AMERICAN THORACIC SOCIETY DOCUMENTS

Preschool Multiple-Breath Washout Testing
An Official American Thoracic Society Technical Statement

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Background: Obstructive airway disease is nonuniformly distributed throughout the bronchial tree, although the extent 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 European Respiratory Society/American Thoracic Society inert gas washout consensus statement led to the 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 yr), where a "window of opportunity" exists for early diagnosis of obstructive lung disease and intervention.

Methods: This preschool-specific technical standards 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 that 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.

Keywords: multiple-breath washout; lung clearance index; peripheral airway function; cystic fibrosis

Contents

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An Executive Summary of this document is available at http://www.atsjournals.org/doi/suppl/10.1164/rccm.201801-0074ST.

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) and 2006 (2), and were supplemented in 2007 by a formal MBW section within an official American Thoracic Society/European Respiratory Society (ATS/ERS) statement on preschool lung function testing (3). The ATS/ERS document highlighted that "few systems for MBW adapted for the preschool age group [were] commercially available," and that remains the case a decade later. The ERS/ATS inert gas washout consensus document (hereafter termed the 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). Although 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 the potential utility of MBW outcomes such as the lung clearance index (LCI) in specific patient groups (e.g., those with cystic fibrosis [CF]), where MBW is being used as an outcome measure for clinical trials. Publications in this area have trebled over each successive 5-year period of the past 15 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 encountered when using these commercially available devices with preschool children 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 toward clarifying the clinical utility of MBW within the preschool age range. It outlines important recommendations on device design for manufacturers and test performance for operators that are specific to preschool children. It recognizes that although 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 toward 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 is 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 in Table 2 and will, it is hoped, prove an incentive to gather the evidence necessary for further advances in standardization within this age group. The 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).

Introduction

In preschool children (2–6 yr of age), conventional lung function tests, such as spirometry, remain technically challenging and relatively insensitive in identifying early airway disease in conditions such as 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 Cystic Fibrosis 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) Standardisation 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).

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 articles contained within Embase and PubMed databases) performed with the keywords MBW, preschool children, preschoolers, LCI, moment ratios, moment analysis, and FRC, as of October 31, 2017. 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

Table 1. Summary of Key Current Recommendations for Manufacturers and Multiple-Breath Washout Operators

Manufacturer Directed	Operator Directed
Compliance with preschool recommendations: commercial MBW systems • 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.	• The end user is encouraged to demand this as part of the marketing material accompanying any device.
Validation of FRC measurement accuracy • *In vitro* validation for preschool MBW systems must include representative FRC volumes of the preschool age range, using respiratory rates and V_T typical for the subjects and lung conditions encountered. • FRC measurement accuracy must not be extrapolated from larger FRC volumes.	
Flow measurement and breath detection • V_T accuracy must be within 3% or 5 ml, whichever is greater. • Flow detection accuracy must be robust and manufacturer assessment based on data mimicking the variability in breathing pattern encountered in this age group. • 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.	
Optimal synchronization of flow and inert gas concentration • Synchronization error must be within 10 ms across the duration of the entire washout. • 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.	• The operator must have the ability to assess the accuracy of inert gas concentration and flow synchronization at the time of testing.
Inert gas choice • 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_6- and N_2-based MBW appear appropriate inert gas choices for preschool children. • Preschool MBW systems must provide the ability to monitor breathing pattern in real time during each test. • 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.	
Equipment-related V_D • Manufacturers must minimize equipment-related V_D. To ensure a consistent approach across age ranges, equipment-related V_D must be kept below 2 ml/kg, as recommended in the recent ERS/ATS consensus guidelines. • 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.	• MBW operators must minimize facemask-associated V_D by ensuring that the smallest appropriately sized facemask is used, and by using therapeutic putty within the facemask to ensure no obstruction to airflow occurs.
Environment for testing	• 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. • Adequate time should be set aside for testing in this age group, particularly for those less than 4 yr of age or who are attending for the first time. An hour is recommended for initial testing. • Familiarization visits for the child and parents to experience equipment, testing procedure, test interface, and environment used during testing are recommended. • 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 • Two operators should be used for MBW testing in this age group, regardless of interface choice.

(Continued)

Table 1. (*Continued*)

Manufacturer Directed	Operator Directed
Testing interface • Both a mouthpiece and nose-clip assembly and a facemask are supported as interface choices for use in preschool-aged children.	• 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.

Special considerations when reporting preschool MBW data
   • Software must clearly state where reported indices values are "based on the average of two values alone."
   • The previously recommended FRC 10% acceptability criterion for LCI reporting is no longer advocated in this age group.
	• The approach to individual and overall test session acceptability in preschool children should be adapted to reflect differences in comparison with older subjects: preschool children require a shorter duration of pretest 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.
	• 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 as when results are reported in software.

