

**Title:** An official ATS Technical Standard: Preschool Multiple Breath Washout Testing**Authors:**

1. Paul D. Robinson<sup>1,2,3</sup>
2. Philipp Latzin<sup>4</sup>
3. Kathryn A. Ramsey<sup>5,6,7</sup>
4. Sanja Stanojevic<sup>8,9,10</sup>
5. Paul Aurora<sup>11,12</sup>
6. Stephanie D. Davis<sup>13</sup>
7. Monika Gappa<sup>14</sup>
8. Graham L. Hall<sup>5,6,15</sup>
9. Alex Horsley<sup>16,17</sup>
10. Renee Jensen<sup>9</sup>
11. Sooky Lum<sup>12</sup>
12. Carlos Milla<sup>18</sup>
13. Kim G. Nielsen<sup>19</sup>
14. Jessica E. Pittman<sup>20</sup>
15. Margaret Rosenfeld<sup>21</sup>
16. Florian Singer<sup>22</sup>
17. Padmaja Subbarao<sup>8,9</sup>
18. Per M. Gustafsson<sup>23,24\*</sup>
19. Felix Ratjen<sup>8,9,25\*</sup>

\*joint senior authors

<sup>1</sup>The Children's Hospital at Westmead, Westmead, Australia

<sup>2</sup>Discipline of Paediatrics and Child Health, University of Sydney, Westmead, Australia

<sup>3</sup>Airway Physiology and Imaging Group, Woolcock Institute of Medical Research, Sydney, Australia

<sup>4</sup>Department of Pediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland

<sup>5</sup>Telethon Kids Institute, Subiaco, Australia

<sup>6</sup>Centre for Child Health Research, University of Western Australia, Subiaco, Australia

<sup>7</sup>Cystic Fibrosis Research and Treatment Centre, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

<sup>8</sup>Division of Respiratory Medicine, Department of Pediatrics, Hospital for Sick Children, Toronto, Canada

<sup>9</sup>Translational Medicine, Research Institute, Hospital for Sick Children, Toronto Canada

<sup>10</sup>Institute of Health Policy Management and Evaluation, University of Toronto, Toronto, Canada

<sup>11</sup>Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom

<sup>12</sup>Respiratory, Critical Care & Anaesthesia section, UCL, Great Ormond Street Institute of Child Health, London, United Kingdom

<sup>13</sup>Section of Pediatric Pulmonology, Allergy and Sleep Medicine; Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, Indiana, USA

<sup>14</sup>Marien Hospital Wesel GmbH, Childrens Hospital and Research Institute, Germany

<sup>15</sup>School of Physiotherapy and Exercise Science, Curtin University, Perth, Australia

<sup>16</sup>Division of Infection, Immunity & Respiratory Medicine, University of Manchester, Manchester, United Kingdom

<sup>17</sup>Manchester Adult CF Centre, University Hospital of South Manchester, Manchester, United Kingdom

<sup>18</sup>Center for Excellence in Pulmonary Biology, Stanford University, Palo Alto, California, USA

<sup>19</sup>Department of Pediatric and Adolescent Medicine, Pediatric Pulmonary Service, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

<sup>20</sup>Department of Pediatrics, Division of Allergy, Immunology, and Pulmonary Medicine, Washington University School of Medicine, St. Louis, Missouri, USA.

<sup>21</sup>Seattle Children's Hospital, University of Washington, Seattle, Washington, USA

<sup>22</sup>University Children's Hospital Zurich, Zurich 8032, Switzerland

<sup>23</sup>Department of Pediatrics, Central Hospital, Skövde, Sweden

<sup>24</sup>The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

<sup>25</sup>University of Toronto, Toronto, Canada

**Corresponding Author:**

Dr. Paul D. Robinson

Department of Respiratory Medicine

The Children's Hospital at Westmead, Locked Bag 4001,

Westmead, NSW, 2145 Australia

Email: paul.robinson1@health.nsw.gov.au

Manuscript count: 7827 words (limit 10000)

Word count of the overview section is not included in the overall word count (as specified by the document committee).

6 Tables and 5 Figures

## **Abstract**

**Background:** Obstructive airway disease is non-uniformly distributed throughout the bronchial tree, although the heterogeneity to which this occurs can vary among conditions. The multiple breath washout (MBW) test offers important insights into pediatric lung disease, not available through spirometry or resistance measurements. The ERS/ATS inert gas washout (IGW) consensus statement led to emergence of validated commercial equipment for the age group 6 years and above; specific recommendations for preschool children were beyond the scope of the document. Subsequently the focus has shifted to MBW applications within preschool subjects (aged 2-6 years) where a “window of opportunity” exists for early diagnosis of obstructive lung disease and intervention.

**Methods:** This preschool-specific technical standard document was developed by an international group of experts, with expertise in both custom-built and commercial MBW equipment. A comprehensive review of published evidence was performed.

**Results:** Recommendations were devised across areas which place specific age-related demands on MBW systems. Citing evidence where available in the literature, recommendations are made regarding procedures that should be used to achieve robust MBW results in the preschool age range. The present work also highlights the important unanswered questions that need to be addressed in future work.

**Conclusions:** Consensus recommendations are outlined to direct interested groups of manufacturers, researchers and clinicians in preschool device design, test performance and data analysis for the MBW technique.

## **Table of Contents**

1. Overview
2. Introduction
3. Methods
4. Background
5. Technical considerations for preschool MBW
6. Physiological and developmental considerations for preschool MBW
7. Reference data for preschool MBW
8. MBW use in preschool interventional research studies
9. Minimal clinically important difference
10. Future work and conclusions
11. Tables
12. Figures
13. References

## 1. Overview

The incorporation of preschool multiple breath washout (MBW) testing into research and clinical practice is growing, as is evident by the increasing number of publications in this area. Initial review articles on preschool MBW appeared in 2005 (1, 2), and were supplemented in 2007 by a formal MBW section within an official ATS/ERS statement on preschool lung function testing (3). The ATS/ERS document highlighted that “few MBW systems adapted for preschool age group (were) commercially available”, and that remains the case a decade later. The ERS/ATS IGW consensus document (hereafter termed ERS/ATS consensus statement), published in 2013, represented an important step forward for the overall technique, providing recommendations for manufacturers and researchers interested in MBW equipment, testing protocol and data analysis (4). Whilst all age groups were mentioned, specific preschool recommendations were limited to brief statements about testing interface and position. Increasing interest in preschool MBW is now being driven by potential utility of MBW outcomes such as the lung clearance index (LCI) in specific patient groups (e.g. cystic fibrosis, CF), where MBW is being used as an outcome measure for clinical trials. Publications in this area were three times greater in number from 2011-2015, compared to the preceding five years (5).

Although commercial MBW equipment exists, these devices have not been developed specifically for the unique requirements of the preschool age group. Several challenges have been encountered using these commercially available devices in preschool children that need to be considered by manufacturers, as well as researchers/clinicians. Higher respiratory rates, lower respiratory flows and lower lung volumes in preschool children place additional demands on equipment performance. This may account for the lower accuracy observed against smaller lung model volumes in some of the previous validation efforts in the literature (6, 7). Success in the infant age range illustrates that these can be overcome (8-10). In addition, initial attempts to replicate high historical preschool feasibility, achieved with custom built research based equipment, within the clinical setting using commercial equipment (or modified versions) have been unsuccessful (11, 12).

This technical standards document aims to build on and complement existing documents in the literature, as part of the process towards clarifying clinical utility of MBW within the preschool age range. It outlines important recommendations on device design for manufacturers and test performance for operators specific to preschool children. It recognizes that whilst there are still areas that require further data for formal standardization, a number of important recommendations can be made that should be implemented to standardize the technique across institutions as the technique moves towards widespread clinical use. Current recommendations are based on consensus, citing evidence where available in the literature, across an international group of experts (Table 1). For aspects where different acceptable options exist, discussion focuses on the advantages and disadvantages of each option. The expertise gathered spans several types of research and commercial MBW equipment. The majority of both research and commercial systems employed to date have been open circuit based systems, although the utility of closed, rebreathing setups are also being explored by some groups (13). Close collaboration between researchers and manufacturers has been a key

aspect in achieving progress to date for MBW, and will be essential for ongoing standardization work within this younger age range.

Important areas that need to be addressed in future work are outlined and summarized (Table 2), and will hopefully prove an incentive to gather the evidence necessary for further advances in standardization within this age group. This overall focus of this document is on MBW and reports mainly on LCI and the experience gained to date through studies in CF, as the most commonly utilized index and disease group, respectively. Other MBW indices have shown promise in CF but understanding of utility remains behind that of LCI. The role of these indices and the general utility of MBW in other respiratory conditions need to be explored in future work. Challenges of defining clinical utility are not discussed within this document; interested readers are directed to existing literature elsewhere (14, 15).

## 2. Introduction

In preschool children (2-6 years of age), conventional lung function tests, such as spirometry, remain technically challenging and relatively insensitive in identifying early airways disease in conditions such as cystic fibrosis (CF) (16-18). MBW for this age group has emerged as a feasible outcome measure for interventional studies and an area of interest for clinicians exploring its utility in clinical care. A recent CF foundation report, based on the discussions within a workshop hosted by the North American CF Foundation and Therapeutics Development Network, concluded that MBW was “a valuable potential outcome measure for CF clinical trials in preschool-aged patients” (15). This was echoed in concurrent recommendations from the European Cystic Fibrosis Society Clinical Trial Network (ECFS-CTN) Standardization Committee, which highlighted the “strong evidence base to support the use... in clinical trials in CF” (14). The vast majority of MBW studies in this age range have focused on CF, with studies of other respiratory conditions including but not focusing on preschool subjects (19, 20).

## 3. Methods

The working group was assembled to develop detailed technical standards for the performance of MBW in the preschool age range, which were lacking in the original ERS/ATS consensus document (4). As with the previous document this work was based largely on consensus, but sought to clearly describe evidence when available. Expert knowledge was supplemented by a comprehensive review of the literature (both of published abstracts and manuscripts contained within Embase and pubmed databases) performed with the keywords multiple breath washout, preschool children, preschoolers, lung clearance index (LCI), moment ratios, moment analysis, and functional residual capacity (FRC), as of December 31, 2016. Members of the working group were selected by the chair (Paul D Robinson) based on involvement with the previous ERS/ATS consensus work, published in 2013, and/or active research or interest in preschool MBW research. International

representation across the main current commercial devices was intentionally targeted. As with the previous ETS/ATS consensus statement, this provided wider applicability for current recommendations to be adopted in future MBW equipment and testing protocols. All potential conflicts of interest were disclosed and managed according to the policies and procedures for ATS projects. Individual sections were drafted by smaller working groups, merged together in the overall draft document by the chair, and all authors provided comment and suggestions for the final document. The methods checklist is presented (Table 3).

## 4. Background

Publications including pediatric MBW testing initially emerged in the 1960s (21, 22), whereas the inclusion of preschool children in studies did not occur until two decades later (23, 24). Initial data collected in the 1980's suggested poor feasibility in preschool children. Using custom built equipment Couriel *et al.* reported feasibility of 49% in 82 subjects aged 3.9-6.8 years (23). Couriel *et al.*, used an airtight snorkel mouthpiece and nose-clip interface and a 2-way breathing valve, and reported “great difficulty” with the technique. The authors stated “fear of the apparatus was the main cause of failure in the younger children” and “major difficulty.... maintaining a leak-free connection to the apparatus for the duration of the test.” Later the same year, Wall reported 80% feasibility in 40 subjects aged 3-6 years, based on *two* acceptable tests (24). Importantly, Wall's testing protocol incorporated distraction using a portable music system and headphones. Wall speculated that replacement of his mouthpiece and nose-clip interface system with a “snug fitting mask system” might allow feasible MBW measurements in even younger children. Incorporation of a facemask-based interface was not reported until 2003; Gustafsson *et al.* used a facemask interface and replaced music with video-based distraction, but did not report feasibility rates (25). Shortly after, Aurora *et al.*, in 2005, used this approach in preschool children and achieved feasibility of 79% across 77 subjects aged 2-6 years, based on stricter criteria of *three* acceptable tests (16).

