FLEXIVENT VERSUS UNRESTRAINEDWHOLE BODY PLETHYSMOGRAPHY

THIS DOCUMENT COMPARES THE FLEXIVENT SYSTEM TO THE UNRESTRAINED WHOLE BODY PLETHYSMOGRAPHY METHOD OF ASSESSING LUNG FUNCTION AND DISCUSSES THE TRADE-OFFS IN TERMS OF LEVEL OF INVASIVENESS AND MEASUREMENT PRECISION.

KEYWORDS: flexiVent, unrestrained plethysmography, respiratory mechanics, enhanced pause, invasiveness

SCIREQ COMPARATIVE ANALYSES provide a direct comparison of the features of SCIREQ's solutions for pre-clinical research to other commercially available products. The information contained in this document applies to flexiWare 7. For more recent versions of the document, please contact us.

1. INTRODUCTION

1.1. Objectives

This document discusses techniques and setups that aim to measure pulmonary function for applications such as the assessment of the bronchoconstrictor response to inhaled methacholine in airway hyper-responsiveness research, monitoring lung function in safety pharmacology applications, or phenotyping of animal models of various lung diseases.

1.2. The flexiVent

The flexiVent is a unique, integrated platform for pre-clinical pulmonary research. Using a fully computer-controlled architecture, it offers, in addition to advanced mechanical ventilation, a wide range of preset or user-defined measurement manoeuvres for a classic, refined, or exhaustive characterization of the respiratory function. Sophisticated measurement and analysis techniques along with leading software features ensure that accurate, reproducible measurements can easily and rapidly be obtained.

The flexiVent seamlessly combines within a single device a wide range of measurements or manoeuvres specifically designed to assess the lung function in one way or another. These include classic resistance and compliance measurements, advanced and detailed respiratory mechanics measurements using the Forced Oscillation Technique, pressure-volume loops, forced expiration measurements, lung volume assessment, and image gating. All of these measurements/manoeuvres require an anaesthetized preparation of the subject and the parameters obtained are derived from the analysis of recorded pressure, volume, and flow signals.

1.3. Unrestrained Whole Body Plethysmography

In unrestrained whole body plethysmography (WBP), a spontaneously breathing animal is free to move within a small, closed box or plethysmograph chamber. Depending on the chamber design, box pressure or flow in and out of the box are measured to capture the subject's breathing pattern. Additional temperature and humidity sensors may be used to partly compensate thermodynamic effects in the chamber. The WBP waveform generated is typically analyzed with descriptive analyzers providing outcomes such as the respiratory rate (RR) as well as estimates of the tidal volume (TV) and minute ventilation (MV). In addition, a dimensionless quantity known as enhanced pause (Penh) can also be calculated to further describe the changes in the subject's breathing pattern.

2. DETAILED COMPARISION

2.1. Invasive versus. Non-Invasive Measurements

According to Bates and Irvin's *Phenotyping Uncertainty Principle* [1], for any physiological measurement, including lung function testing, the

desired level of measurement accuracy and detail must be balanced against the degree of invasiveness needed to perform that measurement. As per that principle, invasive experimental conditions need to be applied in order to measure any physiological outcome in an accurate or detailed manner. On the other side, measurements acquired by non-invasive techniques will suffer in terms of precision of measurement. The flexiVent and WBP differ greatly in the level of invasiveness associated with their measurements and, therefore, many of the key differences presented in this document between these two techniques relate directly to this principle.

WBP is the least invasive technique for assessing lung function that is currently available, requiring no surgery, anaesthesia, nor restraint of the subject. The technique is not technically demanding and lends itself well to repeated or longitudinal studies. In contrast, the flexiVent scores at the other end of the invasiveness spectrum as the technique always necessitates that the subjects be anaesthetized, tracheotomised or orally intubated, and mechanically ventilated. Longitudinal studies are also possible with the flexiVent with repeated intubation.

2.2. Experimental Conditions

It is well known that measurements of respiratory mechanics are highly dependent on the experimental conditions under which they are acquired. As an example, airway resistance has been shown to vary both with the subject's breathing frequency and positive end expiratory pressure (PEEP) [2]. Consequently, accurate and highly reproducible measurements are virtually impossible to obtain unless the influencing factors are tightly controlled.

