Online supplement – Why do SF_6 and N_2 multiple breath washouts give different results?

This online supplement provides additional information to the main body of text including patient demographics, device specifics and more detailed methods and results for the interested reader.

Methods

Comparison of MBW devices

Subjects breathed tidally, inhaling 0.2% SF_6 followed by room air for $SF6_{Inn}$ or medical air followed by 100% O_2 for $N2_{ExD}$. The washout gases were monitored until they reached the conventional 1/40th of the starting concentration ($LCI_{2.5}$), the earlier 1/20th cut off point (LCI_5) was also assessed. $LCI_{2.5}$ is the historical limit of the gas analysers and so is an arbitrary cut off to assess gas mixing and LCI_5 has been used to shorten the testing time to increase feasibility in complex patients [5]. Real time assessments of breathing pattern, leaks or abnormalities were made and traces excluded according to the 2013 consensus statement [6].

Calibration of each device was performed as per manufacturer guidelines and specific standard operating procedures (UKCFGT consortium, Bell 2010 and Standard Operating Procedure: Multiple Breath Nitrogen Washout, Exhalyzer D, Eco Medics AG, Version 1, Jensen *et al.* 2013). MBW data was analysed on specialist offline analysis software (IGOR Pro, simple washout software (SW) for $SF6_{Inn}$ (SimpleWashout Manual, Version 1.5, UKCFGT consortium, Bell 2010) or online analysis software, Spiroware for $N2_{ExD}$ (Standard Operating Procedure: Multiple Breath Nitrogen Washout, Exhalyzer D, Eco Medics AG, Version 1, Jensen *et al.* 2013)). Both software are based on or have been validated using Testpoint™ (Singer, F., et al., *A realistic validation study of a new nitrogen multiple-breath washout system.* PLoS One, 2012. **7**(4): p. e36083).
The equipment deadspace for each device was small (38ml for Inn and 46ml for ExD when using set 3 deadspace reducer). Particular emphasis was placed on the quality control guidelines from the North American and European CF Society Clinical Trials Network LCI Central Over reading Centres.

Statistics: FEV_1 was compared to LCI from both devices using correlations, the Shapiro-Wilk test was used to assess normality and the slopes were analysed using linear regression. Differences in linear regression slopes were assessed using analyses of covariance. All data was analysed in GraphPad Prism 6 (GraphPad Software, Inc).

Simultaneous washout

In order to independently test the washout of both SF_6 and N_2 and remove equipment related variables a simultaneous washout was attempted. As shown in the main text (Figure 2) the Innocor and the Exhalyzer D were attached together using a plastic connector. The serial order of the devices was tested, with no difference between results, but the final placement was for ease of patient attachment. With the flowmeters attached, the devices were calibrated for flow and gas as per the specific SOP's so as to account for the extra resistance and deadspace (73.3ml), deadspace was adjusted for in the ExD set up page.

Testing was performed in much the same way as above but with slight adjustments to deliver both SF_6 in washin and 100% O_2 in washout for the washout of both gases simultaneously.

Washin – Tidal breathing was completed in washin, using a 0.2% SF_6 gas cylinder.

Switch to washout – Once equilibrium with 0.2% SF_6 was detected by the Innocor device (normally around 2 minutes in healthy individuals) "Start WO" button on the Exhalyzer D was pressed. The gas tubing was manually swapped from the 0.2% SF_6 to the Exhalyzer D tubing. 100% O_2 (electronically switched on by the Exhalyzer D) was then breathed for washout of 0.2% SF_6 and 79% N_2.

End of washout – both gases were washed out to 2.5% of their starting concentration (SF_6 monitored on the Innocor device and N_2 on the Exhalyzer D).

Extended washout – An extended washout was performed to observe how low a gas concentration could be achieved beyond the 1/40th end tidal gas concentration. The participant continued to breathe 100% O_2 for a further 2 minutes as a reflection of the washin time of SF_6 to see if N_2 would decrease to its minimal level with similar timings as SF_6. Two further minutes of breathing 100% O_2 did not cause light-headedness in any subject.

Recorded variables - Gas concentration, breath number and time to washout were recorded rather than calculating LCI to avoid any complications related to flow volume and signal synchronisation discrepancies between the devices.

