of flow and inert gas concentration synchronisation, re-measure and manually adjust as necessary
- Ability to review tidal volume tracing to ensure correct identification of breath detection (start and end-points), and manually adjust as necessary
- Ability to review inert gas expirogram for each breath, and manual adjustment if necessary, to ensure correct estimation of
  - End-tidal inert marker gas concentration
  - S_{III} if SBW or if MBW Sn_{III} analysis is being performed
- Ability to examine for and correct any gas-analyser drift occurring during the test. The zero calibration point may be useful as a reference for many of the gases used (N_2, CO_2, He and SF_6) whilst 100% can be used for O_2. Any correction applied should be clearly stated
- If available, monitor end-tidal CO_2 values during MBW to screen for hyperventilation

FRC
- FRC is measured over all breaths of the washout, and updated after each breath, until a defined end-point in time. The end-point used for FRC determination should correspond to the end-test threshold used for ventilation inhomogeneity indices (*e.g.* LCI threshold)
- Exhaled inert gas volume must be corrected for re-inspired gas from the post-gas V_D for each breath
- Reported FRC is that measured at the FRC_{gs}. If other FRC values are reported, *e.g.* FRC_{ao} (*i.e.* FRC_{gs} – pre-gas sampling point V_D) these values should be described appropriately
- Report mean, SD and CoV of three technically acceptable measurements
  - If only two technically acceptable measurements are available, report mean only, and state *e.g.* ''based on two measurements alone''
  - If FRC values are not within 10% of the highest FRC value, then alert the operator. Exclude FRC values which differ by >25% from the median FRC value across the three tests. Excluded tests should not be used for calculation of other MBW indices

Indices of global ventilation distribution inhomogeneity (*e.g.* LCI and moment ratios)
- Correct V_T for external V_D (see section E6.2 in the online supplementary material)
- Use appropriate corresponding FRC for calculation
- Report mean, SD and CoV of three technically acceptable measurements
  - If only two technically acceptable measurements are available, report mean and % difference, and state ''based on two measurements alone''
  - If LCI values are more than 1.0 TO apart (highest *versus* lowest), then alert the operator to perform further tests

Sn_{III} analysis (if performed)
- Calculation of S_{III} and Sn_{III}
  - S_{III} limits set to maximise the phase III used for linear regression, excluding phase II and phase IV contributions, and be manually adjustable, typically 50–95% of the expired volume in adults and 65–95% of the expired volume in children
  - Manual adjustment of the S_{III} for breaths, where marked low frequency noise (or cardiogenic oscillations) or phase IV phenomena occur if automated estimations of S_{III}
  - Expired inert gas concentration used for S_{III} normalisation (*e.g.* mean expired concentration or mean S_{III} concentration) should be clearly stated
- Acceptance criteria for breaths – identify and discard Sn_{III} values of breaths that do not fulfil the following criteria
  - Specific to tidal breathing protocols (*e.g.* paediatrics)
    - Adequate expired volume for Sn_{III} calculation: volume corresponding to S_{III} should be>50% of expiratory V_T
    - The expired volume should not be excessive: volume corresponding to S_{III} should not be>75% of expiratory V_T
    - Note: to try and achieve suitable breaths, an initial tidal breathing range of 10–15 $mL \cdot kg^{-1}$ can be used but may need to be adjusted for the individual patient depending on the expirogram seen
  - Specific to adult protocols using V_T of 1 L
    - Expired volume should be >0.950 L
    - Expired volume should not be >1.4 L
  - A clear S_{III} should be identifiable. Failure to identify S_{III} due to the presence of artefact (*e.g.* breath hold, cardiogenic oscillations, cough) should prompt exclusion of that Sn_{III} value
  - When Sn_{III} values are excluded do not discard the contribution of that breath to other indices (*e.g.* FRC and TO), only the Sn_{III} value
  - Tests should only contribute to overall Sn_{III} analysis if at least two out of three of the breaths remain after Sn_{III} breath exclusion. If >1/3 of Sn_{III} values have been excluded due to above criteria then that entire test should be discarded
    - Number of excluded Sn_{III} values and reasons for exclusion should be reported
- Presentation of Sn_{III} data
  - Data collated from all acceptable breaths of the three technically acceptable MBW tests
  - Acceptable first breath quality on all three tests for subsequent S_{acin} calculation
  - In TO calculation, FRC and V_T are calculated from the same airstream reference point used in ventilation inhomogeneity indices (see the online supplementary material section E6.2)
  - Data displayed graphically as Sn_{III} (y-axis) *versus* TO for each breath (x-axis)
    - Sn_{III} and $Sn_{III} \times V_T$ (*i.e.* V_T-corrected Sn_{III}) displayed for each breath on two separate graphs.
  - These indices rely on the fact that DCDI generates a horizontal asymptote and CDI does not and are therefore only valid in cases where Sn_{III} progression does not show a horizontal asymptote
- Clinical indices calculation
  - S_{acin} calculation
    - Requires three technically acceptable first breath Sn_{III} values
    - S_{acin} calculated as the mean Sn_{III} of the three first breaths minus the S_{cond} contribution (based on the mean TO value of the three first breaths)
  - S_{cond} calculation
    - S_{cond} calculated as the linear regression of Sn_{III} values between approximately 1.5 and 6.0 TO
    - Calculate 95% CI of the S_{cond} regression, reject outlying values and repeat linear regression; data should be pooled from all three runs
- If Sn_{III} analysis is performed with only two or less technically acceptable MBW tests, this should be clearly stated on the report and results interpreted with caution
- SBW S_{III}
  - Report as mean, SD and CoV of three technically acceptable measurements
    - If only two technically acceptable measurements are available, report mean and actual difference, and state ''based on two measurements''
    - VC measurements not within 10% of highest VC value across the SBW tests, then alert the operator
  - Report both S_{III} ($\%\cdot L^{-1}$) and $S_{III} \times$ expiratory VC (%) separately.

