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Employing the Forced Oscillation Technique for the Assessment of Respiratory Mechanics in Adults

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TITLE:

Employing the Forced Oscillation Technique for the Assessment of Respiratory Mechanics in Adults

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SUMMARY:

As the use of forced oscillation technique (FOT) is increasingly utilized to characterize respiratory mechanics, there is a need to standardize methods with respect to nascent technical guidelines and various manufacturer's recommendations. A detailed protocol is provided including FOT assessment and interpretation for two cases to facilitate the standardization of methods.

ABSTRACT:

There is increasing interest in the use of the forced oscillation technique (FOT) or oscillometry to characterize respiratory mechanics in healthy and diseased individuals. FOT, a complementary method to traditional pulmonary function testing, utilizes a range of oscillatory frequencies superimposed on tidal breathing to measure the functional relationship between airway pressure and flow. This passive assessment provides an estimate of respiratory system resistance (Rrs) and reactance (Xrs) that reflect airway caliber and energy storage and dissipation, respectively. Despite the recent increase in popularity and updated Technical Standards, clinical adoption has been slow which relates, in part, to the lack of standardization regarding the acquisition and reporting of FOT data. The goal of this article is to address the lack of standardization across laboratories by providing a comprehensive written protocol for FOT and an accompanying video. To illustrate that this protocol can be utilized irrespective of a particular device, three separate FOT devices have been employed in the case examples and video demonstration. This effort is intended to standardize the use and interpretation of FOT, provide practical suggestions, as well

as highlight future questions that need to be addressed.

INTRODUCTION:

The forced oscillation technique (FOT) or oscillometry was first introduced over 60 years ago[1] and affords measurement of respiratory mechanics *via* externally applied pressure oscillations superimposed during tidal breathing. In brief, pressure and airflow are measured at the mouth by transducers across a range of frequencies. Spectral analysis is then used to determine impedance (Zrs) or the amplitude and phase differences between pressure and airflow at each frequency[2,3]. Zrs represents the sum of forces opposing pressure oscillations and is typically characterized by components of resistance (Rrs) and reactance (Xrs). Rrs reflects the dissipative mechanical properties of the respiratory system (energy dissipation), whereas Xrs reflects dynamic elastance and inertia of the respiratory system (energy storage). Zrs assessment at multiple oscillation frequencies further allows assessment of the uniformity of airflow distribution. For a review of FOT signal processing, physiological principles, and applications please refer to the European Respiratory Society (ERS) Task Force statements[2,4].

FOT is not a substitute for spirometry, rather a complementary assessment of the lung function. It may, however, offer several advantages over spirometric testing including measurements performed during tidal breathing (effort-independent) and potential for assessing the distal or small airways that are not feasible with spirometry[5]. As a result, FOT has gained considerable popularity in the pediatric setting[6,7], as well as for the evaluation of the symptomatic patient with normal or preserved spirometry[8–11]. FOT has also demonstrated clinical utility during bronchoprovocation testing whereby symptoms are more strongly associated with FOT than spirometry[12]. Moreover, FOT necessitates lower doses of bronchoprovocative agents to induce measurable differences in respiratory function[13].

In light of these findings, interest in FOT for clinical practice and research has surged in recent years. In fact, according to a Scopus search conducted in July 2021 for the terms ‘forced oscillation technique’ or ‘impulse oscillometry’, the median number of publications on FOT increased from 35 per year (2000–2010) to 94 per year (2010–2020). Despite this surge of interest, standardization in the acquisition and reporting of FOT data has only recently received greater attention with the recent ERS Technical Standards for Respiratory Oscillometry[4]. At present, several FOT systems are commercially available that vary by pressure signal type (e.g., pseudorandom, train of impulses), recording epoch, frequency range, and resolution[14]. Despite these differences, the acquisition and reporting of FOT data as performed by the technician can follow a universal approach which is the focus of the present manuscript. Herein, a standardized protocol is provided that is consistent with the ERS Technical Standards[4]. This protocol is illustrated through practical examples with research and clinical data acquired in our laboratory. Specifically, the focus is on the application and interpretation of FOT in the clinical evaluation of adult dyspnea.

PROTOCOL:

The following protocol was approved by Rutgers University Institutional Review Board. All volunteers participating in this study have given written informed consent prior to all testing.

1. Pre-test preparation

1.1. Assess the individual for allergies or sensitivity to mouthpiece or nose clip materials, for oral or facial pain preventing proper seal on the mouthpiece, for an ability to follow directions, and for known sensitivity to the bronchodilating agent that will be used.

1.2. Ensure that the individual dresses comfortably and refrains from exercising or ingesting a heavy meal before testing. Refer to local laboratory policies regarding the use of caffeine, tobacco products, or inhaler before testing.

1.3. Perform FOT first in situations of multiple pulmonary function tests requiring deep breaths.

1.4. Perform testing in a quiet and comfortable environment. Prepare supplies and materials prior to the individual's arrival.

1.4.1. Provide an adjustable chair without wheels to ensure that the individual's feet are flat against the floor.

1.4.2. Provide the individual with a disposable anti-bacterial filter and nose clip to be used for testing.

1.4.3. Adhere to local laboratory procedures for donning personal protective equipment when testing.

2. Verification with impedance test load

2.1. Locate the test load object before testing the individual.

NOTE: Static test loads are manufacturer-supplied objects with known impedance (preferably with resistive, elastic, and inertial components) that are specific to each device. Use a test load with an impedance of approximately 15 $hPa \cdot s \cdot L^{-1}$, which exceeds the expected Zrs for adults.

2.2. Ensure that the test load is factory calibrated (if applicable).

NOTE*:* Some test loads require annual factory recalibration, so follow the protocol outlined in the device manual.

2.2.1. Consult the manual or contact the manufacturer if the test load for verification is accidentally dropped or visually appears damaged.

2.3. Open the calibration or verification menu within the software.

2.4. Firmly insert the test load device into the FOT device and complete the verification

procedure according to the manufacturer's recommendations.

2.5. Review and save the verification results.

NOTE*:* A successful verification ensures that the measured values match the test load and will be indicated in the software. If the verification fails or gives errors, ensure that the test load was properly seated into the FOT device and there is no obstruction in the flow. Consult the manual for troubleshooting tips.