Analysis – Analysis was performed after exporting the raw breath tables from both analysis software and matching each breath with the decay of N_2 and SF_6. Deadspace and dynamic delay time corrections were therefore not required. SF_6 and N_2 (per breath) were converted to percentages and then normalised on a Log 10 scale so that they could be plotted on the same graph.

Respiratory Mass Spectrometer – Simultaneous washout

Gas Calibration of the RMS was completed in line with an in house SOP derived from the AMIS 2000 SOP (AMIS 2000, Medical mass spectrometer system, instructions for use available upon request from Innovision, 2010, http://www.innovision.dk/Products/AMIS_2000.aspx). The vacuum pump remained switched on at all times to remove air from the testing chamber. Full calibration at the start of each day and 2 point calibration before each individual test to ensure the secondary electron multiplier was optimising voltages correctly. The calibration gas (±1% accuracy – 4%SF_6, 4% He, 7% CO_2, 21% O_2, 64% N_2) was exposed to the sample line and the RMS identified the sample concentrations. For this testing SF_6 was tuned to high priority.

For testing the RMS gas probe was placed in situ in between the ExD and Inn flowmeters. A specialist 1% SF_6 gas mix was utilised as the RMS and Innocor both technically have the capacity to detect and record this concentration. As the Innocor and Exhalyzer were in place, a sample of subjects completed MBW tests to see the impact and the contribution of the 1% vs. 0.2% on the devices by comparing results against each other. There were no differences in washout times of 0.2% or 1% SF_6 on the Innocor device and the N_2 washout was not notably impacted by 0.2% or 1% SF_6.

MBW Testing was completed as above for simultaneous testing.

For analysis the raw ASCII files were exported, and manipulated in Excel to fit the required column and order requirements for the gas concentrations per breath to be calculated using the simple washout software. For calculation of the end tidal gas concentration of each breath during 1% testing, the RMS gas concentration data needed to be matched with flow. The Innocor raw data file was easy to manipulate as it contains fewer parameters than the Exhalyzer D raw data file, the simple washout software is also more easily manipulated since the IGOR software is designed to handle large datasets in a variety of formats (https://www.wavemetrics.com/products/igorpro/igorpro.htm). First the raw data files were aligned for sample time with the Innocor raw data file. The SF_6 trace in the original Innocor file was then replaced by the SF_6 or the N_2 trace from the RMS. Data was imported into simple washout in the conventional way but now the software showed the RMS gas concentrations. The gas signal per breath was calculated by the highlighting the peak of each breath, generating a mean Cet. Problems with signal synchronisation (gas from RMS and flow from the modified Innocor) meant that calculation of FRC, CEV and in turn LCI were not appropriate. Final results (gas concentrations and breath number to washout) were compared using t tests and plotted together to look at the decay of both SF_6 and N_2.

Device specifics for all methodological sections are described in OLS Table 1. As described in the simultaneous washout and RMS sections, efforts were not made to adjust and account for potential sources of bias from the different hardware or software but instead raw data was utilised to simplify and remove confounding variables.

OLS Table 1 – Device specifics for the MBW comparison, simultaneous washout and RMS simultaneous washout.