BTPS: body temperature, ambient pressure, saturated with water; ASCII: American Standard Code for Information Interchange; S_{III}: phase III slope; SBW: single-breath washout; MBW: multiple-breath washout; Sn_{III}: normalised S_{III}; N_2, nitrogen; CO_2: carbon dioxide; He: helium; and SF_6: sulfur hexafluoride; O_2: oxygen; FRC: functional residual capacity; LCI: lung clearance index; V_D: deadspace volume; FRC_{ao}: FRC at the airway opening; FRC_{gs}: FRC measured at the gas sampling point; CoV: coefficient of variation; V_T: tidal volume; TO: lung turnovers, calculated as cumulative expired volume/FRC; DCDI: diffusion convection-interaction-dependent inhomogeneity; S_{acin}: DCDI contribution to first breath Sn_{III}. CDI: convection-dependent inhomogeneity; Scond: rate of increase of Sn_{III} from 1.5–6 TOs, also CDI contribution to Sn_{III}; VC: vital capacity.

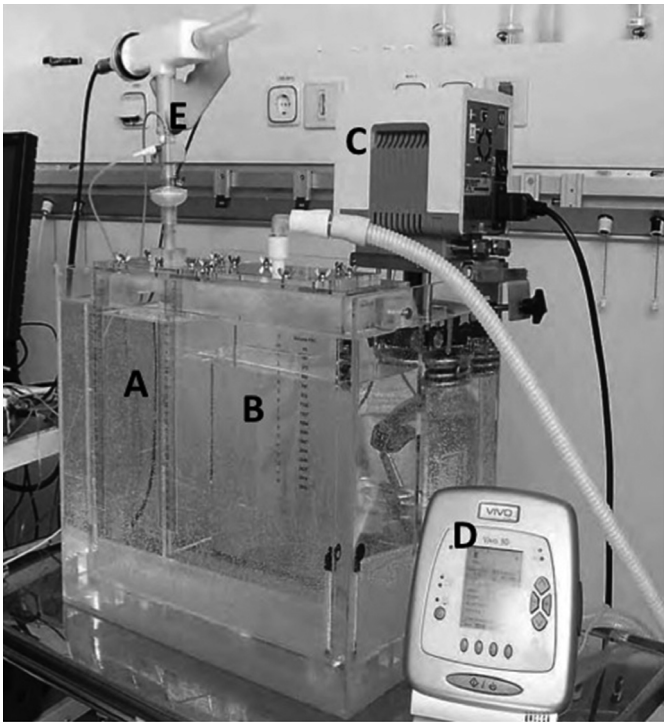

FIGURE 4. Recommended lung model for functional residual capacity (FRC) validation incorporating body temperature, ambient pressure, saturated with water vapour (BTPS) conditions and mimicking *in vivo* clinical testing conditions. The lung model consists of two separate chambers, an inner and an outer chamber. The inner chamber is partially divided (communicating at its inferior aspect) into two compartments: the lung compartment (A) and the ventilated compartment (B). FRC volume is generated by filling the inner chamber with distilled water to a measured height and calculated from known geometric dimensions. Water in the outer chamber is heated (C) such that inner chamber water temperature reaches 37°C, and a portable ventilator (D) is connected to the ventilated compartment of the inner chamber and transmitted hydraulic pressure generates the lung chamber breathing pattern: chosen to simulate physiological tidal volume (V_T)/FRC, V_T and respiratory rates likely to be encountered during intended clinical testing [35]. For example, whilst V_T remains similar (8 mL·kg^{-1}) across age ranges, FRC changes from ~20 mL·kg^{-1} in infants [37] to 40 mL·kg^{-1} in adults [38]. Multiple-breath washout equipment can be attached to the outlet of the lung compartment (E) during validation tests.

Measured FRC can be corrected to represent different points in the airstream: FRC at the airway opening is calculated as FRC measured at the gas sampling point, FRC_{gs}, minus pre-gas sampling point V_D. FRC used in ventilation inhomogeneity index calculations must correspond to a common airstream measurement point (see section E6.2 in the online supplementary material).