## 5. Technical considerations for preschool MBW

The original ERS/ATS consensus statement included a comprehensive list of recommendations for both manufacturers and operators (4). In order to facilitate standardized measurement and practice, technical aspects with particular importance to the preschool age range are discussed in further detail within this section.

### 5.1 Validation of FRC measurement accuracy

Currently no available in-vitro models exist to validate accuracy of ventilation inhomogeneity (e.g. LCI) assessment; therefore validation efforts have mainly focused on FRC measurement accuracy, and recommendations in recent guidelines (4) have provided a framework for assessment. Success in older age groups, however, must not be extrapolated to the preschool age range. Accurate FRC measurement at these

smaller volumes present greater challenges (6, 8); error often increases at smaller volumes (26) and higher respiratory rates (7, 27). In vitro validation must therefore include representative FRC volumes of the preschool age range (typically 30-40 mL/kg in health or an FRC range of 0.4-1.0 liters), using respiratory rates and tidal volumes ( $V_T$ ) typical for the subjects and lung conditions encountered (typically 20-40 breaths/min in health and up to 60 breaths/minute in disease and  $V_T$ /FRC of 0.2-0.4) (28). Dry in-vitro models (10) and those incorporating BTPS conditions (6) offer a two stage approach to assessment. Importantly, in-vitro accuracy may not directly translate to in-vivo accuracy, as suggested by preliminary data from older age groups (29).

## Recommendations

1. In vitro validation for preschool MBW systems must include representative FRC volumes of the preschool age range, using a range of respiratory rates and tidal volumes appropriate for the subjects and lung conditions encountered.
2. FRC measurement accuracy must not be extrapolated from larger FRC volumes.

## 5.2 Flow measurement and breath detection

Breath definition may be based on detection of flow zero-crossings or by integration of flows to detect breath volumes reaching a pre-determined significant value. Additional challenges for the manufacturer in the preschool setting are greater breathing irregularity and the lower tidal flows encountered, compared to older children, with typical peak inspiratory and expiratory flows ranging from 200-400mL/s. To ensure accurate flow detection, and breath identification, in this setting, flow detection thresholds should be set slightly higher than the noise of the flow signal and then back extrapolated to the previous zero flow crossing.  $V_T$ , derived from the flow signal, must be accurate to within 3% or 5 mL whichever is greater (30). This accuracy should not be affected by the gas composition of the breath. For example, dynamic viscosity is 10% higher with 100% O<sub>2</sub> vs. room air, which results in an over-reading of flow with pneumotachographs (PNT) by 10% with 100% O<sub>2</sub> if the PNT is calibrated with room air. In contrast, ultrasonic flow meters measure flow using the Doppler effect; therefore, these devices measure linear velocity and are in principal not sensitive to changing dynamic viscosity, or density (molar mass), within the molar mass range encountered with current MBW inert gas choices.

Manufacturers should be aware that potential errors introduced by higher technical drift and increased sample flow (sampling rate from side stream gas analysis set ups) on measured tidal flows, integrated  $V_T$  and MBW outcomes may be relatively greater than in older subjects. However, a precise threshold for acceptability of technical drift remains unclear.

Greater variability in breathing patterns of preschool children exists, and this should be considered for accurate breath detection. Software must be able to handle pauses in breathing and fragmented breaths that are frequently encountered in this age range. This capability has been demonstrated in different custom-built research software by authors within this working group (16, 31, 32) and is therefore also feasible for



manufacturers. Approaches used must be fully transparent to the user. In breaths split or followed by a pause, if no inspiration has occurred during the pause then it should be viewed as the same breath and not as two separate breaths. Objective thresholds for defining the occurrence of a small inspiration (i.e. minimum breath size) need to be formally defined and the approach used by the manufacturer should be clearly described. Errors in definition of inspiration or expiration may introduce FRC estimation error, given the requirement for accurate measurement of expired inert gas volume during the washout portion of the test, corrected for any re-inspired inert gas. Accurate end-tidal inert gas concentration is also more challenging due to more variable breath size and inconsistent volume and flow profiles of preschool breaths. Small breaths may lead to erroneous early LCI threshold identification, due to falsely low end-tidal inert gas values, and the end-user must be able to examine data closely for this artifact and adjust accordingly.

The manufacturer must carefully assess and report the approach used to ensure its robustness. Assessment requires variability in breathing pattern, which may be best provided by representative *in vivo* data or mimicked using a breath simulator. The latter may also provide a means to validate  $V_T$  accuracy under ambient conditions. Researchers should consider supplying such data to manufacturers, as has occurred with other lung function techniques in the past (e.g. spirometry) (33).

Recommendations:

1.  $V_T$  accuracy must be within 3% or 5 mL, whichever is greater.
2. Flow detection accuracy must be robust and manufacturer assessment must be performed based on data mimicking breathing pattern variability encountered in this age group.
3. Breath detection software must handle the pauses in breathing and fragmented breaths frequently encountered in this age range. The approaches used must be fully transparent to the user.

### 5.3 Optimal synchronization of flow and inert gas concentration

As with MBW systems used in older children, optimal synchronization of flow and inert gas concentration signals for preschool children is essential (4, 34, 35). Furthermore, this precise synchronization needs to be maintained over the entire washout period. Changes in flow, during the breathing cycle, and in dynamic viscosity and gas density, over the course of the washout, can further complicate this process and are of increasing significance the younger the child.

If the point of inert gas measurement (mainstream) or sampling (sidestream) differs from the respiratory flow measurement point, the delay between flow and gas concentration will be affected by variation in flow within the breathing cycle as the flow front moves between those two measurement points (Figure 1). This flow-dependent effect is additional to the delay time that exists in the system due to analyzer response time +/- gas transit time (for sidestream sampling). This flow-dependent effect has now been demonstrated across different

commercial equipment, by separate research groups (7, 26), and leads to an increasing delay between signals as flow decreases. Therefore it is particularly relevant to preschool MBW with its lower tidal flow patterns.

Increasing gas dynamic viscosity and density may reduce sample flow within the sidestream sampling line (e.g. Nafion tubing). The magnitude of this effect, first described more than 30 years ago (36), will depend on the characteristics of the sample line, the sample flow rate and the magnitude of change in viscosity that occurs during the measurement period. This is particularly relevant to N<sub>2</sub> MBW where O<sub>2</sub> concentration varies between 21-100%, and viscosity change may result in an alteration of flow-inert gas delay times of over 10% across the washout portion of the test (37). Successful adjustment for this viscosity affect has been recently described within a commercial MBW system (10).

Based on fixed synchronization approaches, FRC accuracy has been demonstrated to remain within 5% (the specified accuracy threshold) if synchronization error is  $\leq 10$ ms between flow and inert gas concentration. This threshold appears to be consistent across different equipment systems (27, 35, 38) and formed the basis of this ERS/ATS consensus statement recommendation (4). Respiratory rate affects this relationship (37), and the range used must be preschool age specific when this source of error is assessed.

The flow-dependent and viscosity-dependent effects described above suggest that a dynamic approach to synchronization may further improve accuracy and must be considered by manufacturers designing preschool MBW system. Accuracy of the outcome measurement (e.g. FRC) should not be extrapolated to other outcomes, as error magnitude may differ (7). For example, LCI threshold is very dependent on accurate end-tidal inert gas concentration at the end of the washout where viscosity related effects may be greatest, yet relative exhaled inert gas volume contribution to FRC may be far less in this region of the washout. Recommendations to minimize flow-gas delay error to  $\leq 10$ ms error *across the full washout* should be adhered to strictly, and the operator must be able to evaluate the adequacy of synchronization across all data collected.

#### Recommendations:

1. Synchronization accuracy must be within 10ms across the duration of the entire washout.
2. The presence of flow-dependence, gas viscosity and density effects on synchronization of flow and inert gas concentration signals must be assessed within MBW systems evaluated for preschool testing. Manufacturers are encouraged to incorporate dynamic synchronization methods that correct for these factors, if present, to improve MBW system accuracy.
3. The operator must have the ability within the manufacturer's software to assess the accuracy of inert gas concentration and flow synchronization at the time of testing.

## 5.4 Technical considerations regarding inert gas choice

To date, there is no clear evidence to suggest that one particular inert gas is more suitable for the preschool age range from a technical perspective.

Of the three main inert gases used (Helium, SF<sub>6</sub>, and N<sub>2</sub>), most research to date has focused on either SF<sub>6</sub> or N<sub>2</sub>. Helium use has mainly been restricted to the setting of a secondary comparison gas in preschool SF<sub>6</sub> studies, using custom built respiratory mass spectrometer based equipment which facilitates dual gas comparison. Other fast responding helium analyzers are currently lacking.

Indirect inert gas concentration analysis approaches have been developed for both SF<sub>6</sub> and N<sub>2</sub> and are discussed elsewhere (4). One such method deserves further discussion in the setting of preschool MBW. The mainstream molar mass-based approach to SF<sub>6</sub> calculation requires correction of the molar mass signal for the effect of humidity and temperature fluctuation during the breathing cycle. Correction algorithms have been validated for use in young infants (39) but not for preschool children and should not be used in the preschool age range until appropriate validation has been performed. Recent infant-based validation of an improved sidestream based approach, which avoids the need for this correction, holds promise for use in preschool subjects but awaits future validation before firm recommendations can be made (10).

## 6. Physiological and developmental considerations for preschool MBW

### 6.1 Physiological considerations of inert gas choice

To date, there is no clear evidence to suggest that one particular inert gas is more suitable for the preschool age range, with respect to physiological effects and feasibility. Both SF<sub>6</sub> and N<sub>2</sub>-based MBW appear appropriate inert gas choices for preschool children.

#### 6.1.1 Effect of inert gas choice on breathing pattern during testing

Significant deviation from tidal breathing has been reported in infants during both 100% O<sub>2</sub> and 4% SF<sub>6</sub> procedures (40-43). The magnitude of effect is not trivial in infants, with 100% O<sub>2</sub> exposure causing up to a 33% reduction in V<sub>T</sub> (40, 43, 44) . Proposed explanations include the effect of absorption atelectasis on V<sub>T</sub> or a blunted peripheral chemoreceptor response to increased arterial oxygenation on respiratory rate and minute ventilation (V'E) (43, 45). An initial priming period of a lower O<sub>2</sub> concentration (e.g. 40%) has been shown to negate the effect in infants (40). This effect is not as pronounced with SF<sub>6</sub> exposure, at a concentration of 4%, with data describing significant effects on V'E only (41, 42). In a recent direct comparison of the two methods, this effect observed with SF<sub>6</sub> on V<sub>T</sub> was attributed to a technical artifact rather than a true physiological effect

(43). A described effect on respiratory rate in a sedated cohort (41) was not observed in two subsequent non-sedated cohorts (42, 43). The degree to which the effect of 100% O<sub>2</sub> on breathing pattern accounts for the difference observed between SF<sub>6</sub> and N<sub>2</sub>-based MBW indices (e.g. LCI and FRC) remains unclear, but SF<sub>6</sub>-based MBW is currently the preferred method in infants (43).