	Modified Innocor (Innovision 6.11, Odense, Denmark)	**Exhalyzer D (Ecomedics AG, Duerten, Switzerland)**	**AMIS 2000 Respiratory Mass Spectrometer (Innovision, Odense, Denmark)**	**Simultaneous washout (Exhalyzer D and Innocor)**	**RMS Simultaneous washout (Exhalyzer D, Innocor and RMS)**
Flow volume measurement	The flowmeter contains a screen with a pressure output on each side of the screen. By measuring the pressure drop, the flow is calculated. One hertz is one cycle per second, so the sampling frequency of the Innocor is 100 samples per second. There is an automatic offset adjustment prior to each 2x5 strokes. Deviation ±2% relative after a gain calibration, i.e. ±0.02 litre @ 1 litre calibration syringe and ±0.06 litre @ 3 litre calibration syringe.	Two ultrasonic transducers mounted at different sides of the flow channel transmit ultrasonic pulses in an up a downstream direction, the measured transit times determine flow and molecular mass of the gas flow. Ultrasonic transit time detection (10ns time resolution, sampling up to 200Hz). Set 3 Deadspace reducer – flow accuracy ±3% or ±5ml/s, dead space = 20ml. If Flow calibration varies by more than 2% it should be repeated.	n/a – flow signal not completed.	n/a - raw gas concentrations were extracted from the Innocor and Exhalyzer and not incorporated into the flow signal in an attempt to remove equipment bias.	n/a - raw gas concentrations were extracted from the RMS, Innocor and Exhalyzer and not incorporated into the flow signal in an attempt to remove equipment bias.
CO_2 sensor	A photo acoustic analyser measures the effect of absorbed energy on matter by acoustic detection. A laser beam running across an unknown gas hits a known "matter" with known sound wavelengths and the intensity of the sound is proportional to the light the gas gives off, in turn revealing the gas. Principle –photo acoustic spectroscopy. Range - 0-10% Accuracy – ± 1% relative Rise time (10-90%) = <250ms Sample rate 100Hz	CO_2 is measured directly in the patient's breathing circuit. The CO_2 measurement module utilizes advanced, self-calibrating infrared absorption technology to insure accuracy and eliminate the need for routine user calibration. Principle – Mainstream single beam infrared, self-calibrating. Range - 0-150mmHg or 0 to 19.7%. Accuracy – 2mmHg (0-40mmHg), 5% of reading (41-70mmHg) Rise time (10-90%) = <60ms Sample rate 100Hz	See RMS O_2	See Innocor and Exhalyzer D	See RMS

	Modified Innocor (Innovision 6.11, Odense, Denmark)	Exhalyzer D (Ecomedics AG, Duerten, Switzerland)	AMIS 2000 Respiratory Mass Spectrometer (Innovision, Odense, Denmark)	Simultaneous washout (Exhalyzer D and Innocor)	RMS Simultaneous washout (Exhalyzer D, Innocor and RMS)
O_2 sensors	Oxigraph O_2 sensor - The patented Oxigraph sensor uses laser diode absorption spectroscopy in the visible spectrum. Oxygen absorption is in a region of the visible spectrum (760 nm) where there is no interference or absorption by the other gases. As the oxygen concentration increases, the light intensity is attenuated, thereby identifying the concentration. The photo detector response varies linearly with the oxygen concentration. Principle – laser diode absorption spectroscopy – sample gas flow 120ml/min Range – 5-100% Accuracy ± 1% relative Rise time (10-90%) - <250ms Sample rate 100Hz	Oxigraph O_2 sensor - The fast oxygen side stream measurement module measures the oxygen respiration waveform breath-by-breath giving a qualitative indication of ventilation and the oxygen uptake. The patented oxygen sensor uses laser diode absorption method in the infrared spectrum. The lag time and sample flow is compensated by SPIROWARE®. Principle – side stream, laser diode absorption – sample gas flow 200ml/min Range - 2 to 100% Accuracy – 0.3% Resolutions/Linearity – 0.01% and 0.2% Rise time (10-90%) – 80ms Sample rate 100Hz	A high quality quadropole measures mass to charge ratio of all gases specified in the system set up. According to the gas specifications different oscillating electrical charges are applied to four cylindrical rods within the device. Because of the electrical charge applied, gas ions bounce through the rods at different trajectories. The mass to charge ratio can then be calculated for each gas. The signal is multiplied by the secondary electron multiplier (SEM), which enables detection of the gas quantity. Mass range 1-200 atomic mass unit Sensitivity >$2x10^{-3}$A/mili bar Response Time 50msec Sample rate 33Hz	See Innocor and Exhalyzer D	See RMS
N_2 sensor	n/a Attempts were made to indirectly calculate N_2 from the O_2 and CO_2 readings. Issues with the calculation of viscosity and accounting for signal synchronisation mean the testing was aborted.	N_2 is indirectly calculated using the following formula. $N_2 = (100 – O_2 – CO_2)/(1+beta))$ The beta is the relative fraction of Argon/ N_2 in air. Since air contains 0.934% Argon and 78.084% N_2, beta is 0.934/78.084≈0.012.	See RMS O_2	See Innocor and Exhalyzer D	See RMS