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

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

Definition of abbreviations: ATS = American Thoracic Society; EELV = end-expiratory lung volume; ERS = European Respiratory Society; LCI = lung clearance index; MBW = multiple-breath washout; SF_6 = sulfur hexafluoride.

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 in the overall draft document by the chair, and all authors provided comment and suggestions for the final document. The methods checklist is presented in Table 3.

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 1980s suggested poor feasibility in preschool children. Using custom-built equipment, Couriel and colleagues reported feasibility of 49% in 82 subjects aged 3.9–6.8 years (23). Couriel and colleagues used an airtight snorkel mouthpiece and nose-clip interface and a two-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 the 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 and colleagues used a facemask interface and replaced music with video-based distraction, but did not report feasibility rates (25). Shortly after, Aurora and colleagues, 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).

Technical Considerations for Preschool MBW

The original ERS/ATS consensus statement included a comprehensive list of recommendations for both manufacturers and operators (4). To facilitate standardized measurement and practice, technical

Table 2. Important Areas of Interest for Future Work Specific to Preschool Multiple-Breath Washout

Area of Interest	Questions and/or Needs
Shortening the duration of testing for preschool subjects	Analysis of MBW outcomes during the wash-in portion of each test (e.g., FRC and LCI), from two tests alone (vs. three tests), or abbreviated outcomes (e.g., LCI at 1/20th 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 with older subjects.
Effects of pure O_2 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_2 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. Is the relative contribution of this inert gas diffusion effect greater in preschool subjects compared with older age groups? Can inert gas–specific corrections be developed and applied, and do 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	Because of 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 alternative 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 low 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 subsequent calculated MBW indices?
Definition of normal physiological variability in the preschool age range	Better definition of normal V_T and EELV variability in this age range to determine accurate thresholds for breathing pattern stability Optimal definition of suitable target V_T range—defined based on actual weight, ideal body weight, height, or BMI? Definition of normal FRC variability, in comparison with 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_D	Can functional V_D within a bacterial filter or facemask assembly be accurately estimated and corrected for across subjects? What is the contribution of increasing relative V_D to the change in LCI reference values observed across the preschool age range (79)? What is the optimal method for expressing V_D: as a function of weight (ml/kg) or is BMI, percentage of V_T, or 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-related V_D 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)?

Definition of abbreviations: BMI = body mass index; EELV = end-expiratory lung volume; LCI = lung clearance index; MBW = multiple-breath washout.

Table 3. Methods Checklist

	Yes	No
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 prespecified 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

Definition of abbreviations: GRADE = Grading of Recommendations Assessment, Development, and Evaluation; NA = not applicable; PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.
*Required for guidelines but not technical statements.

aspects with particular importance to the preschool age range are discussed in further detail within this section.

Validation of FRC Measurement Accuracy

At present no available *in vitro* models exist to validate the accuracy of ventilation inhomogeneity (e.g., LCI) assessment; therefore validation efforts have focused mainly on FRC measurement accuracy, and recommendations in more 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 presents 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 L), using respiratory rates and V_T values typical for the subjects and lung conditions encountered (typically 20–40 breaths/min in health and up to 60 breaths/min 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 V_Ts appropriate for the subjects and lung conditions encountered.
2. FRC measurement accuracy must not be extrapolated from larger FRC volumes.

Flow Measurement and Breath Detection

Breath definition may be based on detection of zero flow crossings or by integration of flows to detect breath volumes reaching a predetermined significant value. Additional challenges for the manufacturer in the preschool setting are greater breathing irregularity and the lower tidal flows encountered, compared with older children, with typical peak inspiratory and expiratory flows ranging from 200 to 400 ml/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_2 versus room air, which results in an overreading of flow with pneumotachographs by 10% with 100% O_2 if the pneumotachographs are calibrated with room air. In contrast, ultrasonic flow meters measure flow using the Doppler effect; therefore, these devices measure linear velocity and are in principle 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 sidestream gas analysis setups) on measured tidal flows, integrated V_T, and MBW outcomes may be relatively greater in preschool children 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 variations of 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 reinspired inert gas. Accurate end-tidal inert gas concentration is also more

challenging because of the 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 the robustness of its product. Assessment requires variability in breathing pattern, which may be best provided by representative *in vivo* data or mimicked with 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 the 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.

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_2 MBW where O_2 concentration varies between 21 and 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 described within a commercial MBW system (10).

