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: N₂, and argon) and exogenous gases (sulfur hexafluoride: SF₆, 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. Vt 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 N₂ 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 < 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. | 518

SBW: single-breath washout; MBW: multiple-breath washout; FRC: functional residual capacity; BTPS: body temperature, ambient pressure, saturated with water; LCI: lung clearance index; Vt: tidal volume; VC: vital capacity; N₂: nitrogen gas.
FIGURE 1. Example of a typical single-breath washout (SBW) trace. Nitrogen gas (N$_2$) expirogram showing calculation of phase III slope (SIII) in a vital capacity SBW test in a 60-yr-old smoker. SIII is calculated between 25% and 75% of the expired volume (SIII 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.

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 (SIII), explained in figure 1, and magnitude and progression of MBW concentration normalised phase III slopes (SIII) through subsequent breaths (fig. 2); in the latter, SIII 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 SIII in SBW and generates a continuous rise in SIII through subsequent MBW breaths [25]. Diffusion-convection-interaction-dependent inhomogeneity (DCDI), which occurs in the region of the acinar entrance, increases SIII 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 SIII [24] and DCDI contribution to MBW SIII 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 SIII 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 SIII 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 SIII 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 SIII 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, VT 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 Vb effectively introduces a small rebreathing chamber. Pre-gas sampling point Vo is an extension of anatomical Vd.
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 in Bert gas, equipment and software specific. Experimental conditions under which normative data are obtained should be clearly described in manuscripts.

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow measurement</td>
<td>Instantaneous flow accuracy within 5% across the range of flows encountered during clinical testing and volume accuracy within 3% using a precision calibration syringe.</td>
</tr>
<tr>
<td>Sample flow</td>
<td>Ideally, all side-stream washout systems should correct for sample flow. If not performed or achievable, sample flow should be minimised: &lt;20 mL·min⁻¹ for paediatric and &lt;40 mL·min⁻¹ for adult apparatus where gas sample point is proximal to flow metre.</td>
</tr>
<tr>
<td>Volume drift</td>
<td>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 technical causes (e.g. leaks) should be made as part of the routine quality control.</td>
</tr>
<tr>
<td>Gas analyser accuracy</td>
<td>Linearity within 1% relative of full scale (e.g. 0-80% is ±0.8% at 80% N₂) to ensure appropriate assessment of starting concentration, and within 5% relative of any lower value (e.g. 0.25% at 5% N₂) 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.</td>
</tr>
<tr>
<td>Gas analyser rise time</td>
<td>A 10-90% analyser rise time of &lt;100 ms is recommended across all age groups.</td>
</tr>
<tr>
<td>Data sampling frequency</td>
<td>Data sampling should ideally be ≥100 Hz for both flow and inert gas concentration measurement.</td>
</tr>
<tr>
<td>Synchronisation of flow and gas signals</td>
<td>Alignment accuracy within 10 ms or one sample (whichever is longer).</td>
</tr>
<tr>
<td>Equipment-related dead space</td>
<td>Total equipment deadspace for young children should be &lt;2 mL·kg⁻¹ bodyweight, and ideally &lt;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.</td>
</tr>
<tr>
<td>Equipment-related resistance</td>
<td>Should be minimised for both inspiration and expiration to avoid effects on breathing pattern and FRC during test.</td>
</tr>
</tbody>
</table>

The table is expanded with further explanation in section E2.6 in the online supplementary data. N₂: 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 in Bert 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 N₂, inhalation of 100% O₂ 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 N₂ analyser systems (requiring vacuum pumps), indirect N₂ measurement systems have been proposed based on simultaneous O₂ and CO₂ 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 SF₆ or He are also feasible [44–46]. Mainstream MM SF₆ 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 (VTD) 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 (FRCgas) 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
tidal breaths. FRC\textsubscript{gas} is, therefore, often lower than plethysmographic FRC, especially in obstructive lung disease \cite{53}. FRC\textsubscript{gas} = V\textsubscript{IG}/C\textsubscript{et,IG} (initial–final), where: V\textsubscript{IG} is net volume of inert gas expired, and C\textsubscript{et} is end-tidal concentration of inert gas. V\textsubscript{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\textsubscript{D} after the post-gas sampling point (fig. 3, see section E5.2 in online supplementary material).