Calculated FRC may continue to increase through the washout, particularly in subjects with airway disease and in N_2-based MBW (see section entitled Impact of inert gas choice), yet studies rarely disclose when FRC measurement is determined. FRC end analysis threshold should correspond to the end-test threshold used for ventilation inhomogeneity indices (*e.g.* 1/40 of starting end-tidal concentration for lung clearance Index (LCI). The effect of variation in FRC end-point on other FRC-derived indices may be significant. Methodology for reported FRC values should be clearly described.

TABLE 6 Factors that lead to variation in measured indices between centres and recording systems

Factor	Examples
Equipment related	Analyser rise time
	Analyser linearity
	Flow measurement linearity
	Size of equipment related deadspace volume, including distance between gas and flow measurement points
Procedure related	Inert gas used (and concentration)
	Breathing stability
	Age of subjects tested
Analysis related	Algorithms used for calculation of indices
	BTPS correction applied
	Corrections applied for equipment-related deadspace
	Drift correction algorithms
	Synchronisation of flow and gas concentration signals
	Acceptability criteria applied (*e.g.* FRC values within 10%)

BTPS: body temperature, ambient pressure, saturated with water; FRC: functional residual capacity.

Measures of ventilation distribution inhomogeneity

A large number of ventilation distribution indices can be derived from information contained within SBW or MBW [21, 54, 55] (see section E6.1 in the online supplementary material): 1) SBW S_{III}, reflecting combined CDI and DCDI contributions, unless simultaneously performed with marker gases of widely different MM. 2) MBW global ventilation inhomogeneity indices, reflecting efficiency of marker gas clearance. 3) MBW Sn_{III} analysis, distinguishing CDI and DCDI mechanisms. 4) Airway closure and trapped gas volume (V_{TG}) assessment from SBW and MBW, respectively.

Depending on the pathology under study, relationships between MBW-derived indices (*e.g.* S_{acin}, S_{cond} and LCI) may help identify the type of structural changes generating increased ventilation distribution inhomogeneity [56].

Global measures

LCI is the most commonly reported MBW index in current paediatric literature, and defined as the number of FRC lung turnovers (TO; calculated as CEV/FRC) required to reduce alveolar tracer-gas concentration to a given fraction of its starting concentration, historically 1/40 (2.5%) [57]. Alveolar tracer-gas concentration has been estimated in various ways. In paediatric studies C_{et} is widely used, despite potential variability in end-tidal point. Identification of end-test threshold for LCI has not been systematically validated, but we recommend using the first of three consecutive breaths with a C_{et} <1/40 to avoid premature test termination with small breaths. LCI is calculated as the ratio of cumulative expired volume (CEV) to FRC, with CEV defined as the sum of all expiratory V_T over the washout including this first post-threshold. This introduces a small bias (overestimation);

however, the value of interpolated or more complicated curve fit methods to determine exact threshold crossing values is unclear. Alternate methods used should be explicitly stated.

Ideally indices should be assessed at airway opening without external V_D. However, this is, not feasible and V_T should be corrected for equipment V_D as appropriate (see section E6.2 in the online supplementary material). Post-gas sampling point V_D can be reliably estimated from water displacement; however, pre-gas sampling point V_D determination may be challenging, due to streaming within the facemask or filter [58]. Applied pre- and post-gas sampling point corrections should be clearly described. Where V_D correction is implemented, it is advised that both corrected and uncorrected LCI values are reported.

In clinical and modelling studies indices, such as LCI, have small but significant relationships to underlying respiratory patterns (V_T, V_D and FRC) particularly under disease conditions [54, 59, 60]. Effects of variation in respiratory rate and V_T can be minimised using moment analysis (see section E6.4 in the online supplementary material). This describes the degree of skewness of the washout curve to the right, as mean dilution numbers (MDN) or moment ratios [61]. V_D-independent assessment is feasible by correcting CEV for airways V_D ($V_{D,aw}$) and using cumulative expired alveolar volume in calculations (CEV_{alv}; *e.g.* alveolar MDN [59] and alveolar LCI [62]). $V_{D,aw}$, measured using Fowler or Langley methods (see section E4.2 in the online supplementary material) [63, 64], should be based on CO_2 $V_{D,aw}$, or the first few washout breaths of inert gas $V_{D,aw}$, as the latter increases during MBW [25] due to early washout of very well ventilated lung regions with short pathways to the airway opening. However, moment ratio truncation to facilitate between–subject comparison (*e.g.* to 8 TO [65]), may detrimentally affect sensitivity [66], and feasibility. Healthy subjects may also require longer washout periods to reach these higher turnover values, and accurate measurement may be compromised by limited signal resolution and high relative noise at the low gas concentrations encountered.