On the basis of fixed synchronization approaches, FRC accuracy has been demonstrated to remain within 5% (the specified accuracy threshold) if synchronization error is not more than 10 ms between flow and inert gas concentration. This threshold appears to be consistent across various 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 systems. The accuracy of the outcome measurement (e.g., FRC) should not be extrapolated to other outcomes, as the error magnitude may differ (7). For example, LCI threshold is dependent on accurate end-tidal inert gas concentration at the end of the washout, where viscosity-related effects may be greatest, yet the relative exhaled inert gas
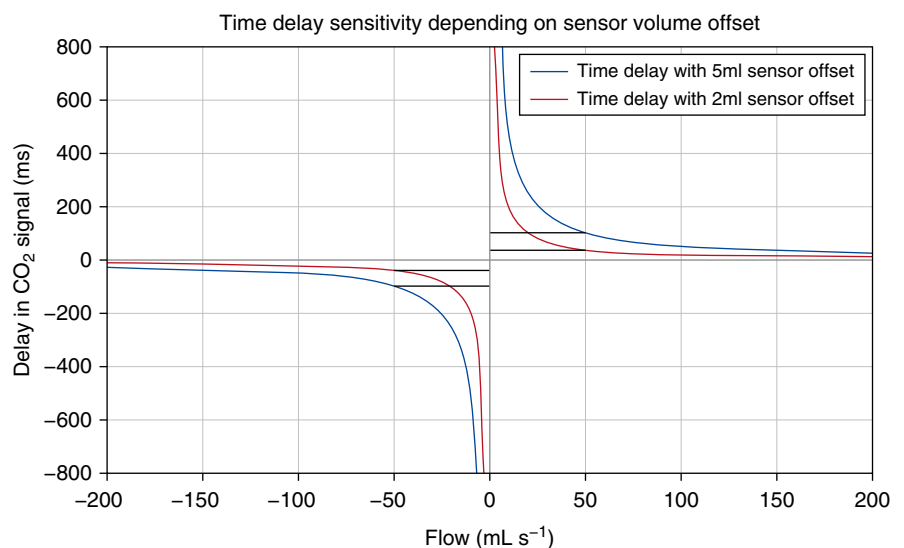
Figure 1. Simulation of flow-dependent delay between flow and gas concentration signals. Visualization of the time delay between two sensors depends on the volume between the two sensors and the flow rate of the measured gas. The simulations were performed assuming both sensors are placed inside a tube with 2 ml (red line) or 5 ml (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: time delay = (volume between sensors) / (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 around.

volume contribution to FRC may be far less in this region of the washout. Recommendations to minimize flow–gas delay error to not exceed 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 10 ms 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.

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, sulfur hexafluoride [SF_6], and N_2), most research to date has focused on either SF_6 or N_2. Helium use has been restricted mainly to the setting of a secondary comparison gas in preschool SF_6 studies, using custom-built respiratory mass spectrometer–based equipment that facilitates dual gas comparison. Other fast-responding helium analyzers are currently lacking.

Indirect inert gas concentration analysis approaches have been developed for both SF_6 and N_2 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_6 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. 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, 40).

Physiological and Developmental Considerations for Preschool MBW

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_6- and N_2-based MBW appear appropriate inert gas choices for preschool children.

Effect of inert gas choice on breathing pattern during testing. Significant deviation from tidal breathing has been reported in infants during both 100% O_2 and 4% SF_6 procedures (41–44). The magnitude of effect is not trivial in infants, with 100% O_2 exposure causing up to a 33% reduction in V_T (41, 44, 45). Proposed explanations include the effect of absorption atelectasis on V_T or a blunted peripheral chemoreceptor response to increased arterial oxygenation on respiratory rate and $\dot{V}_E$ (44, 46). An initial priming period of a lower O_2 concentration (e.g., 40%) has been shown to negate the effect in infants (41). This effect is not as pronounced with SF_6 exposure, at a concentration of 4%, with data describing significant effects on $\dot{V}_E$ only (42, 43). In a direct comparison of the two methods, this effect observed with SF_6 on V_T was attributed to a technical artifact rather than a true physiological effect (44). A described effect on respiratory rate in a sedated cohort (42) was not observed in two subsequent nonsedated cohorts (43, 44). The degree to which the effect of 100% O_2 on breathing pattern accounts for the difference observed between SF_6- and N_2-based MBW indices (e.g., LCI and FRC) remains unclear, but SF_6-based MBW is currently the preferred method in infants (44).

Although these effects of 100% O_2 do not appear to be present in early school-age children (47), the exact stage at which this effect disappears, if in fact it does (48), remains unclear. The effects are likely to act in an age-, dose-, and disease-dependent manner (41, 46, 48, 49), 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_T change of less than 10% and not present consistently across individuals (50). 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_T, respiratory rate, and concurrent end-tidal CO_2 (to detect hyper- or hypoventilation). Display of real-time $\dot{V}_E$ should also be considered. Until further data are available, both SF_6- and N_2-based MBW remain appropriate inert gas choices for preschool children.

RECOMMENDATIONS:

1. In MBW testing of preschool subjects, both SF_6 and N_2 appear to be 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.

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. Although N_2 is inert, in the sense that the human body does not metabolize it, it is a soluble gas, and because of the high partial pressure of the atmosphere, a large amount of N_2 is stored within the body. As a result N_2 will diffuse into the alveoli when the partial pressure of N_2 is lowered, as occurs during N_2-based MBW using 100% O_2 (51, 52). 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_6 and helium (53). The magnitude of diffusion and its effect on subsequent MBW outcomes has not been adequately described to date, beyond initial modeling attempts for N_2 MBW (54).