2.6. Verify the device with the test load daily, or immediately before testing.

3. Test procedure

3.1. Provide standardized instructions and demonstration for the individual.

3.1.1. Let the individual know about the approximate duration of a single acquisition and the number of replicates that will be taken (see step 4.2).

3.1.2. Let the individual know about the sensations that the individual will experience from the oscillations, e.g., fluttering or vibrations in the chest and mouth.

3.1.3. Let the individual know that the device will start oscillations after a brief period of observation to regulate breathing.

3.1.4. Instruct the individual to avoid swallowing during the testing period.

3.1.5. Instruct the individual to sit upright with the feet flat on the floor and the chin facing up for the duration of the testing period.

3.1.6. Instruct the individual to create a seal with the lips and teeth on the mouthpiece *via* a demonstration.

3.1.7. Instruct the individual to keep the tongue relaxed.

3.1.8. Instruct the individual to firmly place open palms against cheeks with fingertips near the temple and thumbs following the mandibular line. Instruct the individual to keep the elbows slightly flared in a comfortable position to ensure chest expansion.

3.1.9. Instruct the individual to maintain regular quiet breathing on the mouthpiece until asked by the technician to stop.

3.2. Perform measurement session

3.2.1. Adhere to hygiene and infection control standards as described for spirometry[15].

3.2.2. Attach the anti-bacterial filter to the device.

NOTE: Use filters that meet ATS/ERS guidelines with a resistance <1.5 $hPa \cdot s \cdot L^{-1}$ at a flow rate less than 14 L/s as verified by the manufacturer.

3.2.3. Provide instructions as described in step 3.1 and ensure that the individual is positioned correctly with the nose clip in place and mouth tightly sealed around the mouthpiece of the device.

3.2.4. After the individual completes several respiratory cycles of stable, passive, and comfortable tidal breathing, ensure that the device automatically begins acquiring data. Alternatively, the technician may trigger data acquisition using the software.

3.2.5. Instruct the individual to come off the mouthpiece after at least three artifact-free breaths are acquired during a single acquisition.

NOTE: To achieve three artifact-free breaths, a minimum recording duration of 30 s is recommended. Some FOT devices' settings will automatically stop at a pre-defined recording duration and/or achievement of a certain number of breaths (see section 4 for details on identifying artifacts).

3.2.6. Adjust the rest intervals between replicate measurements (approximately 60–90 s) as needed to avoid any physical discomfort.

3.3. Optionally, assess the bronchodilator response.

3.3.1. Administer salbutamol to the individual in accordance with standard laboratory procedures for aerosol medications (e.g., metered dose inhaler, nebulizer) and wait for 15 min[16].

NOTE: If using a metered dose inhaler with a spacer, administer four separate doses of 100 µg.

3.3.2. Repeat the same procedures as before (see step 3.2) to obtain post-bronchodilator replicates.

4. Determining acceptable measurements

4.1. Identify artifacts through visual inspection. To do so, monitor the depth (tidal volume; Vt) and rate of breathing (respiratory frequency; f_R) in real-time during acquisition to visually ensure stable and quiet breathing patterns from replicate to replicate.

NOTE: For each replicate, the average Vt, f_R, or their product (minute ventilation, $\dot{V}E$) will be displayed within the software. Compare this value between replicates in order to provide individual feedback on the depth and rate of breathing, if necessary.

4.2. Inspect the replicate manually to exclude artifacts such as cough, swallowing, leak, or other interruptions to flow and pressure traces that can be viewed in real-time.

4.3. Discard any replicates containing negative resistances.

4.4. Review automatic software detection of artifacts.

NOTE: Manufacturers employ software algorithms for detecting artifacts and excluding whole or partial breaths (i.e., inspiration and expiration). Get familiarized with the algorithms applied and report this when summarizing data from a measurement session. Often, these algorithms involve identifying Rrs, Xrs, and breathing patterns outside of normal physiological ranges as well as outliers when comparing breath-by-breath.

4.5. Assess variability

4.5.1. Acquire at least three acceptable replicates (i.e., those containing ≥3 artifact-free breaths). Calculate the within-session coefficient of variation (CoV) for total Rrs at the lowest frequency (e.g., Rrs at 5 Hz).

NOTE: CoV is calculated using the following formula:

$$CoV = \frac{Standard\ Deviation}{Mean}\ x\ 100$$

4.5.2. As the acceptable within-session CoV for adults is ≤10%, obtain additional replicates if the CoV is >10% or proceed to step 5 if CoV is ≤10%.

NOTE: Achieving CoV ≤10% may be difficult in individuals with airway disease.

5. Reporting data

5.1. Include the following details when reporting FOT results.

5.1.1. Include the device name, model, software version, and manufacturer.

5.1.2. Include input stimulus frequency waveform (e.g., pseudo-random noise, multi-frequency) and associated frequency range.

5.1.3. Include the details on subjective and automatic quality control procedures used to determine acceptable replicates and the number of artifact-free replicates included.

5.1.4. Include the repeatability or precision of measurement (CoV) and cut-off.

5.2. Report the mean of the three replicate measurements that were free of artifact and a CoV ≤10% for FOT parameters.

5.2.1. Adhere to laboratory standards regarding which FOT parameters to report.

NOTE: While there is currently no consensus on which FOT variables to include, the ERS Technical Standard provides an example of what parameters might be reported as shown in **Table 1** for the case example results presented below.

5.3. Utilize reference equations from the population being studied using the same FOT device (if available).

NOTE: Many reference equations will assume accurate recording of age, sex, height, and weight[14].

5.4. Optionally, report both the absolute and relative difference if FOT was performed before and after a bronchodilator. Also, include the dose of salbutamol.

6. Quality control and maintenance

6.1. Employ a quality control program using biological controls (i.e., ≥2 healthy non-smoking individuals) that involves routine testing on a periodic basis.

6.1.1. Establish a baseline (mean ± SD) through the acquisition of 10–20 artifact-free replicate measurements on different days (acquired within 2 weeks) from each biological control.

6.1.2. Select a low- (5 Hz) and mid-frequency (20 Hz) parameter for resistance and reactance to follow for quality control. On subsequent routine periodic testing, compare the results to the baseline measures.