	Modified Innocor (Innovision 6.11, Odense, Denmark)	Exhalyzer D (Ecomedics AG, Duerten, Switzerland)	AMIS 2000 Respiratory Mass Spectrometer (Innovision, Odense, Denmark)	Simultaneous washout (Exhalyzer D and Innocor)	RMS Simultaneous washout (Exhalyzer D, Innocor and RMS)
SF_6 sensor	See Innocor CO_2	n/a	See RMS O_2	See Innocor CO_2	See RMS O_2 and Innocor CO_2
Equipment deadspace	Pneumotach 18ml. Hans Rudolph pnemotach – 4700A – 4719series flow range 0-100 L/min Adult green filter – 37ml Paediatric slim line filter – 20ml	Pre-Cap deadspace (volume from patient to mid-CO_2 sensor) SET 1 [ml] (2) SET 2 [ml] (24) SET 3 [ml] (24) Post-Cap deadspace (volume from mid-CO_2 sensor to mid flow sensor) SET 1 [ml] (3.5) SET 2 [ml] (9.5) SET 3 [ml] (22)	n/a – flow not recorded	Deadspace total = 73.3ml (20ml + 3.3ml Exhalyzer D, 18ml Innocor and 30ml bacterial filter).	Deadspace total = 73.3ml (20ml + 3.3ml Exhalyzer D, 18ml Innocor and 30ml bacterial filter).
Analysis software version	Simple washout, IGOR Pro (Wavemetrics Inc., version 6.20B3 or above).	Spiroware 3.1.6.17312	Analysis of each gas concentration was achieved using the online data loading function on the RMS (ATPC turned on, counting breaths and gas Cet from each breath).	Gas concentration, breath number and time to washout were recorded to avoid equipment flow and gas synchronisation issues.	The 1% SF_6 and N_2 washout testing used the simple washout software to estimate the end tidal concentration from raw ASCII files from the RMS.
Calculation of Cet	SW uses an automatic algorithm to determine SF_6 Cet; a smoothed signal where the peak from the first peak working back from the breath-end point.	Calculates Cet from the median concentration between 90-95% of the volume of each expired breath	Online dataloading on the RMA highlighted the mean Cet.	Cet from Innocor and Exhalyzer	An average Cet was calculated using the SW software

	Modified Innocor (Innovision 6.11, Odense, Denmark)	**Exhalyzer D (Ecomedics AG, Duerten, Switzerland)**	**AMIS 2000 Respiratory Mass Spectrometer (Innovision, Odense, Denmark)**	**Simultaneous washout (Exhalyzer D and Innocor)**	**RMS Simultaneous washout (Exhalyzer D, Innocor and RMS)**
Signal to noise ratio	Signal to Noise ratio compares the level of desired signal detection to the amount of background noise. So the Innocor detects SF_6 at 0.2% (in the gas cylinder) at 0.001% (1/1000th) accuracy. >500 @ 21% O_2.	O_2 = 0.3% CO_2 = 5% of the reading 41-70 mmHg	Signal volts should be as high as possible (8-9 volts) in the signal optimisation calibration stage. S:N ~200 RMS auto-optimises itself as the SEM increases and decreases its signal response to different species of gas. Optimal sampling range between 0.1-4% SF_6	See Innocor and Exhalyzer.	See RMS, Innocor and Exhalyzer.
Viscosity	Viscosity correction is not hard coded for in version 6.11 of the Innocor device. Offline attempts were made to correct for viscosity when using the Innocor device with 100% O_2 under guidance from Innovision but difficulties in matching signals meant that this testing was discarded.	No correction, since ultrasonic flow measurement is independent of viscosity. Possible that viscosity should be incorporated because of the sidestream analysis of gas.	Automatic Total Pressure Correction (ATPC) (Ensures the sum of the partial pressures of gases is constant (100% in line with calibration gas cylinder) despite changes to the ion source i.e. with 100% O_2 there would be increased viscosity (gas flow changes) and total pressure of ions but the signal will account for this with ATPC on).	See Innocor and Exhalyzer.	See RMS, Innocor and Exhalyzer.
Sample flow rate	120ml/min	200ml/min	<20ml/min	See Innocor and Exhalyzer	See Innocor, Exhalyzer and RMS.

Results:

All of the participants took part in the main comparison of the Innocor and Exhalyzer D device. Samples of patients took part in the sequential study parts after review of their previous MBW tests and those who had regular tidal breathing and were able to return were selected.

OLS Table 2 – Patient demographics (mean ± SD unless stated).