Several factors are likely to influence the magnitude of the impact of inert gas diffusing across the alveolar–capillary

membrane: *1)* tissue N_2 contribution to the alveolar N_2 fraction is likely to be nonlinear 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_2 elimination and lung perfusion will vary across different lung compartments (55); *2)* the subject's age, given that lung architecture and cardiac output change with age (particularly relevant in preschool subjects, in whom these change more rapidly than in older subjects); and finally *3)* 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_2 contribution to calculated FRC from the 1950s (51) should therefore not be extrapolated to preschool children. In fact, previous attempts to implement corrections based on these data in older school-aged subjects have been reported to be problematic (21). More 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*) (54). However, preliminary *in vivo* studies suggest the effect of tissue N_2 increases with increasing lung volume and subject size, and as lung function worsens (56). Although these findings are physiologically important, it remains to be determined whether correction for tissue N_2 would alter the interpretation of MBW results.

RECOMMENDATION:

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.
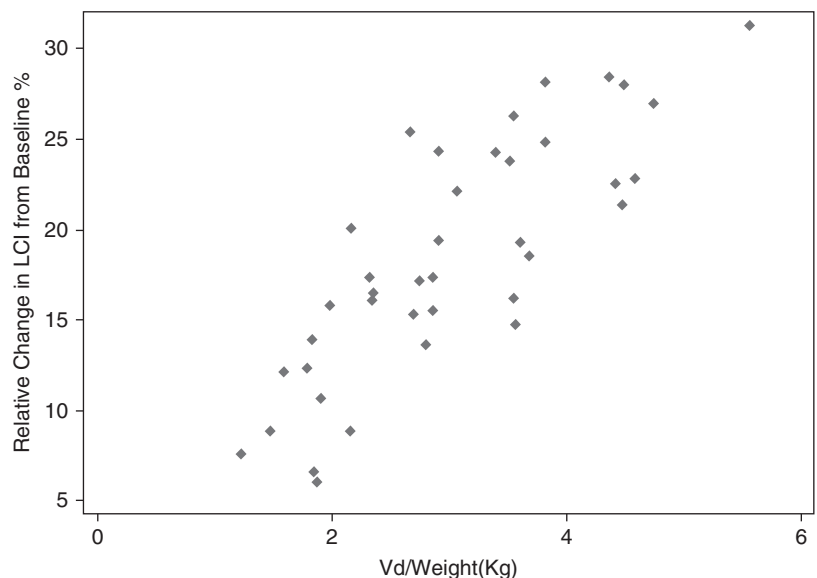
Equipment-related V_D
To reduce equipment-related effects on breathing pattern and end-expiratory lung volume (EELV), manufacturers of

commercial devices must consider equipment-related V_D carefully when designing systems for widespread use in general respiratory clinics. Definitions of the V_D components of an MBW system (equipment, anatomical and physiological) are described in detail in the ERS/ATS consensus statement (4). Studies in animals (57), infants (58), and preschool children and adults (59, 60) have consistently demonstrated detrimental impacts of increased V_D on ventilation inhomogeneity outcomes. Adult data illustrating this increasing detrimental effect of V_D on LCI across the V_D range of 0–5 ml/kg are 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-related 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-related V_D will be most affected. Furthermore, changing relative V_D per kilogram 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 (59). Correction algorithms for LCI have been proposed (59), 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 (61), 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 cumulative expired volume or FRC calculation. Instead, introduced facemask V_D must be minimized by selection of the smallest appropriate size that maintains

Figure 2. Effect of increasing equipment-related V_D on ventilation inhomogeneity. Data displayed are from 10 healthy adult subjects; lung clearance index (LCI) was calculated as the mean of triplicate tests across five V_D values (standard, +50 ml, +100 ml, +150 ml, and +200 ml). 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 1-ml/kg increase in equipment-related V_D. (Based on data contained in Reference 59.)

face seal, and by applying therapeutic putty appropriately (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 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 that the smallest appropriately sized facemask is used, and by using therapeutic putty within the facemask to ensure that no obstruction to airflow occurs.