NOTE*:* Refer to recommended guidance for pulmonary function laboratories[17] for additional details on how to assess and enact quality assurance standards. The frequency of biological control testing (e.g., weekly, monthly) should reflect the volume of testing in the laboratory.

6.2. Follow the manufacturers' recommendations on regular maintenance such as cleaning, air filter change, software updates, and factory calibration.

REPRESENTATIVE RESULTS:

First, a case of a healthy adult is presented as a practical example of data acquisition and how the technician selects individual measurements for reporting (Case Example 1). Second, a clinical example is provided of a patient referred for unexplained dyspnea for FOT acquisition before and after a bronchodilator with emphasis on interpretation (Case Example 2). Note that FOT devices from two different manufacturers have been purposefully used in these case examples to illustrate a universal approach. Additional details are provided in the **Table of Materials**.

Case Example 1

FOT was performed in a healthy 25-year-old Hispanic female (Height: 164 cm, Weight: 84.9 kg). The participant was a never-smoker, denied respiratory symptoms, and had no history of lung disease or other significant past medical history. She had abstained from caffeine (≥8 h) and vigorous exercise (≥24 h). She had a recent spirometric examination that was read as normal without signs of obstruction or restriction: FEV_1/FVC: 0.88, FEV_1: 3.30 L (98% predicted), and FVC: 3.70 L (97% predicted).

After explaining and demonstrating test procedures, three FOT measurements were obtained with approximately 1–2 min between recordings. Visual inspection and the software's quality control algorithm did not identify any artifacts. Rrs at 5 Hz for the first three measurements were then examined to confirm within-session CoV (individual measurements: 3.06, 3.79, 3.46 $hPa \cdot s \cdot L^{-1}$; average: 3.44 $hPa \cdot s \cdot L^{-1}$, standard Deviation: 0.36 $hPa \cdot s\ L^{-1}$, CoV = standard deviation / average = 0.36 / 3.44 = 0.105 * 100 = 10.5%).

Since the CoV of the first three measurements was >10%, additional measurements are necessary. A fourth measurement was obtained (Rrs at 5 Hz = 3.40 $hPa \cdot s \cdot L^{-1}$) and within-session CoV was recalculated using all measurements (individual measurements: 3.06, 3.79, 3.46, 3.40 $hPa \cdot s \cdot L^{-1}$; average: 3.43 $hPa \cdot s \cdot L^{-1}$; standard Deviation: 0.30 $hPa \cdot s \cdot L^{-1}$; CoV = standard deviation / average = 0.30 / 3.43 = 0.087 * 100 = 8.7%)

Now that the within-session CoV criteria are met, average FOT indices were calculated as the average of measurements and illustrated in **Figure 1** and reported in **Table 1**. Additionally, to facilitate comparison to expected values, **Table 2** presents predicted values across all FOT indices (where predicted values are available), lower limits of normal (LLN), upper limits of normal (ULN), % of predicted and Z-scores using standard reference equations that consider age, sex, and weight[14].

Case Example 2

A 48-year-old Caucasian male (Height: 185 cm, Weight: 89 kg) was referred to our center for evaluation of chronic cough and exertional dyspnea without obvious cause (e.g., medication, respiratory or cardiovascular disease, or mental health comorbidity). He was a lifetime never-smoker but endorsed exposure to vapors, gases, dust, and fumes during a 7-month military deployment to Iraq. Complete pulmonary function testing was performed (i.e., body plethysmography, bronchodilator spirometry, and lung diffusing capacity for carbon monoxide) and all results were within normal limits. FOT was performed before and 15 min after administration of bronchodilator (4 puffs of 100 µg salbutamol *via* metered-dose inhaler with spacer) (**Figure 2**). The individual trial data and mean values are presented in **Table 3** pre- and post-bronchodilator administration; as each trial was technically acceptable, the pre- and post-bronchodilator measurements, as well as their absolute and relative difference, are reported in **Table 4**. In addition, predicted values, % of predicted, LLN, and ULN are also reported using standard reference equations that consider age, sex, and weight[14].

We delimited variables reported in **Table 3** and **Table 4** to simplify the illustration of two concepts: 1) determining abnormal versus normal responses, and 2) bronchodilator reversibility. For Rrs measurements, values that exceed the ULN (i.e., elevated resistance) are considered abnormal. Here, pre-bronchodilator Rrs at 4 Hz (3.32 $hPa \cdot s \cdot L^{-1}$) exceeds the ULN (2.59 $hPa \cdot s \cdot L^{-1}$) and is 155% of the predicted value ([3.32 / 2.14] * 100 = 155.14). Following bronchodilator administration, Rrs at 4 Hz was reduced by 45.78% exceeding the 95th percentile reported by Oostveen et al.[14] (i.e., -32% for Rrs at 4 Hz). This response would indicate a positive bronchodilator response in resistance. Additionally, the post-bronchodilator observed value is normalized (i.e., became representative of what is considered a normal value) and is 84.1% of the predicted value ([1.80 / 2.14] * 100 = 84.11).

Xrs at 4 Hz is interpreted differently as observed values are negative. Therefore, abnormal values are those that exceed the LLN (i.e., more negative reactance). Here, the individual had a pre-bronchodilator (-0.98 $hPa \cdot s \cdot L^{-1}$) and post-bronchodilator (-0.83 $hPa \cdot s \cdot L^{-1}$) values that are above the LLN (-1.11 $hPa \cdot s \cdot L^{-1}$). The difference in pre- versus post-bronchodilator was approximately 15%, which is below the 95th percentile reported by Oostveen et al.[14] (i.e., +33.8% in Xrs at 4 Hz). Therefore, all Xrs values are considered normal.

Reactance area (or AX) is the integrated area of low-frequency reactance and, therefore, is a positive value. Abnormal AX values are those that exceed the ULN, reflecting more negative reactance. Like Xrs at 4 Hz, pre-bronchodilator AX (2.77 $hPa \cdot s \cdot L^{-1}$) and post-bronchodilator AX (1.23 $hPa \cdot s \cdot L^{-1}$) are both below the ULN. Although there was a reduction of -55% from pre- to post-bronchodilator value, this falls below the 95th percentile reported by Oostveen et al.[14] (i.e., -56.0% for AX at 4 Hz). Taken together, AX is considered normal as well.