Normalised Sn_{III} analysis

MBW Sn_{III} analysis has a theoretical [67], experimental [68], and lung modelling basis [69–72] from morphometric data in healthy adults [22], to distinguish ventilation inhomogeneity arising from DCDI and CDI mechanisms, expressed as the clinical indices S_{acin} and S_{cond}, respectively [72] (fig. 5). For S_{acin} and S_{cond} determination, S_{III} and gas concentrations must be accurately determined down to breaths with very low concentrations (see section E6.6 in the online supplementary material) and may not be feasible for all washout systems.

S_{III} is dependent on many factors, both linear and non-linear, at least in healthy adult lungs: pre-inspiratory lung volume, inspired and expired volumes, and flow [1, 20, 42, 73–78]. Consequently, these factors should ideally be kept similar between subjects to maintain diffusion-convection front location, and allow changes in indices to be linked to changes in corresponding lung structures. Breath holds at end-inspiration flatten S_{III} and should be minimised [30, 63]. The beating heart generates flow pulses within airways [79] causing cardiogenic gas mixing. Cardiogenic oscillations superimposed onto S_{III} add to signal noise. Automated Sn_{III} calculation algorithms exist [80], but subjective observation is still necessary to review estimated slope accuracy.

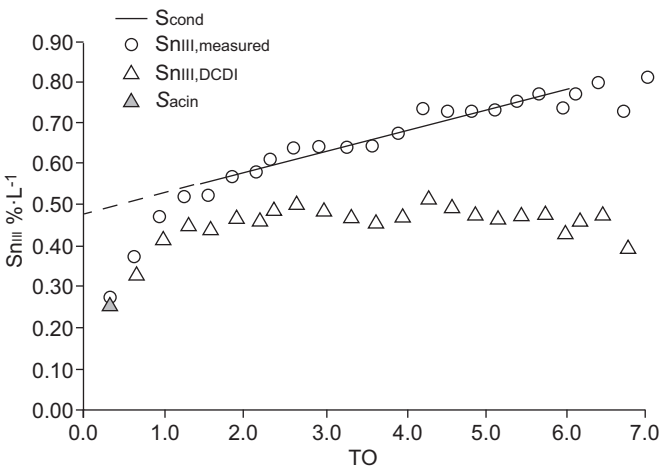

FIGURE 5. Normalised phase III slope (Sn_{III}) analysis. Multiple-breath washout recording illustrating derived phase III slope parameters. Measured concentration Sn_{III}, ($Sn_{III,measured}$) for each breath is plotted against its corresponding lung turnover, calculated as cumulative expired volume/functional residual capacity (TO), value. Progressive Sn_{III} values increase throughout the TO range considered. If this does not occur, the quality of the recording should be closely examined. The index of convection-dependent inhomogeneity (CDI; S_{cond}), is calculated as the increase in measured Sn_{III} per unit TO between ~1.5–6.0 TO per unit TO. For explanation purposes the diffusion convection-interaction-dependent inhomogeneity (DCDI) contribution to the Sn_{III} for each breath is also plotted ($Sn_{III,DCDI}$) This is calculated by subtracting the CDI contribution to Sn_{III} for each breath from the $Sn_{III,measured}$ for each breath. In other words, for each breath, $Sn_{III,measured}$ value equals $Sn_{III,DCDI}$ value minus CDI contribution. S_{acin} is defined as the DCDI contribution to the first breath $Sn_{III,DCDI}$. The complete contribution of the DCDI mechanism reaches a plateau beyond TO 1.5 and is equivalent to the intercept of the S_{cond} regression line. These indices rely on the fact that DCDI generates a horizontal asymptote and CDI does not and are, therefore, only valid in cases where Sn_{III} does not show a horizontal asymptote.

Trapped gas volume

Airway closure occurs in lung units approaching regional RV [81], but may also occur at higher regional lung volumes in infants, older adults [82], obese subjects [83], and in the presence of peripheral airway obstruction. It may be a prominent phenomenon in airway disease. If present, the V_{TG} can be measured during MBW by including five inspiratory capacity breaths after conventional end-test threshold is reached and measuring the volume of lung recruited (see section E6.3 in the online supplementary material). V_{TG} measurement with both resident and exogenous MBW has been established for infants and children [84, 85]. Importantly, this method estimates only the gas volumes recruitable during these large breaths.

Closing volume and closing capacity

Closing volume (CV) and closing capacity require accurate determination of SBW phase III to phase IV transition (fig. 1). CV reflects airway closure occurring preferentially in dependent lung regions and peripheral airway obstruction [81, 86, 87]. Relative merits of these indices have been reviewed elsewhere [88]. Although feasible in adults [89], paediatric

utility of CV is limited [90]. Automated identification of phase IV is feasible [91].