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 statement on pulmonary function testing in preschool children (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 to 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 the best choice may vary with the 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 parent's 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 discreet 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-naive and/or younger preschool subjects, although shorter time periods are feasible in experienced subjects. Detrimental effects of imposed limited
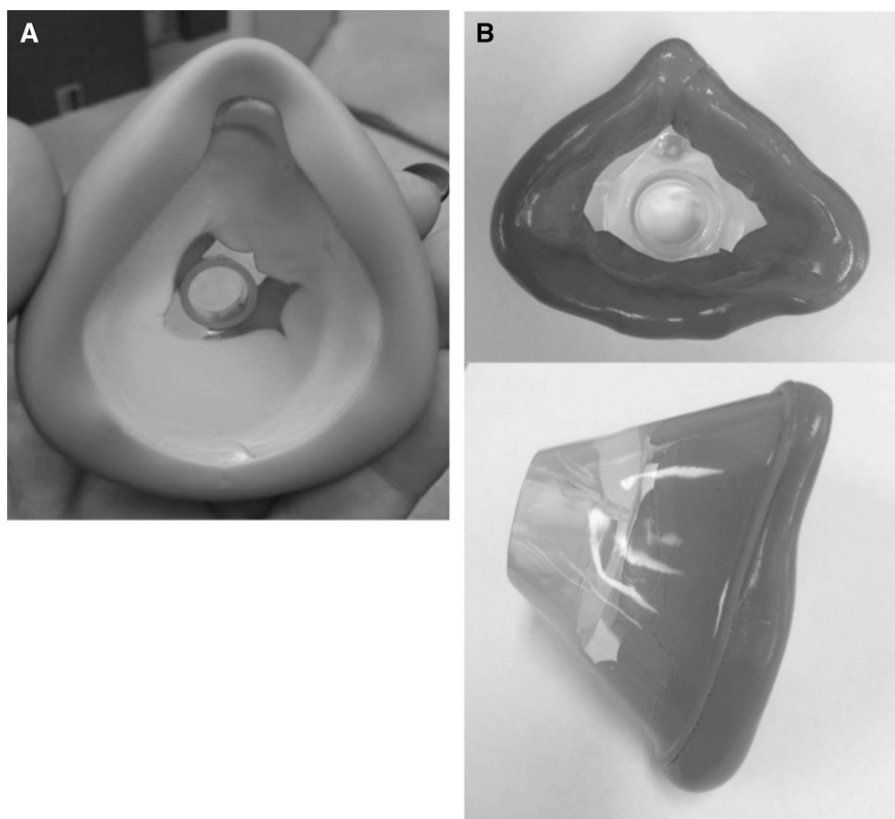
Figure 3. Therapeutic putty use in facemask interfaces to reduce equipment-related V_D. Additional equipment-related V_D introduced by a facemask assembly should be reduced as much as possible. The smallest appropriately sized facemask should be selected. (*A*) Therapeutic putty application will be influenced by the presence of a flange to aid a leak-free seal when applied to the face during testing. In this case, putty is applied solely to reduce V_D within the mask. (*B*) If no flange is present then putty also helps create the seal. A combination of different therapeutic putty consistencies may be required to ensure that the putty maintains its shape and to prevent migration and outflow tract obstruction during testing.

time periods (e.g., 20 min for testing) have been described (12, 62).

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 less than 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.

Preschool Test Feasibility

The feasibility of MBW testing in preschool children has been reported by several studies. In the study by Aurora and colleagues, reporting a success rate of 79% across MBW-naive, 2- to 6-year-old children (16), feasibility was higher in healthy control subjects (84%) compared with subjects with CF (75%), and a clear age-related effect was observed. Feasibility was lowest in 2- to 3-year-old children (50%), more than 80% in those above 3 years, and highest in children 5–6 years of age (87%).

The results of experience with commercial equipment (11, 12, 17, 59, 63–66) are summarized in Table 4. Although 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 *1*) variation in the equipment setup between commercial and custom research equipment; *2*) age ranges tested; *3*) variation in the definition of acceptable data (i.e., two vs. three tests); *4*) 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 *5*) the interface used (i.e., mouthpiece/nose-clip vs. facemask). Potential effects of these individual aspects on feasibility are discussed in detail later in this document. This variation in methodology highlights the importance of implementing the recommendations contained within this technical standards document to standardize the technique moving forward.

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 in Table 5. The use of a

Table 4. Published Preschool MBW Feasibility to Date, Using Commercial MBW Systems

Author (Year)	Subjects	Age Tested (*range, yr*)	MBW-naive (%)	Interface	No. of Acceptable Tests Required	No. of Subjects Attempted	Subjects Successful (%)
Jensen 2014 (65)*	CF	2.9–5.0	0	Facemask	≥2 tests	30	83
Benseler 2015 (59)*	Healthy	2.8–5.9		Facemask	3 tests (successful on both equipment systems)	24	83
	CF	3.3–5.9				27	70
Robinson (64)*	CF, wheeze	2.0–6.9	77	Facemask	3 tests	46	85
					2 tests	46	91
			77	Mouthpiece	3 tests	46	50
					2 tests	46	63
Foong 2015 (66)	Healthy	3.0–6.6	100	Mouthpiece	≥2 tests (first visit)	60	72
			0		≥2 tests (subsequent visit)	19	86
	CF	2.6–6.6	100	Mouthpiece	≥2 tests (first visit)	78	67
			0		≥2 tests (subsequent visit)	59	82
Vilmann 2016 (63)*	Healthy, asthmatic	3–6	100	Mouthpiece	≥2 tests	66	89
					3 tests	66	67
Downing 2016 (11)*	Healthy, PCD, CF, wheeze	2.1–5.9	100	Mouthpiece	≥2 tests within 30 min	116	73
Yammine 2016 (12)†	Asthmatic	3.1–6.7	100	Mouthpiece	3 tests within 20 min	62	24
					2 tests within 20 min	62	60
Stanojevic 2017 (17)*	Healthy, CF	2.5–5.9	100	Facemask	≥2 tests (first visit)	150	66
					≥2 tests (subsequent visits)	150	89