FIGURE AND TABLE LEGENDS:

Figure 1: Respiratory resistance (Rrs) and reactance (Xrs) as a function of oscillation frequency (Hz) in a healthy adult. Mean ± SD of all replicates are plotted for Rrs (blue circles) and Xrs (red squares) at each measured frequency. Each data point represents total or whole-breath measurements. Data were collected using a pseudorandom, relative primes signal type in the 5–37 Hz range. Please see the **Table of Materials** for additional details regarding this device.

Figure 2: Pre- and post-bronchodilator assessment. Respiratory resistance (Rrs; blue) and reactance (Xrs; red) before (open circles) and after (open triangles) bronchodilator administration. Dashed red lines represent the upper and lower limits of normal for Rrs and Xrs, respectively[14]. Data were collected using a pseudorandom signal type in the 4–48 Hz range. Please see the **Table of Materials** for additional details regarding this device.

Table 1: Standard reporting of select FOT parameters: Trials summary. This table illustrates all measurement replicates across trials (T1–T4) and their summary statistics (averages and standard deviations (SD)). The average values across all trials are used to represent the test session. Common parameters are listed under Variable. Resistance (Rrs) and reactance (Xrs) are provided for whole breaths at 5, 11, and 19 Hz, as well as during inspiration at 5 Hz (Rrs5(insp) and Xrs5(insp)). Additional parameters reported include reactance area (AX) at 5 Hz, resonant

frequency (Fres), and tidal volume (Vt).

Table 2: Standard reporting of select FOT parameters: Reference and predicted values. There is currently no consensus on which FOT parameters to include in a basic report; however, the ERS Technical Standard provides an example of what parameters might be reported[4], which are included in the accompanying table. This table illustrates the averaged measurement values reported from the test session as well as the accompanying reference values currently available. Common parameters are listed under Variable. Resistance (Rrs) and reactance (Xrs) are provided for whole breaths at 5, 11, and 19 Hz, as well as during inspiration at 5 Hz (Rrs5(insp) and Xrs5(insp)). Additional parameters reported include reactance area (AX) at 5 Hz and resonant frequency (Fres). For those parameters with reference values available[14], predicted, % predicted, lower and upper limits of normal (LLN, ULN), and Z-score values are also calculated.

Table 3: Interpreting low-frequency resistance (Rrs), reactance (Xrs), and reactance area (AX): Trials summary. This table illustrates all measurement replicates across trials (pre- and post-bronchodilator) and their summary statistics (averages and standard deviations (SD)). The average values across all trials are used to represent the test session's values for baseline averages (pre-bronchodilator) and post-bronchodilator averages.

Table 4: Interpreting low-frequency resistance (Rrs), reactance (Xrs), and reactance area (AX): Reference and predicted values. Low-frequency (4 Hz) Rrs, Xrs, and AX are reported along with the corresponding predicted values, % of predicted, and the lower (LLN) and upper (ULN) limits of normal[14]. Measurements before (Baseline Avg) and after (Post BD Avg) bronchodilator are presented along with their corresponding absolute and relative change (% Change).

DISCUSSION:

The recent ERS Technical Standard on FOT[4] emphasized the need for greater rigor and standardization of measurement. Close adherence to several critical steps before, during, and after testing is necessary. It is recommended that FOT be performed prior to more effort-dependent maneuvers requiring deep breaths such as body plethysmography and diffusing capacity. End-user verification of test load with known impedance is required at least daily or immediately prior to testing. Clear, consistent, and precise instructions given by trained personnel can minimize extrinsic variabilities in data collection. Each research or clinical laboratory should develop its own protocol implementing the minimal coaching techniques recommended by the ERS technical guidelines. It is critical that during each maneuver the end-users can observe, identify, and correct potential errors that may be encountered, such as mouth leaks, glottic closure, coughing, and unstable breathing patterns. Although certain errors may be difficult to evaluate in real-time, end-users should not solely depend on automatic detection from the specific device used. Acceptable criteria set by the manufacturer should be thoroughly reviewed, and additional criteria should adhere to the ERS statements. Though each device will generate a unique report, standardized reporting of FOT parameters is possible and can facilitate comparison across laboratories and studies. Lastly, rigorous quality control procedures, including routine assessment of healthy biological control(s), must be performed in both research and clinical settings.

Strict adherence to a standardized protocol will minimize variability in performance. However, achieving a CoV ≤ 10% may still be difficult, and perhaps not always possible in those with airway disease. It is incumbent on the technician to strive toward minimizing variability and there are several strategies to consider when a CoV ≤ 10% cannot be obtained. Firstly, ensure the measurement is acquired under similar circumstances for each replicate. This includes monitoring the individual's posture, hand placement, and adherence to other instructions. The technician may consider repeating initial instructions, providing additional visual demonstration, and offering the individual a prolonged rest interval. Based on experience, it is found that a common reason for excessive variability includes adopting a different sitting position between replicate measurements whereby individuals may re-position themselves to achieve a more comfortable position or strain to reach the mouthpiece. This is most common when using portable FOT devices designed to be held by the technician where the position of the mouthpiece is not fixed. To address this issue, flexible arm mounts have been purchased, which are designed to hold electronic devices like cameras, that can be quickly secured to a desk or table and accommodate individual positioning. After ensuring performance is appropriate and consistent between replicate measurements, the technician should acquire additional replicates.

Unlike spirometry whereby a maximum of eight attempts is recommended to avoid fatigue, there is no maximum number of replicates recommended for FOT likely because of its effort-independent approach. In practice, some investigators acquire up to eight replicate measurements[18], and a similar rule of thumb of up to 10 measurements is used in our laboratory. Establishing an upper limit is practically important to define the end of a testing session. Doing so is particularly relevant for individuals with respiratory disease whereby CoV greater than 10% may reflect underlying disease processes rather than poor effort. Harkness et al.[18] recently described their experience with these patient populations and suggested that a more liberal cut-off (CoV up to 20%) may still be reportable for clinical interpretation. Each clinic and research laboratory should balance between practical decisions such as time constraint, examinee's ability and fatigue level, as well as the likelihood of achieving the CoV cutoff. One approach to consider is the implementation of a grading system. For example, once at least three artifact-free replicate measurements are obtained from a maximum of 10 attempts, apply a letter grade corresponding to CoV levels – i.e., 'A' ≤ 10%; 'B' > 10% and ≤ 15%; 'C' > 15% and ≤ 20%; and 'D' > 20%. Additional strategies to be considered may include modification of software and hardware acquisition parameters to achieve more complete breaths. For example, some manufacturers have settings to accommodate greater recording durations and/or extended recording epochs to achieve more than the ERS-recommended minimum of three complete breaths. When reporting FOT results, it is imperative to disclose all acquisition parameters to facilitate interpretation and comparison with other published literature. FOT acquisition parameters continue to be actively investigated and result in future modifications to FOT performance and measurement.