IMPACT OF INERT GAS CHOICE

Derived indices may differ depending on the gas used for a number of reasons. Gas diffusion rate is inversely proportional to the square root of the MM, but convective distribution is unaffected. Consequently, diffusion-convection front location is more proximal for lighter gases *versus* heavier gases (*e.g.* He *versus* SF_6 MM is 4 *versus* 146 $g \cdot mol^{-1}$, respectively). Greater series V_D for SF_6, compared to He, generates higher LCI values, irrespective of ventilation distribution itself. In healthy lungs SF_6 S_{III} are greater than He S_{III}, but may reverse in lung pathology [92–94]. In addition, rate of diffusive equilibration in enlarged peripheral air spaces (*e.g.* emphysema) may differ depending on gas choice generating differential S_{III} increase. Homogeneity of gas distribution present at the start of washout may differ depending on whether naturally resident or exogenous gas is used. Measurable differences may be informative. In simultaneous He and SF_6 measurements, disease processes distal to the acinar entrance generate greater abnormality in SF_6 indices, whereas disease processes proximal to the acinar entrance but in the zone of the convection-diffusion front will affect He indices preferentially. However, if disease processes affect SF_6 and He S_{III} to a similar extent, no relative S_{III} difference occurs [95].

Advantages of N_2 washout include widespread availability and affordability of 100% O_2, and avoidance of patient connection to equipment during wash-in periods between tests minimising patient discomfort. N_2 is resident in all lung units including very slowly ventilated lung compartments and may offer improved sensitivity to detect abnormality, compared to other inert gases, which may not equilibrate fully within these regions during wash-in. However, disadvantages also exist. Thresholds at which factors such as age, sleep state and sedation interact with 100% O_2 to affect breathing pattern remain unclear. N_2 is not truly inert and tissue N_2, present due to high atmospheric N_2 partial pressure, diffuses from blood into alveoli along concentration gradients. This diffusion is greatest in well-ventilated lung regions washed out during initial portions of the test, and contribute to exhaled N_2 later in the washout, potentially introducing greater error in longer tests (*e.g.* FRC overestimation). Estimation and correction of tissue N_2 contribution is difficult due to limited available data to base correction [96], and adjustment for tissue N_2 is not currently recommended [97].

Whilst different inert gas concentrations used in the literature are safe (*e.g.* 4% SF_6 and 4% He), additional factors influence inert gas selection. SF_6 may have adverse health effects at higher concentrations [98] and significant greenhouse potential [99]. Feasibility of scavenging following testing is unclear. SF_6 is not universally approved for testing (*e.g.* USA and France). Low density of He renders it more susceptible to leaks during testing, which may aid leak detection. Cost of exogenous gas is increasing in many countries, partly due to increasing logistical requirements when used as a medical gas.

ACCEPTABILITY CRITERIA FOR TESTING

Quality control during testing is critical and extends beyond equipment performance and software feedback to also include close observation by the operator of the subject's behaviour during testing and how this affects the data obtained. Adequate operator training and appreciation of all factors influencing test results is essential. Recommended acceptability criteria for MBW and SBW are summarised in tables 7 and 8.

Multiple-breath washout

Primary index of interest may differ between paediatric and adult testing (*e.g.* LCI and Sn_{III} indices in the current literature, respectively) influencing test termination criteria and acceptability. Recommendations contained within this document attempt to provide a unified approach.

Breathing pattern

Measured FRC reflects lung volume at which washout is commenced (*i.e.* end-expiratory level). Stability of resting lung volumes before and throughout washout is critical [46, 48]. In infants, intrinsic FRC resetting during critical periods, visible as sighs, should prompt test exclusion. In general, large inspiratory breaths during washout may mobilise trapped gas and small inspiratory or expiratory breaths may result in steeper S_{III}. End-tidal volumes below FRC may result in steeper S_{III} and occurrence of phase IV, especially in obstructive lung disease. For Sn_{III} analysis, first breath quality (in particular adherence to target inhalation and exhalation volume) is critical for accurate S_{acin}.

Relaxed tidal breathing has historically been used for global MBW derived indices. Studies introducing adult Sn_{III} analysis used a strict 1 L V_T breathing regimen [101], chosen as a compromise between 1) maintaining physiological breathing conditions, 2) obtaining sufficient phase III to compute its slope, and 3) having sufficient Sn_{III} data points for statistically valid regression from ~TO 1.5–6.0 [101]. This strict protocol is not feasible in all ages, or in more advanced obstructive disease. In addition, due to marked variations in lung size, 1 L may greatly exceed normal V_T and not be appropriate. In an attempt to implement Sn_{III} analysis in younger ages during regular breathing (typically aged $\leqslant$16 yrs), the following criterion for breath acceptability, based on a similar principle, is proposed: each breath must have sufficient phase III to compute S_{III} (at least 50% of V_T). For tests fulfilling this criterion, volume compensation is then performed on Sn_{III}: Sn_{III} is multiplied by FRC (to correct for differences in lung size) and then by V_T/FRC (to account for variations in S_{III} due to changes in breathing pattern). This net multiplication of Sn_{III} by V_T (in L) facilitates comparison among subjects of differing lung sizes, yet needs to be critically interpreted in any particular study setting (see section E6.5 in the online supplementary material). Where implemented, we recommend that both corrected and uncorrected S_{acin} and S_{cond} values are reported, such that *posteriori* analyses are possible, if and when this or other correction methods are validated. Insufficient S_{III} for accurate estimation limits feasibility in infants [105].