Whilst these effects of 100% O<sub>2</sub> do not appear to be present in early school age (46), the exact stage at which this effect disappears is unclear. Effects are likely to act in an age-, dose-, and disease-dependent manner (40, 45, 47), although the impact of the subject's status during testing (i.e. asleep/sedated in infants vs. alert and awake in preschoolers) is another important consideration. Initial preschool specific insight is encouraging, with a detectable effect, which is much smaller in magnitude than described for infants: a V<sub>T</sub> change of <10% and not present consistently across individuals (48). It is not felt to be physiologically relevant by this working group, but the exact effect on MBW outcomes remains unclear.

Preschool MBW equipment must afford the operator the ability to examine and detect these effects. A recommended approach would be to display V<sub>T</sub>, respiratory rate and concurrent end-tidal CO<sub>2</sub> (to detect hyper- or hypoventilation). Display of real time V'E should also be considered. Until further data is available, both SF<sub>6</sub> and N<sub>2</sub>-based MBW remain appropriate inert gas choices for preschool children.

#### Recommendations:

1. In preschool subjects, both SF<sub>6</sub> and N<sub>2</sub>-based MBW appear appropriate inert gas choices for preschool children.
2. Preschool MBW systems must provide the ability to monitor breathing pattern in real time during each test.

#### 6.1.2 Effect of inert gas diffusion across the alveolar-capillary membrane

There are no data currently available to quantify the impact of inert gas diffusing across the alveolar-capillary membrane on measured MBW outcomes in preschool subjects. Whilst N<sub>2</sub> is inert, in the sense that the human body does not metabolize it, it is a soluble gas, and due to the high partial pressure of the atmosphere, a large amount of N<sub>2</sub> is stored within the body. As a result N<sub>2</sub> will diffuse into the alveoli when the partial pressure of N<sub>2</sub> is lowered, as occurs during N<sub>2</sub>-based MBW using 100% O<sub>2</sub> (49, 50). This process of gas diffusion across the alveolar-capillary membrane applies to all gases to differing degrees, and occurs to lesser degrees, and in the opposite direction, with both SF<sub>6</sub> and Helium (51). The magnitude of diffusion and its effect on subsequent MBW outcomes has not been adequately described to date, beyond initial modeling attempts for N<sub>2</sub> MBW (52).

Several factors are likely to influence the magnitude of the impact of inert gas diffusing across the alveolar-capillary membrane: (i) tissue N<sub>2</sub> contribution to the alveolar N<sub>2</sub> fraction is likely to be non-linear in nature, due to the influence of varying concentration gradients through the washout portion of the test, gas exchange rates,

and the fact that time constants for both N<sub>2</sub> elimination and lung perfusion will vary across different lung compartments; (ii) the subject's age, given that lung architecture and cardiac output change with age (particularly relevant in preschool subjects where these change more rapidly than in older subjects); and finally (iii) the degree of ventilation inhomogeneity present and the washout time (both of which are typically lower in preschool subjects). Adult-based data to quantify tissue N<sub>2</sub> contribution to calculated FRC from the 1950s (49) should therefore not be extrapolated to preschool children. In fact, previous attempts to implement corrections based on this data in older school aged subjects have been reported to be problematic (21). Recent modeling work, based on a numeric two compartment lung model, suggested a small effect on measured adult FRC values (1.8%, within the 5% accuracy limit stipulated in the ERS/ATS consensus statement), but a larger relative effect on LCI (6.3%). The relative magnitude of error introduced worsened with increasing ventilation inhomogeneity and, of particular interest to preschool MBW, with decreasing FRC (although this analysis used FRC values for a 10 year old child and not preschool values per se) (52). However, preliminary in vivo studies suggest the effect of tissue N<sub>2</sub> increases with increasing lung volume, subject size and as lung function worsens (54). While these findings are physiologically important, it remains to be determined whether correction for tissue N<sub>2</sub> would alter the interpretation of MBW results.

#### Recommendations

1. Until the magnitude of error introduced and validated correction equations are available for inert gas diffusion across the alveolar-capillary barrier, correction of MBW data for this effect is not recommended.

#### 6.2 Equipment related dead space volume

To reduce equipment-related effects on breathing pattern and end-expiratory lung volume (EELV), manufacturers of commercial devices must consider equipment related dead space volume ( $V_D$ ) carefully when designing systems for widespread use in general respiratory clinics. Definitions of the  $V_D$  components of an MBW system (equipment, anatomic and physiologic) are described in detail in the ERS/ATS consensus statement (4). Studies in animals (55), infants (56), preschool children and adults (57, 58) have consistently demonstrated detrimental impacts of increased  $V_D$  on ventilation inhomogeneity outcomes. Recent adult data illustrating this increasing detrimental effect of  $V_D$  on LCI across the  $V_D$  range of 0-5mL/kg is shown in Figure 2. The fact that there was no clear threshold under which the effect on LCI was no longer seen suggests that  $V_D$  should be minimized wherever possible within an MBW system.

Increasing equipment  $V_D$  leads to increased  $V_D/V_T$ , decreasing effective ventilation, and may trigger a compensatory change in breathing pattern and/or EELV. This is particularly relevant to preschool age subjects as children with the smallest  $V_T$  relative to fixed equipment  $V_D$  will be most affected. Furthermore, changing relative  $V_D/kg$  as a subject grows may influence the interpretation of longitudinal data. No direct effect on breathing pattern was detectable in healthy preschool subjects ( $V_D$  range of 1.49–2.55 mL/kg), and the

threshold at which effects on breathing pattern occur remains unclear (58). Correction algorithms for LCI have been proposed (58), but further work is required before any firm recommendations can be made. Recommendations to correct FRC for  $V_D$  do exist and are routinely applied in practice (4). The impact of increased  $V_D$  on EELV and therefore FRC measurement is currently unclear.

Preschool children are often tested with a facemask, however the  $V_D$  of a facemask is difficult to measure. True effective  $V_D$  is affected by several factors: streaming of gases within the facemask itself (59), the amount of therapeutic putty used, and variation in the  $V_D$  displaced within the facemask during testing by face shape and operator pressure applied. As such, estimates of facemask  $V_D$  should not be incorporated into any corrections applied to the pre-gas sampling point  $V_D$  for CEV or FRC calculation. Instead, introduced facemask  $V_D$  must be minimized by selection of the smallest appropriate size, which maintains face seal and applying therapeutic putty (Figure 3).

Recommendations:

1. Manufacturers must minimize equipment related  $V_D$ . To ensure a consistent approach across age ranges, equipment related  $V_D$  should be kept below 2 mL/kg, as recommended in the recent ERS/ATS consensus guidelines.
2. Efforts to minimize  $V_D$  within an MBW system must not adversely affect overall resistance of the breathing circuit such that breathing pattern is altered.
3. MBW operators must minimize facemask-associated  $V_D$  by ensuring the smallest appropriately sized facemask is used, and by use of therapeutic putty within the facemask ensuring that no obstruction to airflow occurs.

### 6.3 Environment for testing

A detailed description of the desired skills and training of the preschool operator and environment for preschool lung function testing was provided by the ATS/ERS pulmonary function testing in the preschool children statement (3) but a number of important factors were stressed and are worth reinforcing: the importance of a “preschool-aged child friendly” environment; the need for the operator to engage, gain the trust of the child and encourage the child to participate in the test throughout the session without causing distress; adequate allocation of time and patience by operators trained in the techniques to help young children to perform at their best; ability to maintain equipment and understand the procedure well enough to know when a result is or is not acceptable; and additional safety precautions are necessary for preschool subjects, including, but not limited to, the need for constant adult supervision while the child is in the laboratory.

The operator has a crucial role to play to ensure the comfort level of the child. The level of distraction must be enough to take the child’s attention away from his or her breathing, and minimize procedure related anxiety to achieve relaxed stable tidal breathing. To expand further on this last aspect, the movie/show selected should

encourage a calm and relaxed atmosphere and avoid sudden emotions (e.g. excitement, singing, laughter and/or fear). Interactive programs that encourage talking or movement should be avoided. Respiratory function laboratories should have a number of suitable choices from which the child can select, although best choice may vary between child, country and culture. Preschool children should not have direct vision of the online measurement software to prevent “playing with signals” or manipulating visual feedback during the test. The preschool child must sit upright, hands by the side, not elevating their shoulders, and may require a stool to rest their feet. Positioning on a parents lap may help settle the younger preschool child but should not change this careful positioning of the child during testing. Familiarization visits for the child and parents to experience equipment, testing procedure and environment used during testing are strongly recommended, and should also include practice with the test interface used. Two operators should be present during preschool testing: the first operator focuses on the child, the integrity of the interface seal and maintaining adequate distraction by the movie/show to achieve a relaxed stable tidal breathing pattern. The second operator focuses on data collection and providing detailed ongoing feedback to the first operator on quality of data collected. Communication should occur in a discrete way and not disrupt the child’s distraction and relaxed breathing pattern. Adequate time should be allowed for testing: an hour is recommended, especially in MBW naïve and/or younger preschool subjects, although shorter time periods are feasible in experienced subjects. Detrimental effects of imposed limited time periods (e.g. 20 minutes for testing) have been described (12, 60).

#### Recommendations:

1. The environment for preschool MBW testing should be as child friendly as possible. The environment should be quiet, contain suitable preschool furniture and decoration, and accommodate adult supervision during testing.
2. Adequate time should be set aside for testing in this age group, particularly for those <4 years of age or who are attending for the first time. An hour is recommended for initial testing.
3. Familiarization visits are recommended for the child and parents to experience equipment, testing procedure, test interface and the environment used during testing
4. Distraction during the assessment must be enough to take the child’s attention away from his/her breathing, the operator and the immediate surroundings during each test. Appropriate movie/show choice is critical to the process.
5. Two operators should perform MBW testing in this age group, regardless of interface choice.

#### 6.4 Preschool test feasibility

Feasibility of MBW test in preschool children has been reported by several studies. In the study by Aurora et al., reporting a success rate of 79% across MBW naïve 2-6 year old children (16), feasibility was higher in healthy controls (84%) compared with CF subjects (75%) and a clear age-related effect was observed. Feasibility was lowest in 2-3 year old children (50%), >80% above 3 years, and highest in children 5-6 years of age (87%).

The results of recent experience with commercial equipment (11, 12, 58, 61-64) are summarized (Table 4). While it is encouraging to see comparable high rates of feasibility using commercial equipment, these cannot be directly compared as each study differed across several aspects including: (i) variation in the equipment set up between commercial and custom research equipment (ii) age ranges tested (iii) variation in the definition of acceptable data (i.e. two vs. three tests), (iv) the duration allowed for the testing session (i.e. testing duration and whether MBW is performed as the sole test or as part of a research protocol), and (v) the interface used (i.e. mouthpiece/nose-clip vs. facemask). Potential effects of these individual aspects on feasibility are discussed in detail later in the document. This variation in methodology highlights the importance of implementing the recommendations contained within this technical standards document to standardize the technique moving forward.