In this paper, the aim is to highlight the latest technology and usage of FOT, as well as its fundamental procedure of testing in adults. It is, however, important to recognize FOT's associated limitations. First, impedance measurements are particularly suspect to artifacts such as extra-thoracic influences[4]. Therefore, the current protocol focuses on minimizing this

influence, such as ensuring proper cheek support during acquisition. Additionally, interruptions in flow (e.g., tongue covering the mouthpiece, swallowing, errant breaths) preclude accurate measurement and result in fewer valid breaths for Zrs calculations[19]. Second, though FOT is easy to perform from the patient's perspective, identifying these artifacts as well as interpreting the output is challenging for the technician and clinician[20]. For example, current FOT devices produce a considerable amount of data to characterize an individual's respiratory mechanics; however, the paucity of reference values and consensus around key variables are factors that slow its clinical adoption. Similarly, while it is recommended to obtain at least three artifact-free trials[4], if more than three trials are performed and found acceptable, there is no current consensus on the recommended methods to select which of these trials are used to represent the test session. As such, the clinical utility of FOT in a variety of airway diseases continues to be actively investigated. Lastly, from a technical perspective, there is heterogeneity across FOT manufacturers with respect to the following: i) frequency waveforms, ii) algorithms for error detection, and iii) inter-and intra-breath analyses[2,21–24]. Much of the aforementioned limitations may be addressed by following a standardized protocol as well as transparent reporting of output and recording parameters.

Pulmonary function tests traditionally include measurements of lung volumes and capacities, and effectiveness of gas exchange, which require significant instructions, cooperation, and effort from both examiners and examinees. In addition, a mixture of gases at various concentrations is often inhaled during maneuvers, which some might consider invasive techniques. These contrast with FOT, in which mechanical properties of the lungs such as Rrs, elastance, and inertance are examined using less invasive oscillatory frequencies. Thus, FOT can serve as a useful addition to a comprehensive pulmonary function assessment. For example, FOT may afford unique clinical insight in scenarios where symptoms are disproportionate to traditional pulmonary function testing such as those with occupational exposure and/or unexplained dyspnea[9,11]. Additionally, FOT may also be important for screening those at higher risk for future lung diseases such as asymptomatic smokers[25] and those with environmental exposures[26]. Lastly, more recent data has identified that FOT may also be uniquely helpful for day-to-day monitoring of certain disease conditions such as exercise-induced bronchoconstriction[27] and rheumatoid arthritis-related pulmonary symptoms[28]. The present article focuses on FOT's application in the adult population, though FOT's clinical and research utility has been well described in pediatric populations as well[29,30].

Future directions for research should further focus on technical and performance aspects of FOT, such as standardizing data presentation and reporting, as well as characterizing associated variability and repeatability. In clinical settings, FOT can be widely used for the assessment of dyspnea and early detection of chronic airway diseases or systemic diseases-associated pulmonary manifestations in all age groups.

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Figure

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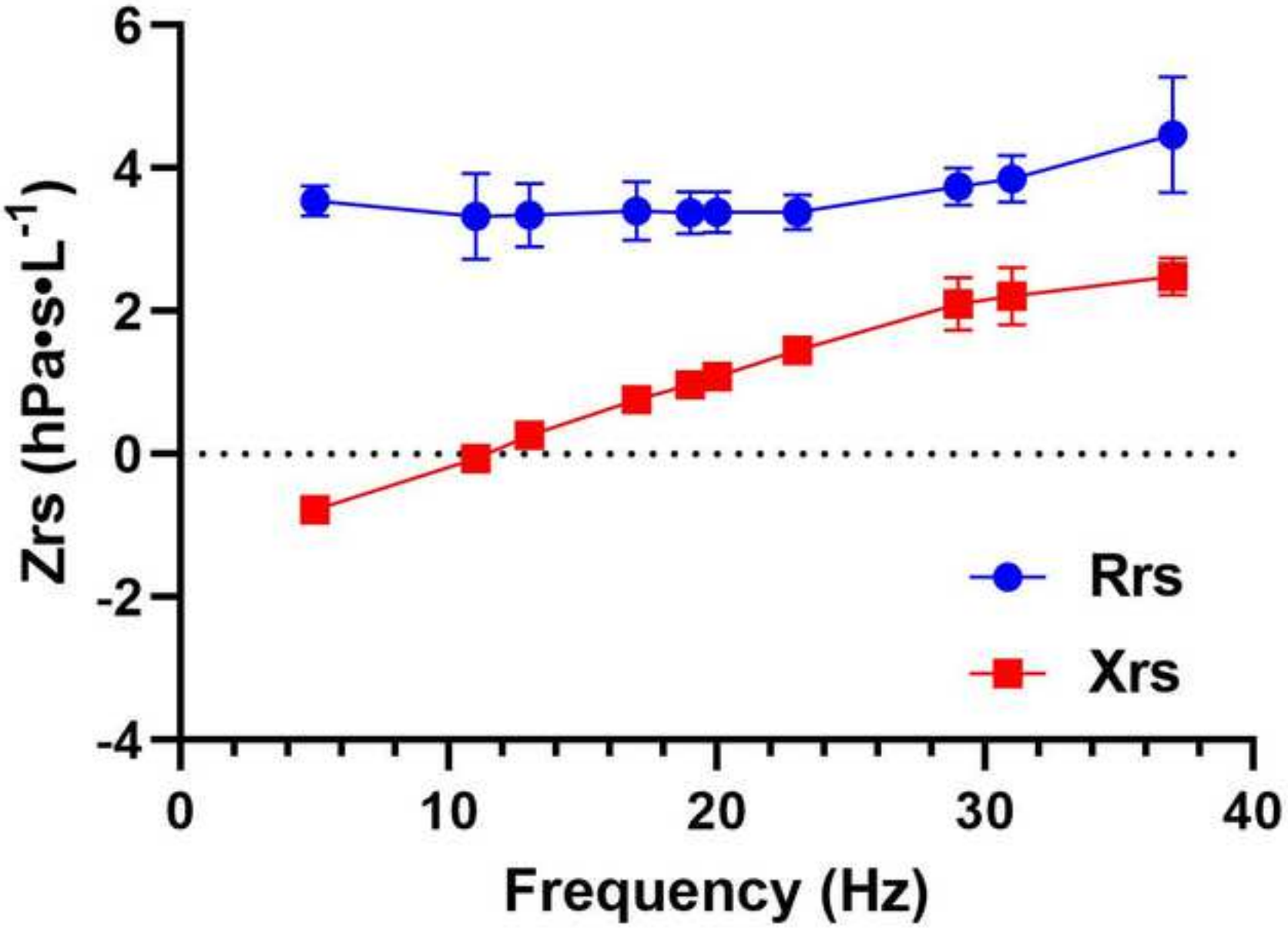