Visual breathing pattern feedback may be useful to guide older adolescents and adults [9] but is problematic in younger subjects, for whom distraction with videos is recommended [6]. Measurements in infants should be performed during quiet nonrapid eye-movement sleep, with or without the use of sedation. No comparative study exists showing the potential effect of sedation on washout indices.

TABLE 7 Multiple-breath washout (MBW) measurement acceptability criteria

- **Testing position**
  - Infants: supine position with head in midline and in sniffing position to optimise upper airway patency
  - Preschool and above: seated position, with head in midline
- **Interface**
  - Infants and preschoolers: suitable facemask with adequate therapeutic putty volume to ensure adequate facemask seal and reduction of the pre-gas sampling point V_D without obstructing the airway opening
    - Nasal mask measurements are feasible during periods of preferential nasal breathing [100] but require further study
    - Box shaped flow–volume loops may indicate an upper airway obstruction or external obstruction of the airway opening by therapeutic putty
  - Older subjects: nose clip and maintain tight mouthpiece seal
- **Three technically acceptable MBW runs should be performed, with acceptability defined by the following criteria**
  - Wash-in phase (or pre-washout phase for N_2 MBW)
    - Stable V_T and end-expiratory lung volume over the preceding 30 s
      - Deviation in end-expiratory lung volume at start of test within 10% of mean V_T of preceding five breaths
      - An irregular small volume breath immediately prior to starting the washout may also lead to error in end tidal estimate of starting alveolar concentration
    - Equilibration of exogenous wash-in gas within the breath cycle (*i.e.* inspiratory *versus* expiratory end tidal concentration)
      - Variability <1% relative to mean inspired concentration (*i.e.* <0.04% if the inspired concentration is 4%)
    - Adequate starting end-tidal inert gas concentration, stable over 30 s (*i.e.* equal to inspired gas concentration)
  - Washout phase
    - Regular breathing pattern
      - Sufficient breath size for adequate phase III slope identification (if S_{nIII} analysis being performed)
      - Breathing protocols of 1 L V_T are recommended in older adolescents (*e.g.* >16 yrs) and adults but may not be feasible in all age groups (*e.g.* V_T 1.0–1.3 L) [101–103]
    - No evidence of significant trapped gas release with larger breaths; release of trapped gas
      - Invalidates S_{nIII} analysis and increases measured LCI
      - May be difficult to avoid in advanced CF lung disease
    - No coughing
    - Specific to infants during critical periods of the wash-in/washout
      - No evidence of apnoeas (may significantly decrease FRC)
      - No evidence of sighs (may significantly elevate FRC)
      - Critical period defined as the 10 breaths prior to achieving equilibration or during the first 10 breaths of the washout
  - Criteria for test termination
    - At least three consecutive breaths with end tidal inert gas concentration values below 1/40 of starting inert gas concentration
    - If S_{nIII} analysis alone, then at least 6 TO must be included
    - If moment analysis is being performed then at least 6 TO should be included, as data collected at 8 TO in normal subjects are likely to be compromised by poor gas signal quality
  - No evidence of leak occurring during the test
    - Resident inert gas (*e.g.* N_2) - leak indicated by the following during the washout phase
      - Sudden spike in N_2 concentration during inspiration (consistent with post-gas sampling point inspiratory air leak)
      - Premature rise in N_2 signal early in expirogram of following breath, where N_2 concentrations should be zero in the initial absolute dead space portion (consistent with pre-gas sampling point inspiratory air leak)
      - $V_{D,aw}$ decrease
      - Sudden step changes of the volume trace
      - Step-up of N_2 concentration plotted *versus* TO
    - Exogenous inert gas: leak indicated by
      - Failure of equilibration between inspiratory and expiratory inert gas concentrations during wash-in (consistent with pre- or post-gas sampling point leak)
      - Sudden drop in inspiratory inert gas concentration during wash-in (consistent with post-gas sampling point leak)
      - $V_{D,aw}$ increase during washout
  - Sufficient interval between runs when using resident inert gases to allow inert gas concentration to return to baseline values
    - Twice the washout time is a conservative recommendation. If a shorter interval is used, then the operator must demonstrate that alveolar concentrations has been resituated [104]
    - This period may be lengthy in advanced obstructive disease
    - Inadequate duration may significantly decrease measured FRC
- **The following should trigger further investigation for artefact but are not a reason to exclude tests alone**
  - Marked volume drift during testing or sudden changes in volume (without other evidence of leak)
  - FRC or LCI variability >10%, measured as the difference between maximum and minimum values
- **Tests where FRC differs by >25% from the median FRC value across the three tests should be automatically rejected**
- **Test equipment and performance must adhere to infection control guidelines**
  - Use of bacterial filters may significantly increase V_D and preclude the use of certain systems in younger age groups

N_2: nitrogen gas; V_D: deadspace volume; V_T: tidal volume; S_{nIII}: normalised phase III slope; LCI: lung clearance index; CF: cystic fibrosis; FRC: functional residual capacity; TO: lung turnovers, calculated as cumulative expired volume/FRC; $V_{D,aw}$: deadspace volume of the conducting airways.