In the WBP technique, the subjects are conscious and freely breathing. They can modify at will their breathing pattern (e.g. frequency, tidal volume) in order to adapt to the experimental conditions. In addition, the activation of protective respiratory reflexes is also possible. Any lung function measurements made in these circumstances are likely to be variable as these experiments are performed under uncontrolled conditions. In such situations, it can become extremely difficult to discern between test conditions or to evaluate what part of a measured change in outcomes is due to a change in lung function or in experimental conditions.

Measurements performed with the flexiVent require an anaesthetized preparation of the subject, as well as mechanical ventilation. This is often viewed as a shortcoming of the technique relative to the WBP technique. However, the positive side of this increased level of invasiveness is that all factors having an influence on the outcomes can be tightly controlled and studied individually. For example, the upper airways are bypassed, ventilation frequency and tidal volume are controlled, lung volume history can be standardized, and a variable positive end expiratory pressure (PEEP) can be applied. As a result, the measurements are not distorted by varying experimental conditions and all changes in measured outcomes, both within a subject and between animals or experimental groups, can entirely be attributed to changes in lung mechanics. The manoeuvre-based approach of the flexiVent also permits a variety of different types of measurements, which settings (e.g. pressure, amplitude, or frequency content) can also be precisely controlled and which sequence can be automated.

2.3. Aerosol Delivery

Aerosol delivery is an important aspect of lung function testing and it is often used, for example, to assess the responsiveness of the respiratory system during broncho-provocation tests. Every standard flexiVent system includes a state-of-the-art Aeroneb vibrating plate nebuliser that is fully computer-controlled, offering programmable nebulisation rate and timing as well as synchronization between aerosol generation and mechanical ventilation. Commercial WBP setups can generally be expected to also include means for aerosol generation.

In WBP, aerosols are dispersed into the plethysmograph chamber that, depending on animal size, has a volume of one to several litres. A bias flow, needed to provide fresh air to the subject, further dilutes the aerosol. This, not only results in a very inefficient use of compound, but also to an exposure of the subject's fur and eyes with a possible non-negligible oral or dermal absorption of the substance as WBP aerosol challenges tend to be lengthy (e.g. 1-3 min).

In the flexiVent system, the nebulizer is mounted directly into the inspiratory branch of the ventilator circuit. The aerosol is therefore delivered with minimal dilution directly into the subject's respiratory tract. Very often, only a few microliters of solution are required per challenge, thus making for a highly efficient use of compounds. The nebulizer can be operated under default time and duty cycle settings (10s, 50%, respectively) or configured to create unique nebulization protocols. An estimate of the dose delivered at the subject opening is also available directly in the operating software [3].

2.4. Raw Data Quality

With WBP, breath-by-breath measurements are continuously derived from a single box pressure or flow signal. As demonstrated in Lundblad et al [4] this signal has two components, one reflecting gas conditioning (heating and humidification) in the lungs and the other reflecting rarefication of alveolar gas due to airway resistance. The relative contributions of these two components change as a subject becomes broncho-constricted. Because of the large chamber volume, the signal-to-noise ratio (SNR) is poor and the signal is sensitive to ambient disturbances.

All measurements provided by the flexiVent are based on at least two raw data signals, permitting the use of reliable, quantitative mathematical system identification techniques. In addition, instead of relying on typical direct flow measurement techniques, which can be both noisy and inaccurate within the flow range of a subject as small as a mouse, the flexiVent employs an indirect measuring technique based on displaced volume, a signal that can easily be measured with great accuracy and minimal noise. Measurements of airway opening pressure and cylinder pressure are also obtained with good SNRs using pressure transducers with accuracy, precision, and response time adapted to the subject.

2.5. Breathing Patterns

Although a qualitative breathing pattern assessment is possible in WBP, as the subject is spontaneously breathing, a more quantitative assessment is limited. This is due to the lack of a true respiratory flow signal. While RR can be accurately determined, TV and MV are estimated based on the assumption that the gas conditioning component dominates the raw data signal. This assumption does not hold true when the subject becomes bronchoconstricted, meaning that volumetric measurements in WBP are valid only under baseline conditions. In comparison, techniques such as head-out or double-chamber plethysmography, which rely on true respiratory flow measurements, offer a more complete breathing pattern assessment.

Because measurements performed with the flexiVent are done in anaesthetized and mechanically ventilated subjects, spontaneous changes in breathing patterns cannot be studied with this method. The flexiVent is however the technique of choice to create and study the effect of specific ventilation profiles, for instance when studying lung injury.