Definition of abbreviations: CF = cystic fibrosis; MBW = multiple-breath washout; PCD = primary ciliary dyskinesia.
*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 Multiple-Breath Washout Test Interface Choice

	Mouthpiece and Nose-Clip	Facemask
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-related V_D	Defined	Difficult to define
Seal (mouthpiece/mask) and risk of leak	Subject determined	Operator determined
Impact of nasal airways	Removed	Unknown

mouthpiece and nose-clip requires 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 because 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. Adult data have demonstrated that nasal breathing may introduce variability to the test result (64). 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 (64); 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 (64). 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 [Sn_{III}] analysis), and ability to factor in practice sessions. Beneficial effects of the latter on both mouthpiece and facemask feasibility have been described (17, 66). 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.

Special considerations when reporting preschool MBW data. Although 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 is available. Special considerations are as follows: 1) the minimal duration of breathing stability before starting the test should be shorter than is expected in school-age and older subjects (30 s adapted to 3–5 breaths); 2) 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 3) operators should recognize that sighs, swallows, and pauses may be more frequently encountered compared with in 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 in 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. 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/ERS 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) (3). This approach for MBW-based FRC measurement accuracy had been recommended in earlier lung volume
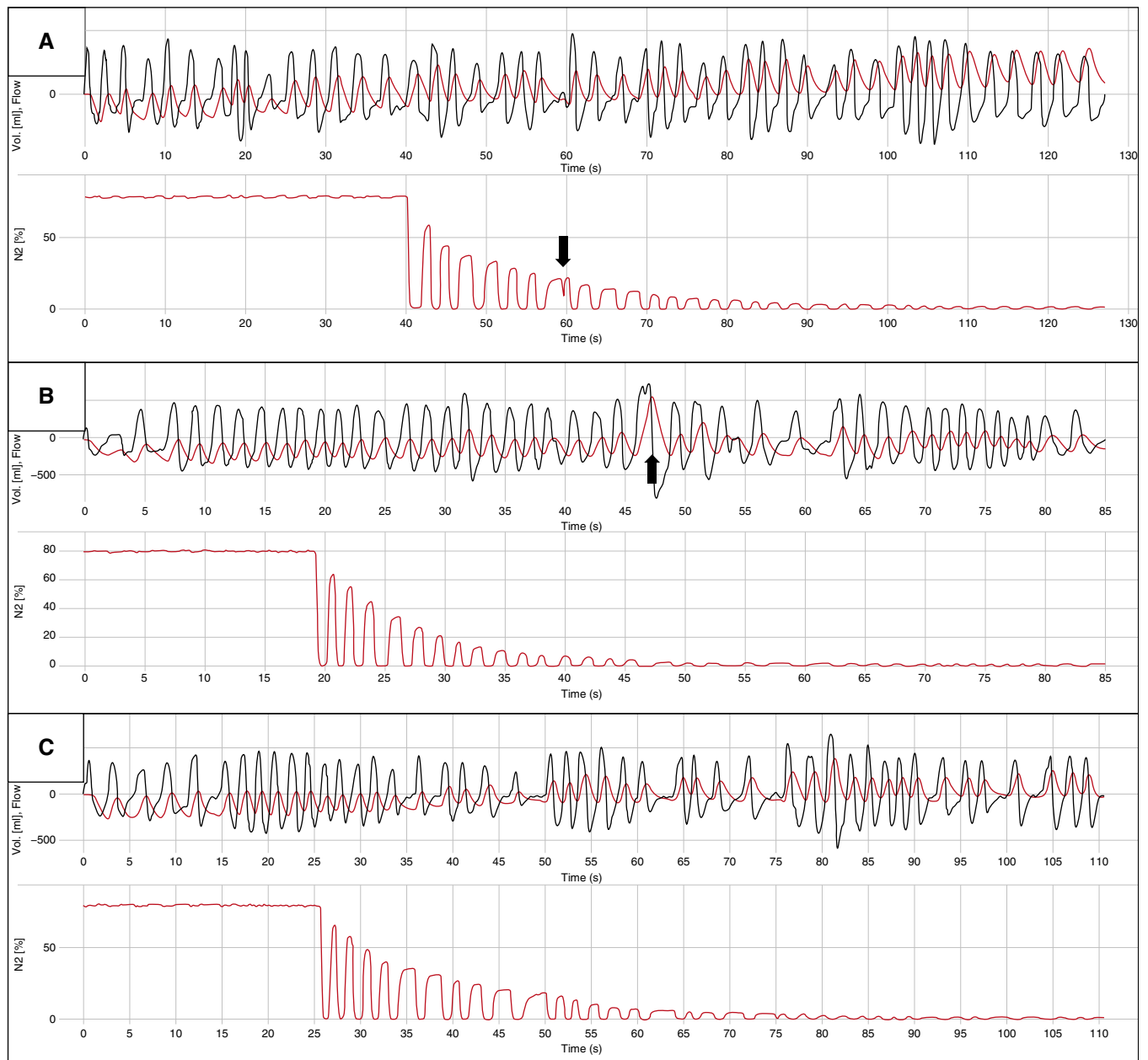
Figure 4. Typical breathing pattern observed in preschool subjects. (*A–C*) Sequential tests from the same test session, recorded using commercial N_2-based multiple-breath washout equipment, in a preschool subject. At the top of each panel, real-time plots of tidal flow (black) and V_T (red) are displayed; the bottom of each panel displays N_2 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 upward arrow, no evidence of resultant trapped gas release).