TABLE 8 Single-breath washout (SBW) measurement quality control

- **Testing position: seated position for all subjects**
  - In general SBW only feasible in older children and adults ⩾12 yrs
- **Interface**
  - Nose clip and maintain a tight mouthpiece seal
- **Three technically acceptable SBW runs should be performed, with acceptability defined by the following criteria**
  - Within-test criteria
    - No evidence of leak (as described for MBW)
    - Inspiratory and expiratory flow maintained between 400 and 500 mL·s^{-1} over first three-quarters of inspiratory and expiratory portions of test manoeuvre
      - Maintaining these flows at end of manoeuvre may be difficult
      - Flow restrictors may be useful to maintain desired flow range
    - Phase III slope default settings to 25–75% of expired volume
      - Manually adjustable to ensure freedom from contamination by phase II, phase IV, and stochastic variations in S_{III} caused by low frequency noise or cardiogenic oscillations
    - Specific to classical VC SBW manoeuvre
      - Inspiratory and expiratory VC breaths within 10% within same test
    - Specific to modified SBW manoeuvre
      - Stable end expiratory level prior to starting the manoeuvre
        - Deviation in end-expiratory lung volume at start of the test within 10% of mean V_T of preceding five breaths
      - Inspiratory volume above FRC reaches 1 L within ± 10%
  - Between-test criteria
    - Specific to classical VC manoeuvre
    - Expiratory VC values within 10% of highest value
    - Specific to modified SBW manoeuvre
      - Inspiratory volumes within 10% of 1 L
      - Expiratory volumes within 10% of each other
    - Re-establish baseline inert gas concentration before starting next test
      - N_2 SBW: end-tidal N_2 concentration returns to baseline levels
      - Exogenous SBW: end-tidal inert gas concentration <1/40 of inspired inert gas concentration
    - Sufficient interval between tests of at least two minutes to stabilise volume history and to reset alveolar N_2 concentrations
- **Test equipment and performance must adhere to infection control guidelines**

MBW: multiple-breath washout; S_{III}: phase III slope; VC: vital capacity; V_T: tidal volume; FRC: functional residual capacity; N_2: nitrogen gas.

Test termination

MBW test termination after alveolar concentration (usually Cet) falls below 1/40 of starting concentration for three consecutive breaths allows standard LCI to be calculated. For standard Sn_{III} analysis and moment ratios MBW should pass beyond 6 and 8 TO, respectively [65].

FRC repeatability

Previously recommended within–session FRC repeatability criteria (within 10%, [106, 107]) have poor feasibility in paediatric testing [108], and may lengthen total testing time significantly. Repeatability within 10% should be viewed as encouraging. Tests should be carefully examined for technical issues if this is not met. Automatic exclusion of tests should occur if FRC differs by >25% from median FRC over three tests. In older children, FRC increases by >20% moving supine to sitting [109], but effects of transition from testing supine infants to seated preschoolers is unclear. Postural effects on ventilation distribution may also depend on severity and topographical location of airways disease. Consideration of these factors should also occur when comparing upright ventilation distribution tests to supine imaging studies.

Single-breath washout

The need to maintain inspiratory and expiratory flows strictly between 400–500 mL·s^{-1} and achieve reproducible VC manoeuvres currently limits feasibility to adults and children >12 yrs [18]. S_{III} volume compensation, using a similar approach to MBW, in this case by multiplying S_{III} by VC, is feasible but not formally validated. It is unclear how much variation in historical predicted S_{III} values [18] is due to physiological intrinsic or technical factors.

FUTURE WORK AND CONCLUSIONS

Important questions remaining unanswered for commercial and research washout systems, SBW and MBW test procedure and subsequent analysis are summarised in table 9. Challenges arise when interpreting washout tests in infants and children where relationships between V_D/V_T and V_T/FRC and calculated indices must be considered. This is particularly relevant when undertaking studies of early lung disease or treatment effects to ensure that reported differences don't reflect alterations in respiratory patterns alone. Longitudinal data for ventilation inhomogeneity indices during normal lung development with age are needed. Influence of sex and ethnic background is unclear.