#### 6.4.1 Testing interface

The advantages and disadvantages of the two testing interface choices in the preschool age range (mouthpiece and nose-clip or facemask) are summarized (Table 5). The use of a mouthpiece and nose-clip require the child to maintain a tight seal around the mouthpiece to prevent leak, which may be difficult in this age group over the extended measurement periods required for MBW testing. The mouthpiece may distract the child, and trigger chewing, which may present additional challenges to the operator in maintaining a stable breathing pattern. If too large, the mouthpiece may also be uncomfortable. The use of a facemask provides some advantages, particularly since the operator is responsible for maintaining an adequate seal. However, facemasks add  $V_D$  to the apparatus, which can be mitigated by the use of therapeutic putty and may help facilitate a leak-free seal. Inserted putty should not obstruct the air stream during breathing, which may be detected by the presence of box shaped flow–volume loops. The route of breathing in a facemask is not fixed and nasal breathing is possible. Recently, adult data has demonstrated that nasal breathing may introduce variability to the test result (65, 66). Nasal and oral breathing have differing effects on relative humidity and temperature of expired gas, and may affect BTPS correction accuracy (67). In addition, the degree of nasal breathing encountered during testing is likely to vary within and between individuals in the preschool age range.

Initial work directly comparing MBW results performed with both interface options in the preschool age range suggests minimal differences overall (62); however, the relatively wide 95% limits of agreement observed suggest mask and mouthpiece should not be used interchangeably. The same study suggested greater feasibility and breathing pattern stability with a facemask interface with a more pronounced difference observed in younger preschool subjects (62). If a facemask is used initially, transition must occur as a subject ages, but the best approach and timing of transition needs to be defined. Ultimately, interface choice may depend on several factors, including age and disease group being tested (i.e. familiarity with interface through regular medical treatments, e.g. use of facemasks with spacers and nebulized medications in young children),



outcomes being assessed (e.g. greater breathing stability is required for concentration normalized phase III slope ( $S_{nIII}$ ) analysis), and ability to factor in practice sessions. Beneficial effects of the latter on both mouthpiece and facemask feasibility have been described (17, 64). Ideally the same interface should be used for the duration of a study. However, this may not be appropriate for longitudinal studies spanning several years as children may outgrow the facemask. Careful consideration of interface choice is necessary when planning and analyzing the data of longitudinal studies where subjects switch interface within the study.

#### Recommendations:

1. Both a mouthpiece and nose-clip assembly and a facemask are supported as interface choices for use in preschool aged children.
2. At the present time these interfaces must not be viewed as interchangeable within this age range, and careful consideration of interface choice is strongly recommended.

#### 6.4.2 Special considerations when reporting preschool MBW data

While technical MBW measurement acceptability criteria found in the ERS/ATS consensus statement are applicable to preschool testing, operators may generally expect a more variable breathing pattern in this age range. Hence, we highlight the following aspects that will require adaptation of previously published criteria applicable to the preschool age range, until the required evidence for formal preschool-specific acceptability criteria are available. Special considerations are (i) the minimal duration of breathing stability prior to starting the test should be shorter than is expected in school age and older subjects (30 seconds adapted to 3-5 breaths); (ii) the acceptable deviation in EELV at start of test should better reflect the inherent greater variability of EELV and  $V_T$  in this age group; and (iii) operators should recognize that sighs, swallows and pauses may be more frequently encountered compared with older children and tests should not be rejected unless these have a resultant action of triggering trapped gas release or leak. Representative figures of acceptable MBW tests typical of the preschool subject are shown (Figure 4).

Obtaining three technically acceptable washout tests may be more challenging in preschool children and may require multiple attempts, with co-operation lost before this target is reached. Recent work has focused on whether comparable information can be obtained from LCI values calculated from two technically acceptable tests (68-73). Firm recommendations cannot be made at this stage, as further comparative data are required for this approach evaluating the sensitivity to detect beneficial effects of interventions. MBW operators are strongly encouraged to obtain three tests, with the aim of achieving at least two technically acceptable tests to report outcomes. Reported LCI and FRC values “based on the average of two values” should be clearly stated. In the ATS 2007 preschool lung function statement, it was recommended that if two tests were used to derive LCI, these should have FRC values within 10% (where the highest value is compared with the lower FRC value). This approach for MBW-based FRC measurement accuracy had been recommended in earlier lung volume measurement consensus documents, not specific to the preschool age range (74, 75). Subsequent

work has shown that when formal 10% FRC reproducibility criteria were applied to preschool data from an experienced preschool MBW center it led to 60% of data being excluded. This suggests that normal FRC variability within the preschool age range is greater than this threshold, and importantly also did not significantly alter LCI estimates (69). The formal FRC 10% acceptability criterion is no longer advocated in this age group for LCI reporting, and requires physiological FRC variability to be better defined before specific preschool criteria for FRC measurement accuracy can be recommended.

The impact of reducing estimates to the mean of two values for other indices (e.g. moment ratios and  $S_{III}$  analysis) remains unclear. The increased variability (e.g. for the second moment ratio, in comparison to LCI) may be a factor affecting suitability for moment analysis (24).  $S_{III}$  analysis requires clear visualization of the expirogram phase III slope, sufficient to estimate its magnitude, for both the first breath and 2/3 of breaths between 1.5 and 6.0 lung turnovers (TO). Given this, greater tidal breathing stability during the test is needed (4). Tidal breath phase III slopes ( $S_{III}$ ) are often shorter in preschool children, compared with older children (76). Recent ERS/ATS consensus document recommendations that  $S_{III}$  should be at least 50% of the expired volume have significant detrimental effects on  $S_{III}$  analysis feasibility in preschool subjects, suggesting that the historical pediatric approach of being measured across 65-95% of the expired volume may be more appropriate (77). For these reasons, the current approach of collating data from three technically acceptable tests for formal calculation may provide a more robust estimate. Initial efforts to explore this have reported a statistically significant effect on measured  $S_{III}$  outcomes, based on two rather than three tests, in preschool but not older pediatric subjects (73, 78).

#### Recommendations:

1. The approach to individual test and overall test session acceptability in preschool children should be adapted to reflect differences in comparison to older subjects: preschool children require a shorter duration of pre-test breathing stability, may have greater variability in EELV and  $V_T$  during normal tidal breathing, and swallows, pauses and sighs may occur more frequently during the test.
2. Until definitive evidence is available for preschool children, MBW operators are strongly encouraged to perform three technically acceptable tests. Outcomes derived from only two acceptable tests must be clearly identified when results are reported in software.
3. The previously recommended FRC 10% acceptability criterion for LCI reporting is no longer advocated in this age group.

### 6.5 Recommendations for commercial software

Recommendations for commercial software development and use for both manufacturers and operators are summarized (Table 6). Quality control should extend beyond equipment performance to include accurate real-time biological feedback to the operator during testing. Real-time software recommendations are applicable to all age groups, but are of increasing importance to the operators in preschool subjects to allow efficient use of

test time, given the limited concentration and co-operation timespan in young children. As in older age groups, this must include real-time displays during both the pre-phase and washout phase of (i) inert gas concentration vs. time, (ii) flow volume loop (ideally with a display of the target  $V_T$  range for the child, typically 8-12mL/kg), (iii) end-tidal  $CO_2$  (if  $CO_2$  is measured) and respiratory rate to detect hypo/hyper-ventilation or rebreathing and (iv) volume and flow vs. time plots to assess breathing pattern stability. Additional features that will improve artifact detection include auto-scaling of the inert gas concentration vs. time plot during the washout period, ideally with additional ability to zoom in and out both during and after each test. The utility of incentive feedback has yet to be established in preschool MBW. Automated start and stop functions during testing may be attractive to the operator but are actively discouraged until formal validation of effectiveness is clearly described.

Recommendations:

1. Software must enable adequate visual quality control of the volume, flow and inert tracer gases for the entire duration of each test.
2. Software quality control cannot be automated until clear evidence based thresholds are available.
3. Incentive software is not recommended in this age range.

## 7. Reference data for preschool MBW

For many years, reference values for MBW indices such as LCI, were assumed to be independent of age. Recent collated data from infancy to adolescence demonstrate an inverse relationship between LCI and height, most pronounced in the first three years of life, with plateauing of the upper limit of normal (ULN) from six years of age (79) (Figure 5). The reasons for elevated LCI values in younger healthy children have not been fully explored, but are most likely multifactorial: ongoing lung and chest wall development and dysanaptic growth between airway (bronchial) and acinar (parenchymal) volume (80) such that the conducting airway space is larger in relation to lung volume which increases respiratory rate and dead space per minute ventilation; use of sedation and supine testing position in infancy; and relatively larger equipment  $V_D$  in younger subjects during testing. Given this, extrapolation of an ULN from older age groups is not recommended.

The available collated reference data (79) were based on data collected using a custom built respiratory mass spectrometer-based MBW system using  $SF_6$  as the inert tracer gas. The described relationship between LCI and height likely exists for all inert gases and systems in early life (81), but reference ranges should not be extrapolated to other MBW systems and across different inert gases (82, 83). Extrapolation could lead to misdiagnosis of abnormal ventilation distribution, and inappropriate tracking of disease progression over time, especially in preschool children (83).

Pooling currently available data in healthy controls (e.g. from studies listed in Table 4) is challenging due to many aspects of the methodological variation discussed in section 6.4. Future pooled reference population data used to derive the ‘normal range’ for individual commercial or research MBW systems must align methodology to address these issues, contain sufficient numbers from the population being tested (i.e. preschool), and report outcome measures collected using standardized protocols, equipment and settings. Training of operators and confirmation of competence to collect good quality data should be part of this process (84). Numbers recommended for spirometry (e.g. 300 subjects) reflect the characteristics of spirometric indices (85) and should not be extrapolated to MBW, where the minimum number has yet to be defined. Collaborative efforts to achieve robust reference data are strongly recommended.

Recommendations:

1. Available reference data from older subjects must not be extrapolated to the preschool age range.
2. Collaborative efforts to achieve robust preschool-specific reference data are strongly recommended.
3. Until robust device specific reference equations are published for commercial systems, research groups must ensure that studies collect an appropriately matched control group.

## **8. MBW use in preschool interventional research studies**

MBW holds exciting promise as an endpoint for early intervention strategies in CF (14, 15), and was recently used as the primary outcome in an international multicenter study in 6-11 year olds (84). To date only one small single-center CF clinical trial has used LCI as an endpoint in the preschool age range (86). This study was able to demonstrate strong feasibility and ability of MBW outcomes to detect a treatment effect despite a small sample size (N=25). Subsequent larger, multicenter trials of hypertonic saline (SHIP and SHIP-CT studies: clinical trial registration numbers NCT02378467 and NCT02950883, respectively, [www.clinicaltrials.gov](http://www.clinicaltrials.gov)) and a cystic fibrosis transmembrane conductance regulator (CFTR) modulator (Vertex: clinical trial registration number NCT02797132) are now underway. Experience to date, within the working group, has highlighted the value of careful and rigorous training, a subsequent certification process targeting the age being tested in the study, central over-reading and ongoing quality control. This approach has achieved high rates of successful testing and acceptable data both in the preschool age range (17) and school aged children (84). A sequential approach to training with initial experience built in older volunteers, prior to preschool specific training, has been beneficial. Standardization of equipment set up and testing protocol across sites within a single study is critical for all age groups, but becomes more important in preschool children due to the extra demands placed on the system and the operators for testing in this age range. Appropriate timelines for site training and certification must be integrated into studies. Testing should be performed prior to any procedures that require active cooperation as it is easier to “wind-up” than “wind-down” the child and it has been reported that deep inhalation may alter bronchial tone (87). Interpretation of results from these ongoing preschool MBW interventional studies will need to consider the unknown aspects of

standardization outlined in this document (e.g., physiologic considerations of inert gases, testing interface, reference data, and minimal clinically important differences).