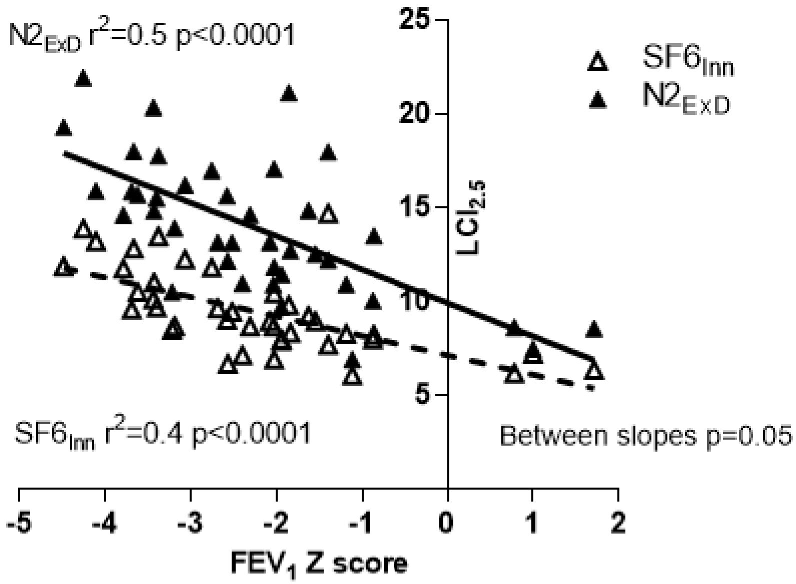
	Comparison of MBW devices	
	CF (47)	HC (42)
Gender	24 (51%) Female	26 (62%) Female
Age (years) (median(range))	16.05(5.9-63.7)	24.32 (5.7-56.1)
Height (cm)	154.6 ± 52.2	158.4 ± 17.9
Weight (kg)	52.2 ± 18	59.7 ± 20.6
FEV_1 (%) (Z Score)	72.9 ± 16.8, -2.29 ± 1.43	Not done
	Simultaneous Washout	
	CF (n=5)	HC (n=8)
Gender	4 (80%) Female	5 (63%) Female
Age (years) (median(range))	24 (12-51)	31.25 (23-50)
Height (cm)	160.4 ± 11.13	171 ± 7.66
Weight (kg)	60.5 ± 18.23	74.2 ± 20.1
FEV_1 (%) (Z Score)	84.02 ± 13.3, -1.3 ± 0.93	Not done
	RMS – simultaneous washout	
	CF	HC (10)
Gender	Not done	6 (60%) Female
Age (years) (median(range))	Not done	28.3 (21.4-35.3)
Height (cm)	Not done	169.9 ± 10.4
Weight (kg)	Not done	66.7 ± 9.9
FEV_1 (%) (Z Score)	Not done	Not done