### TABLE 3

Overall recommendations for washout systems

| Instantaneous flow within 5%, and V\textsubscript{T} and CEV measurement accuracy within 3%. |
| Quality of gas signals allowing determination of FRC, end-tidal gas concentration and S\textsubscript{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\textsubscript{T}: tidal volume; CEV: cumulative expired volume; FRC: forced residual capacity; S\textsubscript{III}: phase III slope; BTPS: body temperature, ambient pressure, saturated with water.

### 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 95–96% of the expired volume
- If S\textsubscript{III} progression is being measured then display the breath-by-breath inert gas expirogram to allow the user to ensure sufficient S\textsubscript{III} is visible (\textgtrsim 50% of the expired V\textsubscript{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\textsubscript{III} 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\textsubscript{T,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\textsubscript{III}: normalised phase III slope; V\textsubscript{T}: tidal volume; CEV: cumulative expired volume; FRC: functional residual capacity; V\textsubscript{T,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, calibrations 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 expirrogram for each breath, and manual adjustment if necessary, to ensure correct estimation of End-tidal inert marker gas concentration
Si if SBW or if MBW SIII analysis is being performed
Ability to examine for and correct any gas-analysers drift occurring during the test. The zero calibration point may be useful as a reference for many of the gases used (N2, CO2, He and SF6) whilst 100% can be used for O2. Any correction applied should be clearly stated
If available, monitor end-tidal CO2 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 Vt for each breath
Reported FRC is that measured at the FRCgs. If other FRC values are reported, e.g. FrCor (i.e. FrCor – pre-gas sampling point Vt) these values should be described appropriately
Report mean, SD and CoV of three technically acceptable measurements

Indices of global ventilation distribution inhomogeneity (e.g. LCI and moment ratios)
Correct Vt for external Vt (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

SIII analysis (if performed)
Calculation of SIII and SIII
Six 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 65–85% of the expired volume in children
Manual adjustment of the SIII for breaths, where masked low frequency noise (or cardiogenic oscillations) or phase IV phenomena occur if automated estimations of SIII
Expired inert gas concentration used for SIII normalisation (e.g. mean expired concentration or mean SIII concentration) should be clearly stated
Acceptance criteria for breaths – identify and discard SIII values of breaths that do not fulfil the following criteria
Specific to tidal breathing protocols (e.g. paediatrics)
 Adequate expired volume for SIII calculation: volume corresponding to SIII should be >50% of expiratory Vt
 The expired volume should not be excessive: volume corresponding to SIII should not be >75% of expiratory Vt
 Note: to try and achieve suitable breaths, an initial tidal breathing range of 10–15 mL/kg can be used but may need to be adjusted for the individual patient depending on the expirrogram seen
 Specific to adult protocols using Vt of 1 L
 Expired volume should be >0.95 L
 Expired volume should not be >1.4 L
 A clear SIII should be identifiable. Failure to identify SIII due to the presence of artefact (e.g. breath hold, cardiogenic oscillations, cough) should prompt exclusion of that SIII value
 When SIII values are excluded do not discard the contribution of that breath to other indices (e.g. FRC and TO), only the SIII value
 Tests should only contribute to overall SIII analysis if at least two out of three of the breaths remain after SIII exclusion. If >1/3 of SIII values have been excluded due to above criteria then that entire test should be discarded
 Number of excluded SIII values and reasons for exclusion should be reported
Presentation of SIII data
Data collated from all acceptable breaths of the three technically acceptable MBW tests
Acceptable first breath quality on all three tests for subsequent Scond calculation
In Scond calculation, FRC and Vt 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 SIII (y-axis) versus TO for each breath (x-axis)
SIII and SIII × Vt (i.e. Vt-corrected SIII) 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 SIII progression does not show a horizontal asymptote
Clinical indices calculation
Scond calculation
Requires three technically acceptable first breath SIII values
Scond calculated as the mean SIII of the three first breaths minus the Scond contribution (based on the mean TO value of the three first breaths)
Scond calculated as the linear regression of SIII values between approximately 1.5 and 6.0 TO
Calculate 95% CI of the Scond regression, reject outlying values and repeat linear regression; data should be pooled from all three runs
If SIII 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 SIII
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”

Clinical indices calculation
Scond calculated as cumulative expired volume/FRC. DCDI: diffusion convection-interrelation-dependent inhomogeneity; Scond: CDI contribution to first breath SIII. CDI: convection-dependent inhomogeneity.