Anatomical distinction between ventilation inhomogeneity represented by S_{cond} and S_{acin} relies on diffusion–convection front location, which has been simulated in an adult lung using available lung structure and airway dimensions. Extending applicability of such indices into childhood and disease processes requires further simulation of the diffusion-convection front based on realistic anatomical data. Beyond *post mortem* data, anatomical and functional data obtained using modern computed tomography scanning techniques or hyperpolarised noble gas magnetic resonance imaging studies may provide this. Simulation studies in realistic lung models could also be used to validate V_T correction of Sn_{III} to compare ventilation inhomogeneity between varying age groups with varying V_D, V_T, and FRC. Until formal validation, studies incorporating Sn_{III} analysis should ideally include matched healthy control data for comparison and report both uncorrected and corrected values. Formal objective quality control thresholds for test acceptance and breath exclusion are also required. Shortening test duration whilst maintaining sensitivity and specificity will enhance feasibility and incorporation into routine clinical testing. Efforts to investigate ways to achieve this are already underway [108, 112].

Inert gas washout provides unique physiological information, which at the very least forms an important complement to current methods in the adult lung function laboratory, while offering improved feasibility and sensitivity compared to spirometry in younger children. A number of important challenges lie ahead for integration into routine clinical care. Standardisation of procedures and development of robust

TABLE 9 Important areas of interest for future studies

Area of interest	Questions and needs
Equipment validation	Feasible validation methods for end-tidal inert gas concentration and phase III slope measurement
Synchronisation of gas and flow signals	Optimal synchronisation method, protocol for measurement, and the thresholds for acceptable synchronisation error remain unclear
BTPS correction	Optimal BTPS correction. Is dynamic BTPS correction required during testing? How are changes in temperature and relative humidity most accurately measured during inspiration and expiration?
Equipment V_D estimation	Accurate estimation of effective external V_D. Streaming may occur with equipment-related V_D. Therefore water displacement measurement of V_D may overestimate influence of $V_{D,ext}$ on breathing pattern. This includes facemasks and in-line bacterial filters
Gas analyser properties	Acceptable maximum response time for different age groups and breathing patterns?
Sample flow (Sidestream gas analysers)	Degree of error introduced by sample flow: What is an acceptable sample flow? Given its age-dependence, should it be considered as a % of V_T? What is the most appropriate method to correct flow and marker gas volume for sample flow?
Tissue N_2	Effective correction for effect of tissue nitrogen diffusing into alveoli during washout. What is the error introduced into subsequent indices (FRC, LCI and Sn_{III} analysis)?
N_2-based MBW	At what age does 100% O_2 no longer have a detrimental effect on breathing pattern?
Use of sedation in infants	Effect of sedation on ability of infants to actively maintain FRC or effect on breathing pattern? This has been speculated upon but remains unproven [110, 111]
Measures of global ventilation inhomogeneity	Can test duration be shortened whilst preserving acceptable sensitivity? Flexibility of current MBW end-test thresholds (*e.g.* evaluation of 1/20 for LCI and 6 TO for moment ratios) How many tests are needed to give an accurate estimate? [108] Can wash-in data also be utilised to calculate indices? Utility of interpolation or curve fitting methods to determine exact end-of-test for LCI Validation of pre- and post-gas sampling point V_D corrections
MBW Sn_{III} analysis	Validity of paediatric correction of Sn_{III} by V_T to account for differences in tidal V_T and breathing pattern Most appropriate inert gas reference concentration for normalisation of S_{III} Formal objective criteria for exclusion of outlying Sn_{III} values Can accurate estimates be obtained from two tests?
Considerations for FRC, CEV and TO	Influence of geometric choice within the airstream and the time point chosen for FRC determination during the washout on FRC, CEV and TO on subsequently reported ventilation inhomogeneity indices
Importance of FRC repeatability	FRC repeatability recommendations here are based on consensus and further research is needed to define these in future studies; the impact of FRC variability on Sn_{III} indices is unclear
SBW S_{III} analysis	Validity of paediatric correction of S_{III} by expiratory VC to account for differences in lung size
Normative data	Normative data needs to be collected for indices across different age, sex and ethnic groups. Standardisation of procedures is essential if results are to be comparable across centres and between devices. Differences in results obtained among gases with different molecular masses are expected; formal comparisons are lacking
Commercial devices	Development of robust accurate commercial devices which can be used across wide age ranges

BTPS: body temperature, ambient pressure, saturated with water; V_D: deadspace volume; N_2: nitrogen gas; MBW: multiple-breath washout; Sn_{III}: normalised phase III slope; FRC: functional residual capacity; CEV: cumulative expired volume; TO: lung turnovers, calculated as CEV/FRC; SBW: single-breath washout; S_{III}: phase III slope; $V_{D,ext}$: external equipment deadspace volume; V_T: tidal volume; LCI: lung clearance index; VC: vital capacity.

appropriately validated affordable commercial equipment is essential. This will only be achievable if manufacturers work in collaboration with researchers, as we seek to address the important issues and questions that remain unanswered. This standardisation document provides the basis for this future work.

STATEMENT OF INTEREST

Statement of interest for P.D. Robinson, M. Gappa, G.G. King, J.J. Pillow, and F. Ratjen can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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