Figure

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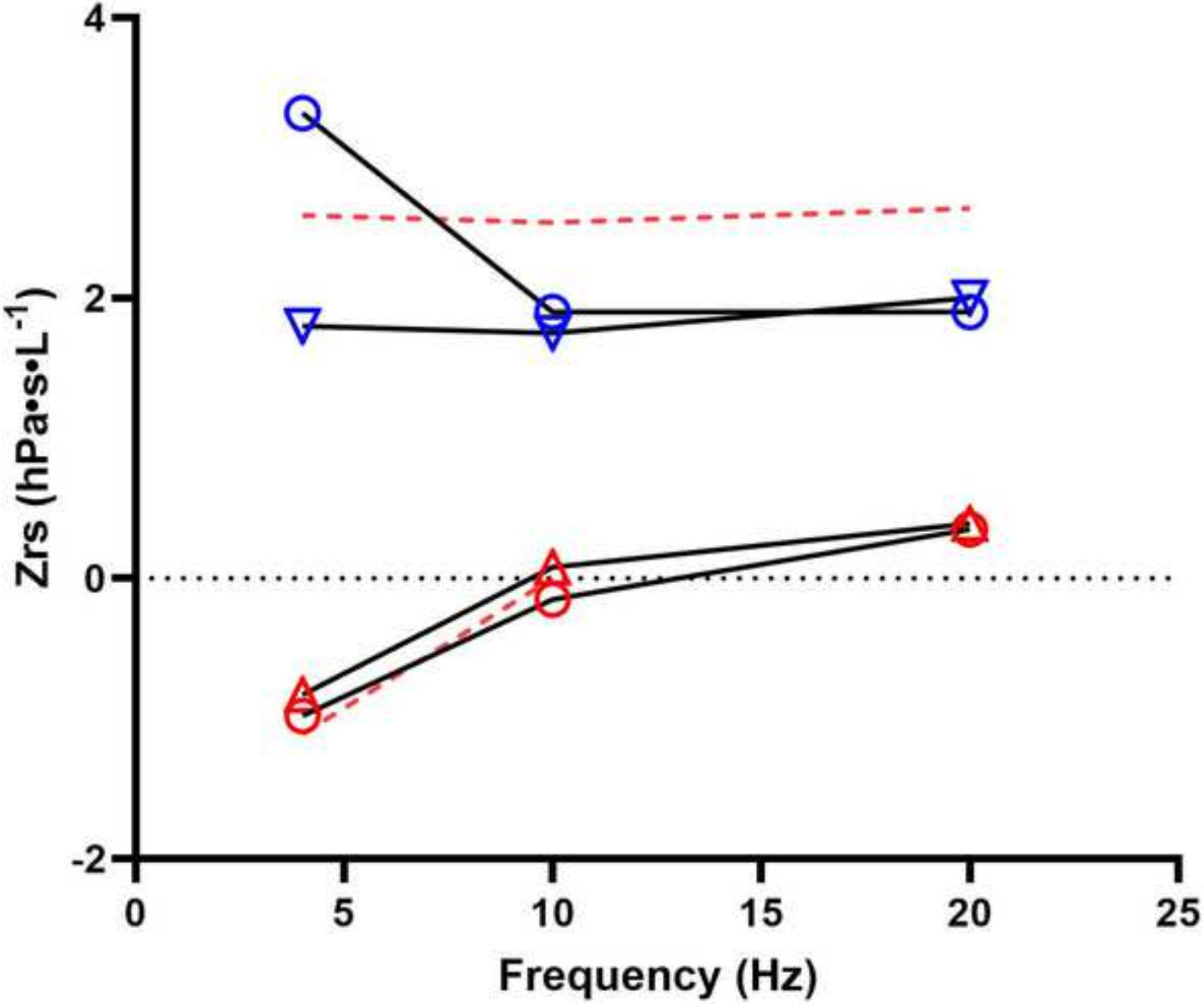

Table

Variable	T1	T2	T3	T4	Avg	SD
Rrs5	3.06	3.79	3.46	3.40	3.43	0.30
Rrs5 (insp)	3.30	3.45	3.34	3.64	3.43	0.15
Rrs11	2.77	4.02	3.08	2.89	3.19	0.57
Rrs19	2.92	3.71	3.30	3.13	3.27	0.33
Rrs5-19	0.14	0.08	0.15	0.26	0.16	0.08
Xrs5	-0.90	-0.76	-0.69	-0.90	-0.81	0.11
Xrs5 (insp)	-1.44	-0.91	-0.86	-1.08	-1.07	0.26
Xrs5 (exp)	-0.63	-0.46	-0.55	-0.77	-0.60	0.13
Delta Xrs5	-0.81	-0.45	-0.31	-0.31	-0.47	0.24
Xrs11	-0.04	-0.09	0.00	-0.09	-0.06	0.04
Xrs19	0.92	0.86	1.12	0.94	0.96	0.11
AX	2.83	2.57	2.05	2.98	2.61	0.41
Fres	11.27	11.62	10.99	11.57	11.36	0.29
Vt	0.90	0.98	0.95	0.61	0.86	0.17