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 Sn_{III} analysis) remains unclear. The increased variability (e.g., for the second moment ratio, in comparison with LCI) may be a factor affecting suitability for moment analysis (24). Sn_{III} analysis requires clear visualization of the expirogram phase III slope, sufficient to estimate its magnitude, for both the first breath and two-thirds of breaths between 1.5 and 6.0 lung turnovers.

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). ERS/ATS consensus document recommendations that S_{III} should be at least 50% of the expired volume have significant detrimental effects on Sn_{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 Sn_{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 with older subjects: preschool children require a shorter duration of pretest 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.

Recommendations for Commercial Software

Recommendations for commercial software development and use for both manufacturers and operators are summarized in 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 working with preschool subjects to allow efficient use of test time, given the limited concentration and co-operation time span of young children. As in older age groups, this must include real-time displays during both the prephase and washout phase of *1)* inert gas concentration versus time; *2)* flow–volume loop (ideally with a display of the target V_T range for the child, typically 8–12 ml/kg); *3)* end-tidal CO_2 (if CO_2 is measured) and respiratory rate to detect hypo/hyperventilation or rebreathing; and *4)* volume and flow-versus-time plots to assess breathing pattern stability. Additional features that will improve artifact detection include autoscaling of the inert gas concentration versus 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.

Table 6. Recommendations for Commercial Software Development and Use for Manufacturers and Operators

Manufacturer Directed	Operator Directed
• Real-time biological feedback data must be displayed during both prephase and washout phases of each test. • Additional operator ability to zoom in and out both during and after each test to detect subtle artifact • Real-time inert gas concentration plot against time must be displayed. • Autoscaling of display during the washout to facilitate artifact detection	• 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
• 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). • Display of target V_T range appropriate for the subject • Real-time display of expirogram for each breath during the washout portion of each test	• Close inspection of whether the V_T 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. • Flow–volume loop display useful in preschool testing to detect obstruction of the facemask outflow tract, with any therapeutic putty used to reduce equipment-related V_D
• End-tidal CO_2 should be displayed to assess for hyper/hypoventilation. • Calculation and display of respiratory rate during each test	• End-tidal CO_2 should remain within the normal range (typically defined as 4–6%) through both the prephase (or wash-in) and washout portions of each test.
• Single real-time V_T (both inspiratory and expiratory) vs. time must be displayed to monitor breathing pattern and stability of end-expiratory lung volume.	• Evidence of breathing pattern and EELV stability must be present before starting each test (defined as present for 3–5 breaths).
• Manual start and stop options of the washout portion of each test must be provided.	• Until automated start and stop functions of testing have been validated, manual option to start and stop each test must be used.

Definition of abbreviation: EELV = end-expiratory lung volume.

2. Software quality control cannot be automated until clear evidence-based thresholds are available.
3. Incentive software is not recommended in this age range.

Reference Data for Preschool MBW

For many years, reference values for MBW indices, such as LCI, were assumed to be independent of age. Collated data from infancy to adolescence demonstrate an inverse relationship between LCI and height, most pronounced in the first 3 years of life, with plateauing of the upper limit of normal from 6 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-related V_D in younger subjects during testing. Given this, extrapolation of an upper limit of normal from older age groups is not recommended.

The available collated reference data (79) were based on data collected by 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 control subjects (e.g., from studies listed in Table 4) is challenging because of many aspects of the methodological variation discussed in the section PRESCHOOL TEST FEASIBILITY (above). 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.