## 9. Minimal clinically important difference

To date the minimal clinically important difference (MCID) in preschool subjects for LCI or any other MBW index as well as respective intra-subject and inter-subject variability remain poorly understood (14, 15, 88). In addition the relationship between improvement in LCI and other surrogate endpoints such as FEV<sub>1</sub> or rate of FEV<sub>1</sub> decline remains unclear. More information is urgently needed in this area, including comprehensive, longitudinal studies in health and disease to elucidate how MBW outcomes change over time within individuals in the preschool age range. Data outlining variability over time has recently started to emerge. Aurora *et al* described a mean (95% CI) within–subject change of 0.0 (-0.2 to 0.2) units in healthy subjects measured at two time points (preschool and early school age), which were on average 3.7 (range 1.3–6.6) years apart (89). Stanojevic *et al* observed the same stability in healthy preschool subjects measured over several time points across a 12 month period. LCI within a comparison preschool CF cohort increased by 0.4 units/year, in contrast to FEV<sub>1</sub> which did not change (17). Pooling of data from treatment studies within specific disease groups may offer opportunities to accelerate this process and the use of MBW in children with CF undergoing exacerbation treatment is an example of the benefits of this approach (90).

## 10. Future work and conclusions

Important areas for future work in this age group are summarized in Table 2. Replicating the strong feasibility of research-based equipment, whilst maintaining its sensitivity as an outcome measure in pediatric obstructive lung disease (16, 86), is the challenge faced by emerging commercial equipment. Many of the commercial systems available today were designed for older subjects, and need extensive testing, and in some cases modifications, before they are suitable for use in preschool children. As such, the flexibility to conduct future studies may be inhibited due to limitations within the commercial software. Custom research based software, developed for research-built MBW equipment over recent years, will also play a key supportive role in this progress. Commercial software developers must recognise that modifications may have a significant impact on MBW outcomes, and ensure that the full impact of software changes, for a range of patient demographics, are evaluated and transparently documented prior to formal commercial release. It remains unclear whether a specific inert gas choice is warranted in this age range. Until there is evidence of significant detrimental effects or lack of validation with one particular choice, no firm recommendations for a specific choice can be made and a number of choices will be supported. A choice of interface is also supported based on current evidence and experience. Efforts to optimise feasibility and breathing pattern stability in younger children with facemasks must be accompanied by development of effective strategies to define optimal training regimens and timing of transition to a mouthpiece and noseclip interface, so that detrimental effects on breathing stability and MBW outcomes are minimized at later ages. The latter in particular may be best accomplished using a co-ordinated

effort to perform comparison studies across multiple sites and equipment to ensure results obtained are generalizable.

Future work must establish clear objective criteria for an acceptable test and overall test session in this age group, to minimize subjective assessment. These efforts to standardize reporting have commenced (91), but require future work, especially in preschool children. The medium term aim must be to provide an accurate tool, which can be feasibly incorporated into routine clinical care. Regional regulatory approval (e.g. FDA approved) for devices fulfilling the criteria outlined in this document, with demonstrated strong feasibility for testing across the preschool age group, will be an essential step in that process.

Future technological advances may offer opportunity for optimizing MBW device design and must be encouraged. An example of one such area is advances in mainstream O<sub>2</sub> analysis which may one day negate the need for sidestream O<sub>2</sub> analysis and adjustments for associated sample flow rate (92). The development of mainstream analysis for not just one but all gases measured also offers opportunity to reduce analyzer response time further (e.g. to 10ms in this case). This is important given the increased susceptibility of preschool MBW to sources of technical error. Current recommendations (<2mL/kg) for equipment related V<sub>D</sub> target alignment with the recommendations of older age groups, but further minimization through targeted design and better appreciation of streaming within bacterial filters may facilitate meeting the <1mL/kg effective V<sub>D</sub> advocated for infant systems (93). Whether new facemasks that prevent nasal breathing would be beneficial to address issues raised in this document needs to be determined. Device design must also incorporate the needs of infection control, an increasingly important area in conditions such as CF, where MBW interest is currently greatest. Validation approaches should aim to extend to MBW outcomes beyond FRC alone, as FRC accuracy cannot be extrapolated to other outcomes (e.g. LCI). While many challenges remain, MBW testing in preschool children already provides an exciting approach to detect and monitor early lung disease. Implementation of the recommendations contained within this technical standards document are essential for standardization and validation in this age range and will increase the utility of the test in the future.

## 11. Tables

**Table 1. Summary of key current recommendations for manufacturers and MBW operators**

<i>Manufacturer directed</i>	<i>Operator directed</i>
<p><i>Compliance with preschool recommendations: Commercial MBW Systems</i></p> <ul style="list-style-type: none"> <li>Manufacturers must provide sufficient information and complete transparency to the end user regarding their ability to comply with the preschool recommendations contained within this document.</li> </ul>	<ul style="list-style-type: none"> <li>The end user is encouraged to demand this as part of the marketing material accompanying any device.</li> </ul>
<p><i>Validation of FRC measurement accuracy</i></p> <ul style="list-style-type: none"> <li>In vitro validation for preschool MBW systems must include representative FRC volumes of the preschool age range, using respiratory rates and <math>V_T</math> typical for the subjects and lung conditions encountered.</li> <li>FRC measurement accuracy must not be extrapolated from larger FRC volumes.</li> </ul>	
<p><i>Flow measurement and breath detection</i></p> <ul style="list-style-type: none"> <li><math>V_T</math> accuracy must be within 3% or 5mL, whichever is greater.</li> <li>Flow detection accuracy must be robust and manufacturer assessment based on data mimicking the variability in breathing pattern encountered in this age group.</li> <li>Breath detection software must handle the pauses in breathing and fragmented breaths frequently encountered in this age range. The approaches used must be fully transparent to the user.</li> </ul>	
<p><i>Optimal synchronization of flow and inert gas concentration</i></p> <ul style="list-style-type: none"> <li>Synchronization error must be within 10ms across the duration of the entire washout.</li> <li>The presence of flow-dependence, gas viscosity and density effects on synchronization of flow and inert gas concentration signals must be assessed within MBW systems evaluated for preschool testing. Manufacturers are encouraged to incorporate dynamic synchronization methods that correct for these factors, if present, to improve MBW system accuracy.</li> </ul>	<ul style="list-style-type: none"> <li>The operator must have the ability to assess the accuracy of inert gas concentration and flow synchronization at the time of testing.</li> </ul>
<p><i>Inert Gas Choice</i></p> <ul style="list-style-type: none"> <li>To date, there is no clear evidence to suggest which inert gas is most suitable for the preschool age range, with respect to technical, physiological effects and feasibility. Both SF<sub>6</sub> and N<sub>2</sub>-based MBW appear appropriate inert gas choices for preschool children.</li> <li>Preschool MBW systems must provide the ability to monitor breathing pattern in real time during each test.</li> <li>Until the magnitude of error introduced and validated correction equations are available for inert gas diffusion across the alveolar-capillary barrier,</li> </ul>	

<p>correction of MBW data for this effect is not recommended.</p>	
<p><i>Equipment-related Dead Space Volume (<math>V_D</math>)</i></p> <ul style="list-style-type: none"> <li>Manufacturers must minimize equipment related <math>V_D</math>. To ensure a consistent approach across age ranges, equipment related <math>V_D</math> must be kept below 2 mL/kg, as recommended in the recent ERS/ATS consensus guidelines.</li> <li>Efforts to minimize <math>V_D</math> within an MBW system must not adversely affect overall resistance of the breathing circuit such that breathing pattern is altered.</li> </ul>	<ul style="list-style-type: none"> <li>MBW operators must minimize facemask-associated <math>V_D</math> by ensuring the smallest appropriately sized facemask is used, and by use of therapeutic putty within the facemask ensuring no obstruction to airflow occurs.</li> </ul>
<p><i>Environment for testing</i></p>	<ul style="list-style-type: none"> <li>The environment for preschool MBW testing should be as child friendly and safe as possible, including ensuring it is quiet, contains suitable preschool furniture and decoration, and accommodates adult supervision during testing.</li> <li>Adequate time should be set aside for testing in this age group, particularly for those &lt;4 years of age or who are attending for the first time. An hour is recommended for initial testing.</li> <li>Familiarization visits for the child and parents to experience equipment, testing procedure, test interface and environment used during testing are recommended.</li> <li>Adequate distraction during the assessment is essential and must be enough to take the child's attention away from his/her breathing, the operator and the immediate surroundings during each test. Appropriate choice of movie is critical to the process.</li> <li>Two operators should be used for MBW testing in this age group, regardless of interface choice.</li> </ul>
<p><i>Testing Interface</i></p> <ul style="list-style-type: none"> <li>Both a mouthpiece and nose-clip assembly and a facemask are supported as interface choices for use in preschool aged children.</li> </ul>	<ul style="list-style-type: none"> <li>At the present time these interfaces must not be viewed as interchangeable within this age range, and careful consideration of interface choice is strongly recommended.</li> </ul>
<p><i>Special considerations when reporting preschool MBW data</i></p> <ul style="list-style-type: none"> <li>Software must clearly state where reported indices values are "based on the average of two values alone".</li> <li>The previously recommended FRC 10% acceptability criterion for LCI reporting is no longer advocated in this age group.</li> </ul>	<ul style="list-style-type: none"> <li>The approach to individual and overall test session acceptability in preschool children should be adapted to reflect differences in comparison to older subjects: preschool children require a shorter duration of pre-test breathing stability, may have greater variability in EELV and <math>V_T</math> during normal tidal breathing, and swallows, pauses and sighs may occur more frequently during the test.</li> <li>Until definitive evidence is available for preschool children, MBW operators are</li> </ul>



	strongly encouraged to perform three technically acceptable tests. Outcomes derived from only two acceptable tests must be clearly identified as when results are reported in software.
<p><i>Recommendations for online software requirements for manufacturers</i></p> <ul style="list-style-type: none"> <li>• Software must enable adequate visual quality control of the volume, flow and inert tracer gases for the entire duration of the each test.</li> <li>• Software quality control cannot be automated until clear evidence based thresholds are available.</li> <li>• Incentive software is not recommended in this age range.</li> </ul>	
<p><i>Reference data for MBW in preschool age range</i></p> <ul style="list-style-type: none"> <li>• Available reference data from older subjects must not be extrapolated to the preschool age range.</li> <li>• Collaborative efforts to achieve robust preschool-specific reference data are strongly recommended.</li> </ul>	<ul style="list-style-type: none"> <li>• Until robust device specific reference equations are published for commercial systems, research groups must ensure that studies collect appropriately matched healthy control data.</li> </ul>

Footnote: CO<sub>2</sub>, carbon dioxide; EELV, end expiratory lung volume; FRC, functional residual capacity; MBW, multiple breath washout; N<sub>2</sub>, nitrogen; SF<sub>6</sub>, sulfur hexafluoride; V<sub>D</sub>, dead space volume; V'E, minute ventilation; V<sub>T</sub>, tidal volume.