2.6. Lung Function Measurement

Many software providers offer analyzers that calculate a dimensionless entity referred to as enhanced pause (Penh) from the WBP waveform. Penh is an empirical parameter that essentially characterizes the shape of the expiratory box pressure or flow waveform. Although changes in Penh were originally thought to be indicative of bronchoconstriction, a number of studies [4-6] have demonstrated that it is not a reliable, quantitative measure of bronchoconstriction.

At baseline, the gas conditioning term (see section 2.4) dominates the WBP box pressure or flow signal. However, as the subject's airways constrict, the gas rarefication term becomes increasingly important and ultimately dominates the signal. It is hence not meaningful to create dose response curves relating Penh baseline values to those obtained during constriction.

For respiratory mechanics measurements, the flexiVent uses the Forced Oscillation Technique (FOT). As mentioned earlier, this technique is based on the analysis of pressure, volume, and flow, captured in response to an oscillatory airflow test signal. In this active measurement technique, single or multiple frequency test signals are imposed at the subject's airway opening to assess the mechanical properties of the respiratory system, as a whole or to distinguish between central airway resistance and tissue

mechanics. FOT measurements can be combined in each subject with other types of measurements offered with the flexiVent system to provide the most comprehensive, specific, and reproducible assessment currently available. Several SCIREQ application notes are available to explain how these measurements are best used for different applications.

2.7. Data Validation

Data validation, either simple or complex, is useful to obtain useable and consistent data that can be trusted. Simple data validation strategies can detect failure of an algorithm (e.g. when breath detection fails during coughing or swallowing). More advanced forms of data validation use quantitative criteria to classify data points. This kind of validation is possible only when mathematical data analysis techniques are employed and permits the calculation of parameters such as coefficients of determination or coherence.

WBP analyzers rely on a single raw data signal and employ basic and/or empirical signal processing techniques. Data validation in this technique is thus limited to simple exclusion criteria. In contrast, mathematical data analysis techniques are used in the flexiVent. These lend themselves well to a variety of quantitative data validation strategies, which have been integrated in the flexiVent operating software for some of the most comprehensive data validation capabilities on the market.

2.8. Practical Considerations

As would be expected from any advanced data acquisition package, the flexiWare software includes preconfigured experiment templates, a powerful scripting feature that permits automation of virtually all system functions, as well as user-configurable data export and advanced study management and data review modules. Advanced users can also customize perturbation waveforms to create their own application-specific flexiVent measurement manoeuvres.

WBP software (iox2) features include customized experiment configurations, automated protocols, and highly configurable analyzers. This software also stores and reanalyzes large data files easily to produce an analysis specific to researchers' needs.

3. SUMMARY & CONCLUSIONS

The fundamental differences between WBP and the flexiVent were reviewed in this document. Many of the differences between the two systems stem from the level of invasiveness associated with each measurement technique and the implications on the accuracy of the outcomes.

WBP is the least invasive technique for lung function testing. However, the quality and detail it provides is extremely limited; RR being the only parameter that can be measured reliably under a wide range of circumstances. The flexiVent, on the other hand, offers highly detailed, specific, and reproducible data, with the challenge of working in an invasive experimental setting.

Consequently for any given application, a decision has to be made between the level of invasiveness and the desired measurement accuracy. This decision should be based on a clear understanding of the measurement techniques, their outcomes and shortcomings, as well as on the needs of the research objectives or applications.

Considerations such as the efficiency of experimentation, or regulatory constraints could also guide the decision process. For example, basic respiratory research or drug development objectives involving the phenotyping or the characterization of animal models of lung diseases tend to be best addressed with detailed accurate measurement, which provide an insightful and comprehensive assessment. In addition, the accuracy and reproducibility of the measurements often translate into smaller group sizes, a reduced risk of false positive or false negative results, and ultimately, a greater overall efficiency.

On the other hand, regulatory authorities mandate measurements in conscious subjects for safety pharmacology core battery evaluations. Techniques like WBP, or slightly more invasive ones such as head-out or double-chamber plethysmography, are suited for this application, at least at the first level of evaluation or as a screening tool. Accurate measurements provided by the flexiVent could still prove very useful as second tier measurements since breathing pattern changes are sensitive to both direct (e.g. bronchoconstriction) and indirect (e.g. sedation, hypotension) pharmacological or toxicological effects on the respiratory system [7].

4. LINKS

To learn more about these products, please visit the following pages of our website:

http://www.scireq.com/flexivent/
http://www.scireq.com/plethysmographs

5. PUBLICATIONS

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