MBW Use in Preschool Interventional Research Studies

MBW holds exciting promise as an end point for early intervention strategies in CF (14, 15), and was used as the primary outcome in an international multicenter study in 6- to 11-year-olds (84). To date,
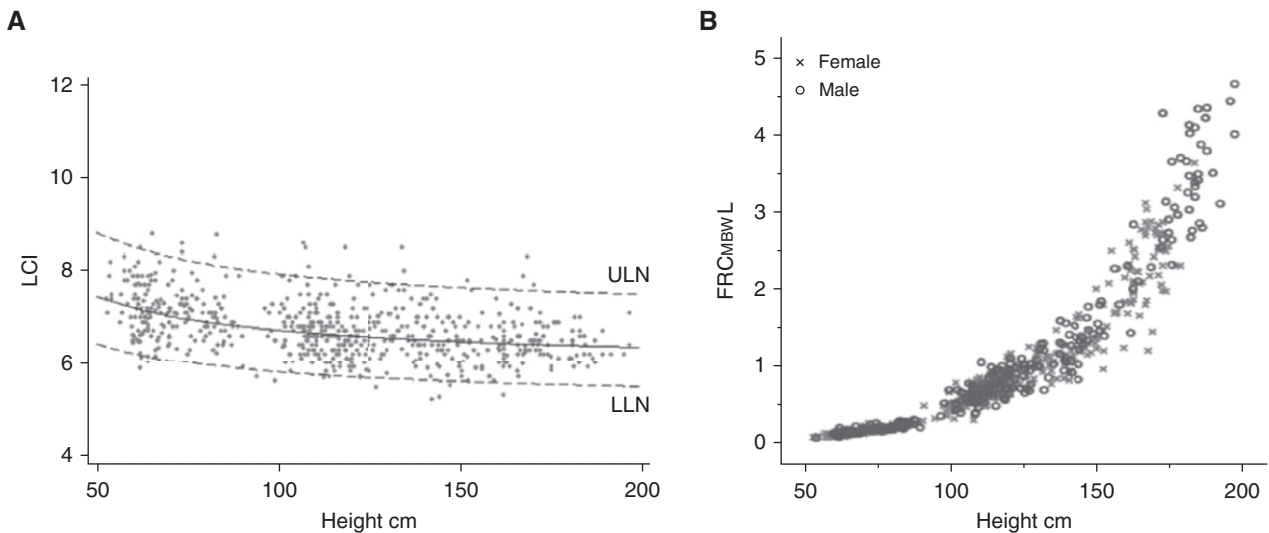
Figure 5. Changes in multiple-breath washout (MBW) indices—lung clearance index (LCI; *A*) and FRC (*B*)—across the pediatric age range. Data taken from a cohort of 497 subjects (ranging in age from infancy to 19 yr) tested on 659 occasions using custom-built research MBW equipment and sulfur hexafluoride as the inert tracer gas of interest. (*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 125 cm. These data must be viewed as inert gas– and equipment-specific. (Reproduced by permission from Reference 79. This material has not been reviewed by European Respiratory Society before release; therefore the European Respiratory Society may not be responsible for any errors, omissions, or inaccuracies, or for any consequences arising therefrom, in the content.)

only one small single-center CF clinical trial has used LCI as an end point 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) and a cystic fibrosis transmembrane conductance regulator (CFTR) modulator (Vertex: clinical trial registration number NCT02797132) have commenced since. 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 overreading, 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, before preschool-specific training, has been beneficial. Standardization of equipment setup and testing protocol across sites within a single study is critical for all age groups, but becomes more important in preschool children because of 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 before 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., physiological considerations of inert gases, testing interface, reference data, and minimal clinically important differences).

Minimal Clinically Important Difference

To date the minimal clinically important difference in preschool subjects for LCI or any other MBW index, as well as respective intrasubject and intersubject variability,

remain poorly understood (14, 15, 88). In addition, the relationship between improvement in LCI and other surrogate end points such as FEV_1 or rate of FEV_1 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 have started to emerge. Aurora and colleagues described a mean (95% confidence interval) 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 and colleagues 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_1, 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).

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, while 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 because of limitations within the commercial software. Custom research-based software, developed for research-built MBW equipment, will also play a key supportive role in this progress. Commercial software developers must recognize 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, is evaluated and transparently documented

before 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 optimize feasibility and breathing pattern stability in younger children with facemasks must be accompanied by the development of effective strategies to define optimal training regimens and timing of transition to a mouthpiece and nose-clip 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 coordinated 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., U.S. Food and Drug Administration 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_2 analysis, which may one day negate the need for sidestream O_2 analysis and adjustments for associated sample flow rate (92). The development of mainstream analysis for not just one but all gases measured also offers the opportunity to reduce analyzer response time further (e.g., to 10 ms in this case). This is important given the increased susceptibility of preschool MBW to sources of technical error. Current recommendations (<2 ml/kg) for equipment-related V_D 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 less than 1-ml/kg effective V_D 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 need for 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). Although 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 is essential for

standardization and validation in this age range and will increase the utility of the test in the future. ∎

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