**Table 2. Important areas of interest for future work specific to preschool MBW**

Area of interest	Questions and/or needs
Shortening the duration of testing for preschool subjects	Analysis of MBW outcomes during the washin portion of each test (e.g. FRC and LCI), from two tests alone (vs. three tests), or abbreviated outcomes (e.g. LCI at 1/20 <sup>th</sup> threshold) offers potential for shortening overall test session duration. This is of particular interest in preschool subjects given the more limited timeframe for cooperation compared to older subjects.
Effects of pure O <sub>2</sub> exposure on breathing pattern and EELV in preschool subjects	Further work is required to clarify the magnitude of the effect of inert gas choice on breathing pattern during testing, and whether changes affect MBW indices. The age threshold at which the detrimental effects of pure O <sub>2</sub> exposure reported in infants disappears needs to be clarified as well as the magnitude of effect on MBW outcomes.
Inert gas diffusion across the alveolar-capillary barrier	Further work is required to clarify the magnitude of inert gas diffusion across the alveolar-capillary membrane for the common inert gases used and the potential impact on MBW indices. Whether relative contribution of this inert gas diffusion effect is greater in preschool subjects compared with older age groups. Whether inert gas specific corrections can be developed and applied and if these need to be age specific.
Artifact definition and exclusion	Definition of normal preschool breathing pattern and the level at which artifact occurrence should lead to test rejection.
Accuracy of flow and volume measurement	Due to the lower flows and faster respiratory rates encountered, the relative errors introduced by sample flow, technical drifts and BTPS correction may be greater in younger subjects. Given the challenges of FRC validation for preschool specific equipment, is an alternate approach to BTPS correction warranted? The current fixed BTPS correction approach may introduce a greater relative error in preschool subjects due to the smaller flows and faster respiratory rates encountered, as well as the variable contributions of nasal and oral breathing to relative humidity and temperature of expired gas (67). Should a dynamic approach be considered? There is a lack of information about how BTPS conditions change during preschool MBW.
Breath detection	Optimal approach to breath detection in an age range where breath pauses, low flows and volumes are more frequently encountered, as well as the magnitude of effect on MBW outcomes if not addressed. Should the operator have the ability to correct breath detection errors when they occur? How should minimum breath volume be defined and how does the threshold chosen affect accuracy of subsequently calculated MBW indices?
Definition of normal physiological variability in the preschool age range	Better definition of normal V <sub>T</sub> and EELV variability in this age range to determine accurate thresholds for breathing pattern stability. Optimal definition of suitable target tidal volume range - defined based on actual weight, ideal body weight, height or BMI? Definition of normal FRC variability, in comparison to older age groups, so that preschool specific recommendations for FRC measurement accuracy can be made.
Interface transition	Strategies to reduce the magnitude of effect on MBW indices when changing interfaces between mouthpiece and nose-clip assembly and facemask. Definition of best approach and timing of transition.
Equipment related V <sub>D</sub>	Can functional V <sub>D</sub> within a bacterial filter or facemask assembly be accurately estimated and corrected for across subjects? What is the contribution of increasing relative V <sub>D</sub> to the change in LCI reference values observed across the preschool age range (79). What is the optimal method for expressing V <sub>D</sub> : as a function of weight (mL/kg) or is BMI, percentage of V <sub>T</sub> or is height more appropriate?

	Do other indices, which are less sensitive to the effects of $V_D$ (e.g. slope index, moment ratios and alveolar LCI), offer improved utility to LCI?
Preschool specific reference data	What is the true effect of lung development across the preschool age range once the impact of other factors such as changing relative equipment dead space volume have been removed?
Minimal clinically important difference	What is a significant difference in an individual with a particular lung disease? What difference signals a clinically important deterioration or risk for relapse or exacerbation? What is a significant difference in a clinical trial (i.e. on a group level)?

Footnote: BMI, body mass index; BTPS: body temperature, ambient pressure, saturated with water; EELV, end expiratory lung volume; FRC, functional residual capacity; LCI, lung clearance index; MBW, multiple breath washout;  $O_2$ , oxygen;  $V_D$ , dead space volume;  $V_T$ , tidal volume

**Table 3. Methods checklist**

	<b>Yes</b>	<b>No</b>
Panel assembly		
Included experts from relevant disciplines with experience across the main current commercial equipment exploring utility in the preschool age range	X	
Included individual who represents the views of patients and society at large		X
Included methodologist with appropriate expertise		NA*
Literature review		
Performed in collaboration with a librarian	X	
Searched multiple electronic databases	X	
Reviewed reference lists of retrieved studies	X	
Evidence synthesis		
Applied pre-specified inclusion and exclusion criteria	X	
Evaluated included studies for sources of bias	X	
Explicitly summarized benefits and harms	X	
Used PRISMA1 to report systematic review		X
Used GRADE to describe quality of evidence		X
Generation of recommendations		
Used GRADE to rate the strength of recommendations		NA

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses;

GRADE: Grading of Recommendations Assessment, Development and Evaluation; NA: not applicable.

\*Required for guidelines but not a technical standards document.

**Table 4. Published preschool MBW feasibility to date using commercial MBW systems**

Author	Subjects	Age tested, (range, years)	MBW naive	Interface	N acceptable tests required	N subjects attempted	% Subjects successful
Jensen 2014#	CF	2.9 – 5.0	0%	Facemask	≥ 2 tests	30	83
Benseler 2015#	Healthy	2.8 – 5.9		Facemask	3 tests (successful on both equipment systems)	24	83
	CF	3.3 – 5.9				27	70
Robinson 2015#	CF, Wheeze	2 – 6 years	83%	Facemask	3 tests	48	81
					2 tests		88
				Mouthpiece	3 tests	48	50
					2 tests		63
Foong 2015	Healthy	3.0 – 6.6	100%	Mouthpiece	≥ 2 tests (first visit)	60	72
			0%		≥ 2 tests (subsequent visit)	19	86
	CF		100%	Mouthpiece	≥ 2 tests (first visit)	78	67
			0%		≥ 2 tests (subsequent visit)	59	82
Vilmann 2015#	Healthy, Asthmatics	3 – 6	100%	Mouthpiece	≥ 2 tests	66	89
					3 tests	66	67
Downing 2016#	Healthy, PCD, CF, Wheeze	2.1 – 5.9	100%	Mouthpiece	≥ 2 tests within 30 minutes	116	73
Yamine 2016¶	Asthmatic	3.1 – 6.7	100%	Mouthpiece	3 tests within 20 minutes	62	24
					2 tests within 20 minutes	62	60
Stanojevic 2017#	Healthy, CF	2.5 – 5.9	100%	Facemask	≥ 2 tests (first visit)	150	66
					≥ 2 tests (subsequent visits)	150	89

# Modified from off the shelf equipment in an attempt to improve suitability for preschool testing. ¶ Additional time restriction criteria of 20 minutes specified for total test session duration.

**Table 5. Advantages and disadvantages associated with MBW test interface choice**

	<b>Mouthpiece and Nose-clip</b>	<b>Facemask</b>
Factors affecting ability to distract during testing	Mouthpiece stimulation within the oral cavity (e.g. chewing) Need for reminders to maintain mouthpiece seal	Avoids oral stimulation. Pressure to face may distract if too great
Equipment dead space volume	Defined	Difficult to define
Seal (Mouthpiece/Mask) and risk of leak	Subject determined	Operator determined
Impact of nasal airways	Removed	Unknown

**Table 6. Recommendations for commercial software development and use for manufacturers and operators.**

<i>Manufacturer directed</i>	<i>Operator directed</i>
<ul style="list-style-type: none"> <li>• Real time biological feedback data must be displayed during both pre-phase and washout phases of each test.</li> <li>• Additional operator ability to zoom in and out both during and after each test to detect subtle artifact.</li> </ul>	<ul style="list-style-type: none"> <li>• Close inspection of display for artifact during each test must be performed by a dedicated operator. Further review after each test is completed. Rejection of tests containing artifact.</li> </ul>
<ul style="list-style-type: none"> <li>• Real time inert gas concentration plot against time must be displayed.</li> <li>• Auto-scaling of display during the washout to facilitate artifact detection.</li> </ul>	
<ul style="list-style-type: none"> <li>• Real time flow volume loop must be displayed for each breath (i.e. Flow vs. Volume plot for each breath), referenced to a specified number of previous breaths (e.g. at least 5 breaths).</li> <li>• Display of target <math>V_T</math> range appropriate for the subject.</li> <li>• Real time display of expirogram for each breath during the washout portion of each test.</li> </ul>	<ul style="list-style-type: none"> <li>• Close inspection of whether the <math>V_T</math> size is appropriate for the subject (typically defined as 8-12 mL/kg) must be performed. A clearly visible phase III slope of the expirogram is supportive of this.</li> <li>• Flow vs. volume loop display useful in preschool testing to detect obstruction of the facemask outflow tract with any therapeutic putty used to reduce equipment <math>V_D</math>.</li> </ul>
<ul style="list-style-type: none"> <li>• End-tidal <math>CO_2</math> must be displayed to assess for hyper/hypo ventilation.</li> <li>• Calculation and display of respiratory rate during each test .</li> </ul>	<ul style="list-style-type: none"> <li>• End-tidal <math>CO_2</math> should remain within the normal range (typically defined as 4-6%) through both the pre-phase (or washin) and washout portions of each test .</li> </ul>
<ul style="list-style-type: none"> <li>• Single real time <math>V_T</math> (both inspiratory and expiratory) vs. time must be displayed to monitor breathing pattern and stability of end expiratory lung volume.</li> </ul>	<ul style="list-style-type: none"> <li>• Evidence of breathing pattern and EELV stability must be present prior to starting each test (defined as present for 3-5 breaths).</li> </ul>
<ul style="list-style-type: none"> <li>• Manual start and stop options of the washout portion of each test must be provided.</li> </ul>	<ul style="list-style-type: none"> <li>• Until automated start and stop functions of testing have been validated, manual option to start and stop each test must be used.</li> </ul>



## 12. Figures

### Figure 1. Simulation of Flow-dependent delay between flow and gas concentration signals

Foot note: Visualization of the time delay between two sensors depending on the volume between the two sensors and the flow rate of the measured gas. The simulations were performed assuming both sensors are placed inside a tube with 2ml (red line) respectively 5ml (blue line) of volume separating them. Response times of the sensors were assumed to be similar. The time delay plotted is the time difference by which a gas, flowing at a constant rate, reaches each sensor. Mathematically the flow dependence of the time delay can be calculated by the following relationship:  $\text{time delay} = (\text{volume between sensors}) / (\text{flow rate})$ . Delays are symmetric, but depending on the direction of the flow, one sensor will measure the gas first and the other one second or the other way round, respectively. Acknowledgment: Mr Jeremy Wolfensberger, Division of Respiratory Medicine, Department of Pediatrics, University of Bern, Bern, Switzerland.

### Figure 2. Effect of increasing equipment related dead space volume on ventilation inhomogeneity

Footnote: Data displayed from 10 healthy adult subjects where LCI was calculated as mean of triplicate tests across five  $V_D$  values (standard, +50mL, +100mL, +150mL and +200mL). The change in LCI relative to baseline (i.e. standard  $V_D$ ) is expressed as a percentage (y-axis) and  $V_D$  is expressed in terms of body weight (x-axis). The magnitude of effect observed on LCI suggests a 10% increase in LCI for each 1mL/kg increase in equipment  $V_D$ . Based on data contained within Benseler et al (58).

### Figure 3. Therapeutic putty use in facemask interfaces to reduce equipment related dead space volume

Footnote: Additional equipment related dead space volume ( $V_D$ ) introduced by a facemask assembly should be reduced as much as possible. The smallest appropriately sized facemask should be selected. Therapeutic putty application will be influenced by the presence of a flange to aid a leak free seal when applied to the face during testing (A). In this case, putty is solely applied to reduce  $V_D$  within the mask. If no flange is present then putty also helps create the seal (B). A combination of different therapeutic putty consistencies may be required to ensure putty maintains its shape and prevent migration and outflow tract obstruction during testing.