Table

Variable	Predicted	LLN	ULN	Baseline Avg	% of Predicted	Z Score
Rrs5	3.76	-	4.11	3.43	91%	-0.34
Rrs5 (insp)	-	-	-	3.43	-	-
Rrs11	2.74	-	3.18	3.19	116%	-0.33
Rrs19	3.52	-	3.92	3.27	93%	-0.3
Rrs5-19	0.14	-	-	0.16	118%	0.05
Xrs5	-1.37	-1.50	-	-0.81	59%	1.32
Xrs5 (insp)	-	-	-	-1.07	-	-
Xrs5 (exp)	-	-	-	-0.60	-	-
Delta Xrs5	-	-	-	-0.47	-	-
Xrs11	-0.14	-0.26	-	-0.05	36%	0.22
Xrs19	-	-	-	0.96	-	-
AX	4.08		5.11	2.61	64%	-0.64
Fres	12.73	-	13.14	11.36	89%	-

Table 3

	Pre-Bronchodilator					Post-Bronchodil		
Variable	T1	T2	T3	Avg	SD	T1	T2	T3
Rrs	3.34	3.21	3.42	3.32	0.11	1.81	1.89	1.69
Xrs	-1.25	-0.72	-0.98	-0.98	0.26	-0.42	-1.32	-0.74
AX	2.50	2.02	2.79	2.44	0.39	0.73	1.95	1.01

lator

Avg	SD
1.80	0.10
-0.83	0.45
1.23	0.64

Table 4

Variable	Predicted	LLN	ULN	Baseline Avg	% of Predicted	Post BD Avg	% of Predicted
Rrs	2.14	NA	2.59	3.32	155%	1.80	84%
Xrs	-0.97	-1.11	NA	-0.98	101%	-0.83	86%
AX	2.15	NA	3.08	2.44	113%	1.23	57%

Absolute Change	% Change
1.52	-45.78%
-0.15	15.31%
1.21	-49.59%

Editor

Please note that novelty is not a requirement for publication and reviewer comments questioning the novelty of the article can be disregarded. Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please revise the manuscript to thoroughly address these concerns. Additionally, please describe the changes that have been made or provide explanations if the comment is not addressed in a rebuttal letter. We may send the revised manuscript and the rebuttal letter back to peer review.

We thank the Editor for the opportunity to respond to these critiques to improve our submission. We have responded (in bold font) below with a point-by-point response to your comments as well as those from the Reviewers that we hope have addressed any concerns.

Specific Comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

   We have reviewed the manuscript for spelling/grammar and believe it is now ready for review.

2. Please provide an abstract between 150-300 words to clearly state the goal of the protocol. The current abstract is 124 words.

   The abstract has been revised and is now 187 words.

3. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly.

   The protocol section has been revised to reflect the imperative tense throughout. Where appropriate, we have added a 'note' to add additional details.

4. Please avoid the usage of the phrase "should be" throughout the Protocol.

   We have removed all use of 'should be' throughout the protocol and have replaced with imperative tense. Where we could not replace the phrase "should be", we used one protocol note.

5. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Readers of all levels of experience and expertise should be able to follow your protocol.
   a. Step 2: If this step needs to be filmed, please provide all the steps required to insert test load including calibration/recalibration, verification, as well as device verification. We need action items to show how this was done.

      This section has been revised to be explicit with specific steps. We have also added a step on review of calibration results.

   b. Step 3.2.2: How was the flow rate and filter resistance measured and adjusted?

      We have revised the statement to clarify that filters used must meet minimum standards per recommended guidelines. Manufacturers are expected to confirm their produced filters meets these standards.

   c. Step 3.2.4: How were the measurements acquired? If using a device attached to the mouthpiece, please provide all the steps needed to acquire these measurements on the device.

We have added additional language to clarify how measurements acquire. All of the steps that precede acquisition are described in 3.1

d. Step 3.3.1: What is the concentration of salbutamol used?

The following has now been added,

3.3.1.1 Note: If using a metered dose inhaler with spacer, administer four separate doses of 100 µg.

e. Step 4.1.1: Please mention all the steps needed to measure and monitor breathing depth and rate of breathing.

Only visual inspection is possible at this time with current devices. We have revised this statement accordingly.

f. Step 4.2.1: How was the CoV calculated?

Formula has now been provided.

6. Please include a single line space between each step, substep, and note in the protocol section. Please highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

We have edited the protocol to ensure proper spacing. Recommended essential steps are highlighted (yellow) in the protocol for the video.

7. Line 245: The value 3.06 is struck out. Please see if this is correct.

This has now been removed. We made an oversight that Reviewer 2 pointed out and this has now been corrected.

8. Please remove the embedded figure(s) and table(s) from the manuscript.

Now removed.

9. Please upload all tables individually as a .xlsx file.

Tables have now been moved to .xlsx files.

10. Please also include any limitations of the technique in the Discussion with citation (wherever applicable).

We have now substantively revised this section.

11. Please do not abbreviate journal names in references.

References have been updated accordingly.

Reviewer #1:

I think this is well-written, easy to follow and useful for respiratory labs who are currently using FOT or want to start using FOT. I only have a few minor suggestions/correction.

We thank Reviewer 1 for their time spent reviewing this manuscript and for the helpful suggestions. Please see our responses below in bold.

Minor Concerns:

1. Wrong paper cited: "Some investigators have acquired up to 8 measurements". It has ref 17, but it should be ref 18.

   Thank you for identifying this error. We have now corrected this mistake and updated the references accordingly.

2. For reporting for FOT parameters, is importing to show the upper/lower limits of normal or z-scores. This makes interpretation easier, are these values normal or abnormal? Also, I think it would be clearer to just report the mean values of all trials instead of every single trial mean (Table 1). My suggestions would be to include (mean raw value, predicted value, z-score, CoV).

   We would like to thank the reviewer for providing this comment. However, the Reviewer has both suggested we add more detail, as well as simplify what we are presenting. As such, we believe what is most important is that we have thoroughly and thoughtfully come to a decision for what we are presenting and why. We have discussed as a team and have updated our results tables. In the protocol section 5 (Reporting Data), we suggest to follow the ERS Technical Standards which are illustrative only of which FOT indices might be reported given that there is no consensus yet for which indices must be included – these are the variables or FOT indices we present in Table 1. Even as pointed out by the ERS guidelines, the final report of each laboratory should be decided by the end users given their specific purpose for using FOT (e.g., clinical vs. research).