BTPS: body temperature, ambient pressure, saturated with water; ASCII: American Standard Code for Information Interchange; SIII: phase III slope; SBW: single-breath washout; MBW: multiple-breath washout; SIII: normalised SIII; N2: nitrogen; CO2: carbon dioxide; He: helium; and SF6: sulfur hexafluoride; O2: oxygen; FRC: functional residual capacity; LCI: lung clearance index; Vt: deadspace volume; FrCor: FRC at the airway opening; FrCor: FRC measured at the gas sampling point; CO2FRC coefficient of variation; Vt: tidal volume; TO: lung turnover calculated as cumulative expired volume/FRC. DCDI: diffusion convection-interrelation-dependent inhomogeneity; Scond: CDI contribution to first breath SIII. CDI: convection-dependent inhomogeneity.
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, FRCps, minus pre-gas sampling point Vd. 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 N₂-based MBW (see section entitled Impact of inert gas choice), yet studies rarely disclose when FRC measurement is determined.

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 (VT)/FRC, VT and respiratory rates likely to be encountered during intended clinical testing [35]. For example, whilst VT 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.

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

**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 SnIII analysis, distinguishing CI and DCDI mechanisms. 4) Airway closure and trapped gas volume (VTG) assessment from SBW and MBW, respectively.

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 Cet 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 Cet <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 VT over the washout including this first post-threshold. This introduces a small bias (overestimation);

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

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

BTPS: body temperature, ambient pressure, saturated with water; FRC: functional residual capacity.
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 VD. However, this is not feasible and VT should be corrected for equipment VD as appropriate (see section E6.2 in the online supplementary material). Post-gas sampling point VD can be reliably estimated from water displacement; however, pre-gas sampling point VD 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 VD 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 (VT, VD and FRC) particularly under disease conditions [54, 59, 60]. Effects of variation in respiratory rate and VT 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]. VD-independent assessment is feasible by correcting CEV for airways VD (VDaw) and using cumulative expired alveolar volume in calculations (CEValv; e.g. alveolar MDN [59] and alveolar LCI [62]). VDaw, measured using Fowler or Langley methods (see section E4.2 in the online supplementary material) [63, 64], should be based on CO2 VDaw, or the first few washout breaths of inert gas VDaw, 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 SnIII analysis
MBW SnIII 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 DCIDI and CDI mechanisms, expressed as the clinical indices Sacin and Scond, respectively [72] (fig. 5). For Sacin and Scond determination, SnIII 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.

SnIII 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 SnIII and should be minimised [30, 63]. The beating heart generates flow pulses within airways [79] causing cardiogenic gas mixing. Cardiogenic oscillations superimposed onto SnIII add to signal noise. Automated SnIII calculation algorithms exist [80], but subjective observation is still necessary to review estimated slope accuracy.

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 VTG 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). VTG 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·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$_6$ SIII are greater than He SIII, 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 SIII 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 SIII to a similar extent, no relative SIII 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 SnIII 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 SIII. End-tidal volumes below FRC may result in steeper SIII and occurrence of phase IV, especially in obstructive lung disease. For SnIII 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 SnIII 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 SnIII data points for statistically valid regression from $\sim$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 SnIII analysis in younger ages during regular breathing (typically aged $\leq$16 yrs), the following criterion for breath acceptability, based on a similar principle, is proposed: each breath must have sufficient phase III to compute SIII (at least 50% of $V_t$). For tests fulfilling this criterion, volume compensation is then performed on SnIII: SnIII is multiplied by FRC (to correct for differences in lung size) and then by $V_t$/FRC (to account for variations in SIII due to changes in breathing pattern). This net multiplication of SnIII 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 SIII 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.
Three technically acceptable MBW runs should be performed, with acceptability defined by the following criteria