#### Figure 4. Typical breathing pattern observed in preschool subjects.

Footnote: Sequential tests (A, B, C) from the same test session, recorded using commercial N<sub>2</sub> based MBW equipment, in a preschool subject. In the upper part of each panel, real time plots of tidal flow (black) and volume (red) are displayed, whilst the lower part of the panel displays N<sub>2</sub> concentration. These technically acceptable tests are representative of the variable breathing pattern encountered in preschool subjects and also contain examples of swallows (solid downward arrow) and sighs (solid upwards arrow, no evidence of resultant trapped gas release).

#### Figure 5. Changes in MBW indices across the pediatric age range

Footnote: Data taken from a cohort across infancy to 19 years of age across a cohort of 497 subjects tested on 659 occasions using custom built research MBW equipment and SF<sub>6</sub> as the inert tracer gas of interest. Reproduced from Lum *et al.* (79) with the permission of the publisher. In figure A, the solid line denotes the predicted (50th centile) LCI for height and the dashed lines denote the upper limit of normal (ULN; 97.5th centile) and lower limit of normal (LLN; 2.5th centile). The typical height range of a preschool child is 75 to 125cm. This data must be viewed as inert gas and equipment specific.

Acknowledgement Wording: This material has not been reviewed by European Respiratory Society prior to release; therefore the European Respiratory Society may not be responsible for any errors, omissions or inaccuracies, or for any consequences arising there from, in the content. Reproduced with permission of the European Respiratory Society ©: European Respiratory Journal Jun 2013, 41 (6) 1371-1377; **DOI:** 10.1183/09031936.00005512

## 13. References

1. Aurora P. Multiple-breath washout in preschool children--FRC and ventilation inhomogeneity. *Paediatr Respir Rev* 2006; 7 Suppl 1: S14-16.
2. Gustafsson PM. Inert gas washout in preschool children. *Paediatr Respir Rev* 2005; 6: 239-245.
3. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E, Pillow JJ, Seddon PC, Silverman M, Sly PD, Stocks J, Tepper RS, Vilozni D, Wilson NM. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med* 2007; 175: 1304-1345.
4. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs S, King G, Lum S, K.A. M, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson B, Gustafsson PM. ERS/ATS Consensus statement for inert gas washout measurement using multiple and single breath tests. *Eur Respir J* 2013; 41: 507-522.
5. [cited 2016 12/31/2016]. Available from: [www.pubmed.com](http://www.pubmed.com).
6. Singer F, Houlitz B, Latzin P, Robinson P, Gustafsson P. A realistic validation study of a new nitrogen multiple-breath washout system. *PLoS One* 2012; 7: e36083.
7. Gonem S, Singer F, Corkill S, Singapuri A, Siddiqui S, Gustafsson P. Validation of a photoacoustic gas analyser for the measurement of functional residual capacity using multiple-breath inert gas washout. *Respiration* 2014; 87: 462-468.
8. Schmidt A, Yammine S, Proietti E, Frey U, Latzin P, Riedel T, Singer F. Validation of multiple-breath washout equipment for infants and young children. *Pediatr Pulmonol* 2015; 50: 607-614.
9. Shawcross A, Murray CS, Goddard N, Gupta R, Watson S, Horsley A. Accurate lung volume measurements in vitro using a novel inert gas washout method suitable for infants. *Pediatr Pulmonol* 2016; 51: 491-497.
10. Gustafsson PM, Robinson PD, Lindblad A, Oberli D. Novel methodology to perform sulfur hexafluoride (SF6)-based multiple-breath wash-in and washout in infants using current commercially available equipment. *J Appl Physiol* 2016; 121: 1087-1097.
11. Downing B, Irving S, Bingham Y, Fleming L, Bush A, Saglani S. Feasibility of lung clearance index in a clinical setting in pre-school children. *Eur Respir J* 2016; 48: 1074-1080.
12. Yammine S, Summermatter S, Singer F, Lauener R, Latzin P. Feasibility of nitrogen multiple-breath washout in inexperienced children younger than 7 years. *Pediatr Pulmonol* 2016; 51: 1183-1190.
13. Horsley AR, O'Neill K, Downey DG, Elborn JS, Bell NJ, Smith J, Owers-Bradley J. Closed circuit rebreathing to achieve inert gas wash-in for multiple breath wash-out. *ERJ Open Research* 2015; 2016: 00042-02015.
14. Kent L, Reix P, Innes JA, Zielen S, Le Bourgeois M, Braggion C, Lever S, Arets HG, Brownlee K, Bradley JM, Bayfield K, O'Neill K, Savi D, Bilton D, Lindblad A, Davies JC, Sermet I, De Boeck K, European Cystic Fibrosis Society Clinical Trial Network Standardisation C. Lung clearance index: evidence for use in clinical trials in cystic fibrosis. *J Cyst Fibros* 2014; 13: 123-138.
15. Subbarao P, Milla C, Aurora P, Davies JC, Davis SD, Hall GL, Heltshe S, Latzin P, Lindblad A, Pittman JE, Robinson PD, Rosenfeld M, Singer F, Starnes TD, Ratjen F, Morgan W. Multiple-Breath Washout as a Lung Function Test in Cystic Fibrosis. A Cystic Fibrosis Foundation Workshop Report. *Ann Am Thorac Soc* 2015; 12: 932-939.
16. Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, Stroobant J, Carr S, Stocks J. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. *Am J Respir Crit Care Med* 2005; 171: 249-256.
17. Stanojevic S, Davis SD, Retsch-Bogart G, Webster H, Davis M, Johnson RC, Jensen R, Pizarro ME, Kane M, Clem CC, Schornick L, Subbarao P, Ratjen FA. Progression of Lung Disease in Preschool Patients with Cystic Fibrosis. *Am J Respir Crit Care Med* 2017; 195: 1216-1225.
18. Hardaker K, Hulme K, Gustafsson P, Cooper P, Fitzgerald D, Selvadurai H, Robinson P. Higher lung clearance index (LCI) reflects other measures of clinical respiratory status in cystic fibrosis (CF) preschool children. *Respirology* 2016; 21 Suppl: 41.

19. Macleod KA, Horsley AR, Bell NJ, Greening AP, Innes JA, Cunningham S. Ventilation heterogeneity in children with well controlled asthma with normal spirometry indicates residual airways disease. *Thorax* 2009; 64: 33-37.
20. Zwitserloot AM, Fuchs SI, Gappa M. Lung clearance index in pediatric patients with asthma. *Eur Respir J* 2013; 42: P4314.
21. Kjellman B. Ventilatory efficiency, capacity and lung volumes in healthy children. *Scand J Clin Lab Invest* 1969; 23: 19-29.
22. Herdegen L, Janousková A, Böswart J. Distribution of gas ventilating the alveoli in asthmatic children. *Cesk Pediatr* 1965; 20: 213.
23. Couriel JM, Schier M, Hutchison AA, Phelan PD, Landau LI. Distribution of ventilation in young children with cystic fibrosis. *Pediatr Pulmonol* 1985; 1: 314-318.
24. Wall MA. Moment analysis of multibreath nitrogen washout in young children. *J Appl Physiol* 1985; 59: 274-279.
25. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. *Eur Respir J* 2003; 22: 972-979.
26. Thamrin C, Latzin P, Sauteur L, Riedel T, Hall GL, Frey U. Dead-space estimation from CO<sub>2</sub> versus molar mass measurements in infants. *Pediatr Pulmonol* 2007; 42: 920-927.
27. Buess C, Fuchs SI, Gappa M. Influence of tracer gas delay-time on FRC and Lung clearance index from Multiple-breath nitrogen washout. *Am J Respir Crit Care Med* 2012; 185: A1691.
28. Mathers LH, Frankel LR. Pediatric emergencies and resuscitation. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. *Nelson Textbook of Pediatrics*. Philadelphia: Saunders; 2007. p. 389.
29. Raaijmakers L, Jensen R, Stanojevic S, Merkus P, Ratjen F. In vitro and in vivo comparison of two Multiple Breath Nitrogen Washout devices. *Eur Respir J* 2015; 46: PA1262.
30. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319-338.
31. Horsley A, Smith L, Samaraj P, Bell NJ. Rapid and reliable analysis of multiple breath washout using custom built offline software. *J Cystic Fibrosis* 2017; 16: S32.
32. Yammine S, Lenherr N, Nyilas S, Singer F, Latzin P. Using the same cut-off for sulfur hexafluoride and nitrogen multiple-breath washout may not be appropriate. *Journal of applied physiology* 2015; 119: 1510-1512.
33. Hankinson JL, Gardner RM. Standard waveforms for spirometer testing. *Am Rev Respir Dis* 1982; 126: 362-364.
34. Latzin P, Thompson B. Validation of multiple-breath washout equipment: from bench to clinic and possible pitfalls. *Respiration* 2014; 87: 456-458.
35. Summermatter S, Singer F, Latzin P, Yammine S. Impact of Software Settings on Multiple-Breath Washout Outcomes. *PLoS One* 2015; 10: e0132250.
36. Brunner JX, Wolff G, Cumming G, Langenstein H. Accurate measurement of N<sub>2</sub> volumes during N<sub>2</sub> washout requires dynamic adjustment of delay time. *J Appl Physiol* 1985; 59: 1008-1012.
37. Horsley A, Macleod K, Gupta R, Goddard N, Bell N. Enhanced photoacoustic gas analyser response time and impact on accuracy at fast ventilation rates during multiple breath washout. *PLoS One* 2014; 9: e98487.
38. Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, Davies JC, Cunningham S, Alton EW, Innes JA. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. *Thorax* 2008; 63: 135-140.
39. Latzin P, Sauteur L, Thamrin C, Schibler A, Baldwin D, Hutten GJ, Kyburz M, Kraemer R, Riedel T, Frey U. Optimized temperature and deadspace correction improve analysis of multiple breath washout measurements by ultrasonic flowmeter in infants. *Pediatr Pulmonol* 2007; 42: 888-897.
40. Singer F, Yammine S, Schmidt A, Proietti E, Kieninger E, Barben J, Casaulta C, Regamey N, Gustafsson P, Frey U, Latzin P. Ventilatory response to nitrogen multiple-breath washout in infants. *Pediatr Pulmonol* 2014; 49: 342-347.
41. Banton GL, Hall GL, Tan M, Skoric B, Ranganathan SC, Franklin PJ, Pillow JJ, Schulzke SM, Simpson SJ. Multiple breath washout cannot be used for tidal breath parameter analysis in infants. *Pediatr Pulmonol* 2016; 51: 531-540.