   We had originally intended to focus our two tables on the most relevant parameters given their respective case examples. However, we agree that for consistency across both of our case examples (and with the illustrative example provided in the ERS Technical Guidelines), we have updated both tables to report the following information for the selected FOT indices (if applicable and available based on different FOT devices, measurements and software): Predicted value, LLN, ULN, Z-score, % of Predicted, Baseline value, Post BD value, Absolute change, and % Change. Plus, in a separate table for each case example, we are still maintaining the reporting of individual trial data with averages and standard deviations. As for the CoV calculation, this is already detailed in the case example text.

   These modified tables support our manuscript's multiple purposes. First, we provide a practical, hands on protocol for performing the FOT procedure. Second, we provide a more in-depth evaluation of the "behind the scenes" steps taken leading to the final reported outcomes. This level of detail can be very informative to other researchers, technicians and/or those who simply want to learn more about the FOT procedure. Accordingly, we have updated our original two tables to now present across 4 different tables the following as presented below. The updated tables have been provided as separate excel files as requested.

Data Presented	Case Example #1	Case Example #2
The "behind the scenes" data from each trial leading to the final reported FOT indices	**Table 1**	**Table 3**
The reported FOT indices plus parameters suggested by the ERS Technical Guidelines	**Table 2**	**Table 4**

3. Another suggestion would be to add grades to the quality of the FOT measurements or add that CoV <15% would also be acceptable if no artefacts are present. Like also mentioned in the discussion a CoV of 10% might not be feasible in a patient with severe airways disease. CoV is not only a QC measure, it is also characteristic of airways disease. For example, Grade A would be when there are at least 3 or more artefact-free breaths per trial and CoV<10%, grade B CoV <15, Grade C <20 etc…That can also be easily reported on. You would try to get at least grade A or B.

   We agree that CoV may reflect quality as well as airways disease, and now recognize that we could call attention to this point more clearly. Additional information is provided in the protocol as well as the discussion. A grading system is an excellent suggestion which we have now incorporated into the discussion. The following has now been added:

   > ***"An approach to consider is implementation of a grading system. For example, once at least three artefact-free replicate measurements are obtained from a maximum of 10 attempts, apply a letter grade corresponding to CoV levels – i.e., 'A' ≤ 10%; 'B' > 10 and ≤ 15%; 'C' > 15 and ≤ 20%; and 'D' >20%."***

4. Biological calibration performed weekly might be a lot (too much) for respiratory labs. Acceptable is when personal perform an initial collection of a minimum of 10 tests on different days to establish the BioQC range preferably within 2 weeks. After that, biological calibration performed monthly should be sufficient.

   This section has now been revised accordingly. Specifically, we now state:

   > ***"Employ a quality control program using biological controls (i.e., ≥ 2 healthy non-smoking individuals) that involves routine testing on a periodic basis. First establish a baseline (mean±SD) through acquisition of 10-20 artefact-free replicate measurements on different days from each biological control. Select a low- and mid-frequency parameter (Rrs and Xrs at 5Hz and 20Hz) for resistance and reactance to follow for quality control. On subsequent routine periodic testing, compare results to baseline measures."***

Reviewer #2:
Generally, the manuscript is well written. There is a lack of respiratory oscillometry protocols and standards available, so the protocol outlined does serve as a useful tool. However, additional information to detect artefacts will be useful. Examples of troubleshooting may also be helpful to readers (such as inability to obtain CoV ≤10).

We thank Reviewer 2 for their critique and time spent reviewing our work. We appreciate the suggestions and have responded in bold below. Please note that we have added considerably more troubleshooting examples to the manuscript for identifying artefacts and minimizing variability. If accepted, this will also be an important focus of the video recording.

Major Concerns:
The author described two case studies but did not describe the Oscillometry device used. Although the spectral frequency range were mentioned, it appears that these results were collected by two different device. This was confusing to follow. In the protocol, section 5 describes how the oscillometry data should be reported, but the two case studies results were some critical information that were outlined in the protocol such as frequency waveform, device name, model, software version and manufacturer. Many information in the protocol are described from the 2020 ERS Technical Standards Guideline. While they should be aligned, the author should include information that they may have learned during oscillometry testing.

It is correct that we used two different devices in our case examples and propose a third device for the video. We felt strongly that doing so would illustrate a universal approach to FOT rather than one that is device specific.

In our initial submission, we deliberately omitted these details regarding device name and model as we believed that was the requirement of the Journal. To address this concern, we contacted the Editor and received the following response:

> ***"…manuscripts with commercial terms cannot be published by the journal. The details of the frequency waveform can be included in the protocol while the details regarding device name, model, manufacturer, etc. can be mentioned in the table of materials which can then be referenced in the protocol. Please avoid the use of ™/®/© symbols in the table of materials."***

As such, we have updated our 'Table of Materials' attachment to include software version as well as referenced this table in the manuscript for the readers.

Minor Concerns:
Some details described in the protocol were vague (see notes below):

We have made significant revisions to the protocol in response to feedback from the Editor, Reviewer 1, and your suggestions below.

1. Rest intervals between measurements - lack example,

Additional details are now provided.

2. Zrs of the anti-bacterial filter used during testing should be accounted for - this seems to be the standards in PFTs already.

We agree this is fairly standard guidance with respect to filters. However, the ERS Technical Standard recommends *"…to regularly measure the resistances of the filters and to compensate for the combined resistances of the oscillometric system + filter*." In our experience with three separate FOT devices, only one device is able to directly measure the resistance of the filter. If accepted, we will illustrate this process in the video. We have moved this step to quality control procedures.

3. Generally two or more biological controls are recommended in the event of variance in such individual, such as illness.

We agree, this section has now been updated.

4. Case Example #1 why was 3.06 excluded instead of reporting 4 acceptable measurements? The CoV would be < 10%.
Thank you for pointing this out. This was an oversight on our part.

5. Case Example #2 The discussion between measurement at 4Hz and 5Hz is confusing. Please choose one set of reference when reporting.

We have now removed reference to 5 Hz criteria.