**Wash-in phase** (or pre-washout phase for N₂ MBW)
- Stable VT and end-expiratory lung volume over the preceding 30 s
- Deviation in end-expiratory lung volume at start of test within 10% of mean VT 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 SnIII analysis being performed)
- Breathing protocols of 1 L VT are recommended in older adolescents (e.g. >16 yrs) and adults but may not be feasible in all age groups (e.g. VT 1.0–1.3 L) [101–103]
- No evidence of significant trapped gas release with larger breaths; release of trapped gas
- Invalidates SnIII 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 SnIII 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. N₂) - leak indicated by the following during the washout phase
  - Sudden spike in N₂ concentration during inspiration (consistent with post-gas sampling point inspiratory air leak)
  - Premature rise in N₂ signal early in expirogram of following breath, where N₂ concentrations should be zero in the initial absolute dead space portion (consistent with pre-gas sampling point inspiratory air leak)
  - VD,Daw decrease
  - Sudden step changes of the volume trace
  - Step-up of N₂ 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)
  - VD,Daw 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 VD and preclude the use of certain systems in younger age groups

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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]. Sii volume compensation, using a similar approach to MBW, in this case by multiplying Sii by VC, is feasible but not formally validated. It is unclear how much variation in historical predicted Sii 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 VD/VT and VT/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 Scod and Sacin 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 VT correction of Snii to compare ventilation inhomogeneity between varying age groups with varying VD, VT, and FRC. Until formal validation, studies incorporating Snii 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**
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TABLE 9
Important areas of interest for future studies

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<tr>
<th>Area of interest</th>
<th>Questions and needs</th>
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<tr>
<td>Equipment validation</td>
<td>Feasible validation methods for end-tidal inert gas concentration and phase III slope measurement</td>
</tr>
<tr>
<td>Synchronisation of gas and flow signals</td>
<td>Optimal synchronisation method, protocol for measurement, and the thresholds for acceptable synchronisation error remain unclear</td>
</tr>
<tr>
<td>BTPS correction</td>
<td>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?</td>
</tr>
<tr>
<td>Equipment V0 estimation</td>
<td>Accurate estimation of effective external V0. Streaming may occur with equipment-related V0. Therefore water displacement measurement of V0 may overestimate influence of V0,ext on breathing pattern. This includes facemasks and in-line bacterial filters</td>
</tr>
<tr>
<td>Gas analyser properties</td>
<td>Acceptable maximum response time for different age groups and breathing patterns?</td>
</tr>
<tr>
<td>Sample flow (Sidestream gas analysers)</td>
<td>Degree of error introduced by sample flow. What is an acceptable sample flow? Given its age-dependence, should it be considered as a % of Vt? What is the most appropriate method to correct flow and marker gas volume for sample flow?</td>
</tr>
<tr>
<td>Tissue N2</td>
<td>Effective correction for effect of tissue nitrogen diffusing into alveoli during washout. What is the error introduced into subseqent indices (FRC, LCI and SIII analysis)?</td>
</tr>
<tr>
<td>N2-based MBW</td>
<td>At what age does 100% O2 no longer have a detrimental effect on breathing pattern?</td>
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<tr>
<td>Use of sedation in infants</td>
<td>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]</td>
</tr>
<tr>
<td>Measures of global ventilation inhomogeneity</td>
<td>Can test duration be shortened whilst preserving acceptable sensitivity?</td>
</tr>
<tr>
<td>MBW SIII analysis</td>
<td>Validity of paediatric correction of SIII by Vt to account for differences in tidal Vt and breathing pattern</td>
</tr>
<tr>
<td>Considerations for FRC, CEV and TO</td>
<td>Most appropriate inert gas reference concentration for normalisation of SIII</td>
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<tr>
<td>Importance of FRC repeatability</td>
<td>Formal objective criteria for exclusion of outlying SIII values</td>
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<tr>
<td>SBW SIII analysis</td>
<td>Can accurate estimates be obtained from two tests?</td>
</tr>
<tr>
<td>Normative data</td>
<td>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</td>
</tr>
<tr>
<td>SBW SIII analysis</td>
<td>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 SIII indices is unclear</td>
</tr>
<tr>
<td>Normative data</td>
<td>Validity of paediatric correction of SIII by expiratory VC to account for differences in lung size</td>
</tr>
<tr>
<td>Commercial devices</td>
<td>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</td>
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</table>

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