42. Jost K, Egger B, Kieninger E, Singer F, Frey U, Latzin P. Changes in minute ventilation after exposure to 4% sulfur hexafluoride (SF<sub>6</sub>) in infants. *Pediatr Pulmonol* 2017; 52: 151-153.
43. Gustafsson PM, Bengtsson L, Lindblad A, Robinson PD. The Effect of Inert Gas Choice on Multiple Breath Washout in Healthy Infants - Differences in Lung Function Outcomes and Breathing Pattern. *Journal of applied physiology* 2017; jap 00524 02017.
44. Schibler A, Schneider M, Frey U, Kraemer R. Moment ratio analysis of multiple breath nitrogen washout in infants with lung disease. *Eur Respir J* 2000; 15: 1094-1101.
45. Katz-Salamon M, Jonsson B, Lagercrantz H. Blunted peripheral chemoreceptor response to hyperoxia in a group of infants with bronchopulmonary dysplasia. *Pediatr Pulmonol* 1995; 20: 101-106.
46. Jost K, Lenherr N, Singer F, Schulzke SM, Frey U, Latzin P, Yammine S. Changes in breathing pattern upon 100% oxygen in children at early school age. *Respir Physiol Neurobiol* 2016; 228: 9-15.
47. Bureau MA, Lupien L, Begin R. Neural drive and ventilatory strategy of breathing in normal children, and in patients with cystic fibrosis and asthma. *Pediatrics* 1981; 68: 187-194.
48. Foong R, Gallagher C, Hall G, Ramsey KA. The effect of hyperoxia on tidal breathing in preschool children. *Paediatr Pulmonol* 2015; 50: S411.
49. Lundin G. Nitrogen elimination during oxygen breathing. *Acta Physiol Scand Suppl* 1953; 111: 130-143.
50. Battino R, Rettich T, Tominaga T. The Solubility of Nitrogen and Air in Liquids. *J Phys Chem Ref Data* 1984; 13: 563-601.
51. Hlastala MP, Meyer M, Riepl G, Scheid P. Solubility of helium, argon, and sulfur hexafluoride in human blood measured by mass spectrometry. *Undersea Biomed Res* 1980; 7: 297-304.
52. Nielsen N, Nielsen JG, Horsley AR. Evaluation of the impact of alveolar nitrogen excretion on indices derived from multiple breath nitrogen washout. *PLoS One* 2013; 8: e73335.
53. Lundin G. Nitrogen elimination from the tissues during oxygen breathing and its relationship to the fat: muscle ratio and the localization of bends. *J Physiol* 1960; 152: 167-175.
54. Kane M, Stanojevic S, Jensen R, Ratjen F. Effect of Tissue Nitrogen Excretion on Multiple Breath Washout Measurements. *Am J Respir Crit Care Med* 2016; 193: AA4491.
55. Schmalisch G, Proquitte H, Roehr CC, Wauer RR. The effect of changing ventilator settings on indices of ventilation inhomogeneity in small ventilated lungs. *BMC Pulm Med* 2006; 6: 20.
56. Schulzke SM, Deeptha K, Sinhal S, Baldwin DN, Pillow JJ. Nasal versus face mask for multiple-breath washout technique in preterm infants. *Pediatr Pulmonol* 2008; 43: 858-865.
57. Verbanck S, Thompson BR, Schuermans D, Kalsi H, Biddiscombe M, Stuart-Andrews C, Hanon S, Van Muylem A, Paiva M, Vincken W, Usmani O. Ventilation heterogeneity in the acinar and conductive zones of the normal ageing lung. *Thorax* 2012; 67: 789-795.
58. Benseler A, Stanojevic S, Jensen R, Gustafsson P, Ratjen F. Effect of equipment dead space on multiple breath washout measures. *Respirology* 2015; 20: 459-466.
59. Saatci E, Miller DM, Stell IM, Lee KC, Moxham J. Dynamic dead space in face masks used with noninvasive ventilators: a lung model study. *Eur Respir J* 2004; 23: 129-135.
60. Robinson PD. Feasibility of squeezing multiple breath washout testing into busy clinical laboratories. *Pediatr Pulmonol* 2016; 51: 1271-1273.
61. Vilmann L, Buchvald FF, Nielsen KG. Multiple Breath Nitrogen Washout (MBWN2) and Fractional Exhaled Nitric Oxide (FeNO) in Healthy and Asthmatic Preschool Children. *Am J Respir Crit Care Med* 2016; 193: A4487.
62. Robinson PD, Hardaker KM, Gustafsson PM, Cooper P, Fitzgerald D, Selvadurai H. Effect of Interface on Multiple Breath Washout feasibility and results in young children. *Am J Respir Crit Care Med* 2015; 191: A2330.
63. Jensen R, Stanojevic S, Webster H, Gibney K, McDonald N, Subbarao P, Ratjen F. Feasibility of Longitudinal Multiple Breath Washout Measurements in Preschool Children. *Am J Respir Crit Care Med* 2014; 189: A1032.
64. Foong RE, Ramsey KA, Gallagher C, Turkovic L, Hall GL. Feasibility of using mouthpieces for multiple breath washout testing in young children with cystic fibrosis. *Pediatr Pulmonol* 2015; 50: S345.
65. Lum S, Stocks J, Kozłowska W, Aurora P. Effects of using a mask vs. mouthpiece on the multiple breath inert gas washout technique. *Eur Respir J* 2015; 46: PA1582.
66. Moore C, Benseler N, Jensen R, Stanojevic S, Ratjen FA. The Effect Of Nasal Vs. Oral Breathing On The Lung Clearance Index Measurement. *Am J Respir Crit Care Med* 2015; 191: A2328.

67. Ingelstedt S. Studies on the conditioning of air in the respiratory tract. *Acta Otolaryngol Suppl* 1956; 131: 1-80.
68. Yammine S, Singer F, Abbas C, Roos M, Latzin P. Multiple-breath washout measurements can be significantly shortened in children. *Thorax* 2013; 68: 586-587.
69. Robinson PD, Stocks J, Aurora P, Lum S. Abbreviated multi-breath washout for calculation of lung clearance index. *Pediatr Pulmonol* 2013; 48: 336-343.
70. Green K, Ejlertsen JS, Madsen A, Buchvald FF, Kongstad T, Kobbernagel H, Gustafsson PM, Nielsen KG. Abbreviation modalities of nitrogen multiple-breath washout tests in school children with obstructed lung disease. *Pediatr Pulmonol* 2016; 51: 624-632.
71. Klingel M, Stanojevic S, Jensen R, Webster H, Davis S, Retsch-Bogart G, Ratjen F. Comparison of Reporting Two Versus Three Trials of the Lung Clearance Index. *Am J Respir Crit Care Med* 2016; 193: AA4492.
72. Ramsey KA, Foong RE, Harper AJ, Stick S, Hall G. Relationships between lung clearance index and clinical markers of early cystic fibrosis lung disease are unchanged when only two trials are assessed. *Am J Respir Crit Care Med* 2016; 193: A4494.
73. Hardaker KH, Jayasuriya G, Gustafsson PM, Cooper P, Fitzgerald DA, Selvadurai H, Robinson PD. Can Scond and Sacin be estimated from two multiple breath washout tests across childhood? *Eur Respir J* 2015; 46: PA1267.
74. Clausen JL, Wanger J, participants ftW. Consensus Statement on the Measurement of Lung Volumes in Humans. *ATS/NHLBI Consensus Document* 2003.
75. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson D, Macintyre N, McKay R, Miller MR, Navajas D, Pellegrino R, Viegi G. Standardisation of the measurement of lung volumes. *Eur Respir J* 2005; 26: 511-522.
76. Narayanan M, Owers-Bradley J, Beardsmore CS, Mada M, Ball I, Garipov R, Panesar KS, Kuehni CE, Spycher BD, Williams SE, Silverman M. Alveolarization continues during childhood and adolescence: new evidence from helium-3 magnetic resonance. *Am J Respir Crit Care Med* 2012; 185: 186-191.
77. Hardaker K, Cooper P, Fitzgerald D, Gustafsson P, Jayasuriya G, Selvadurai H, Robinson P. Feasibility of concentration normalized phase III slope (SnIII) analysis across the paediatric age range using updated acceptability criteria. *Respirology* 2015; 20: 93.
78. Bigler A, Yammine S, Singer F, Riedel T, Latzin P. Feasibility of automated slope III and Scond analysis in children. *Pediatr Pulmonol* 2015; 50: 805-813.
79. Lum S, Stocks J, Stanojevic S, Wade A, Robinson P, Gustafsson P, Brown M, Aurora P, Subbarao P, Hoo AF, Sonnappa S. Age and height dependence of lung clearance index and functional residual capacity. *Eur Respir J* 2013; 41: 1371-1377.
80. Martin TR, Feldman HA, Fredberg JJ, Castile RG, Mead J, Wohl ME. Relationship between maximal expiratory flows and lung volumes in growing humans. *J Appl Physiol* 1988; 65: 822-828.
81. Chakr VC, Llapur CJ, Sarria EE, Mattiello R, Kisling J, Tiller C, Kimmel R, Poindexter B, Tepper RS. Ventilation homogeneity improves with growth early in life. *Pediatr Pulmonol* 2012; 47: 373-380.
82. Poncin W, Singer F, Aubriot AS, Lebecque P. Agreement between multiple-breath nitrogen washout systems in children and adults. *J Cyst Fibros* 2017; 16: 258-266.
83. Jensen R, Stanojevic S, Gibney K, Salazar JG, Gustafsson P, Subbarao P, Ratjen F. Multiple breath nitrogen washout: a feasible alternative to mass spectrometry. *PLoS one* 2013; 8: e56868.
84. Ratjen F, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, Milla CE, Robinson PD, Waltz D, Davies JC, VX14-809-109 investigator group. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. *The lancet Respiratory medicine* 2017; 5: 557-567.
85. Quanjer PH, Stocks J, Cole TJ, Hall GL, Stanojevic S, Global Lungs I. Influence of secular trends and sample size on reference equations for lung function tests. *Eur Respir J* 2011; 37: 658-664.
86. Subbarao P, Stanojevic S, Brown M, Jensen R, Rosenfeld M, Davis S, Gustafsson P, Ratjen F. Lung Clearance Index as an Outcome Measure for Clinical Trials in Young Children with Cystic Fibrosis: A Pilot Study using Inhaled Hypertonic Saline. *Am J Respir Crit Care Med* 2013.
87. Orehek J, Nicoli MM, Delpierre S, Beaupre A. Influence of the previous deep inspiration on the spirometric measurement of provoked bronchoconstriction in asthma. *Am Rev Respir Dis* 1981; 123: 269-272.

88. Stanojevic S, Ratjen F. Physiologic endpoints for clinical studies for cystic fibrosis. *J Cyst Fibros* 2016; 15: 416-423.
89. Aurora P, Stanojevic S, Wade A, Oliver C, Kozłowska W, Lum S, Bush A, Price J, Carr SB, Shankar A, Stocks J. Lung clearance index at 4 years predicts subsequent lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 2011; 183: 752-758.
90. Sonneveld N, Stanojevic S, Amin R, Aurora P, Davies J, Elborn JS, Horsley A, Latzin P, O'Neill K, Robinson P, Scrase E, Selvadurai H, Subbarao P, Welsh L, Yammine S, Ratjen F. Lung clearance index in cystic fibrosis subjects treated for pulmonary exacerbations. *Eur Respir J* 2015; 46: 1055-1064.
91. Jensen R, Stanojevic S, Klingel M, Pizarro ME, Hall GL, Ramsey K, Foong R, Saunders C, Robinson PD, Webster H, Hardaker K, Kane M, Ratjen F. A Systematic Approach to Multiple Breath Nitrogen Washout Test Quality. *PLoS One* 2016; 11: e0157523.
92. Ciaffoni L, O'Neill DP, Couper JH, Ritchie GA, Hancock G, Robbins PA. In-airway molecular flow sensing: A new technology for continuous, noninvasive monitoring of oxygen consumption in critical care. *Sci Adv* 2016; 2: e1600560.
93. Frey U, Stocks J, Coates A, Sly P, Bates J. Specifications for equipment used for infant pulmonary function testing. ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/ American Thoracic Society. *Eur Respir J* 2000; 16: 731-740.