OLS Table 3 – Coefficient of variation in primary outcomes

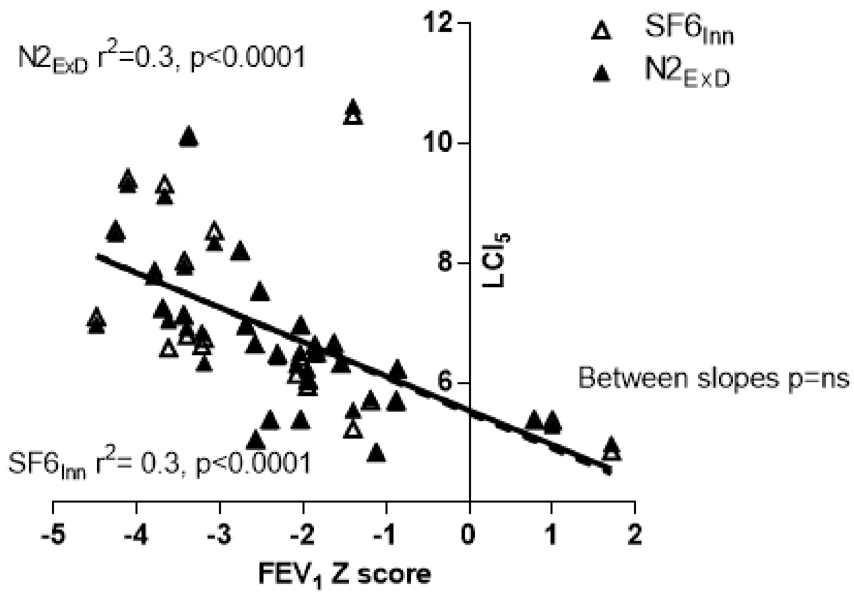
	Comparison of MBW devices	
	CF (47)	HC (42)
LCI CV% (SF6 and N2) p value	3.7(0.02-13.02) and 3.06(0.1-1.74) p>0.05	2.85(0.2-11.8) and 2.87 (0.1-10.9) p>0.05
FRC CV% (SF6 and N2) p value	3.2(0.2-4.6) and 3.5(0-8.4) p>0.05	2.8(0.2-9.1) and 2.85(0-14.3) p>0.05
CEV CV% (SF6 and N2) p value	3.2(0.05-12.6) and 5.4(0.03-13.1) p=0.03	3.2(0.03-10.7) and 3.7(0.2-12.1) p>0.05
	Simultaneous Washout	
	CF (n=5)	HC (n=8)
Washout breath number (n) (SF6 and N2) p value	24.8 ± 8.7 vs. 59.5 ± 28.3, p=0.063	32.0 ± 11.9 vs. 46.3 ± 13.7, p=0.001
Washout breath number CV% (SF6 and N2) p value	1.6(0-8) and 5.3(4.3-8.3) p>0.05	4.7(0-10.9) and 3.9(2.9-14.3) p>0.05
	RMS – simultaneous washout	
	CF	HC (10)
Washout breath number (n) (SF6 and N2) p value	Not done	34.5 ± 12.4 vs. 45.8 ± 19.1, p=0.005
Washout breath number CV% (SF6 and N2) p value	Not done	5.1(1.6-14) and 3.0(0.8-5.9) p>0.05

Results - Comparison of MBW devices

100% of HC participants and 93% of CF patients were able to complete at least 2 acceptable MBW tests on both machines. (3 CF patients' traces were excluded at analysis due to poor quality). The LCI coefficients of variation (CV%) were small and not statistically different between devices (HC Inn 2.85(0.2-11.8) vs. HC ExD 2.87(0.1-10.9) p>0.05 and CF Inn 3.7 (0.02-13.02) vs. CF ExD 3.06 (0.1-7.4) p>0.05) or between HC and CF subjects (p>0.05). LCI values obtained from $SF6_{Inn}$ and $N2_{ExD}$ were tightly correlated with FEV_1 Z score (OLS Figure 1) but the correlation slopes differed significantly, steeper with $N2_{ExD}$ (OLS Figure 1; p=0.05). LCI_5 was still higher in CF compared to HC but there was no difference in slopes between gases (OLS Figure 2 and Table 1) confirming that the latter part of the washout curve is most affected by the difference in behaviour of the two gases (Figure 2 and 3 main paper).



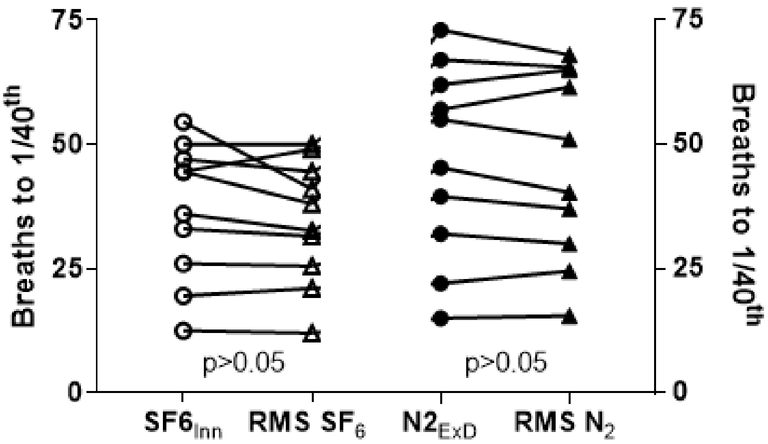
OLS Figure 1 – Plot of $LCI_{2.5}$ (lung clearance index taken from the traditional end point of 1/40th of the starting concentration) vs. Forced Expiratory Volume in one second (FEV_1) Z Score in cystic fibrosis patients from the Innocor ($SF6_{Inn}$, open triangles) and the Exhalyzer D ($N2_{ExD}$, closed triangles). The r^2 values for each device are statistically significant ($r^2=0.4$ for $SF6_{Inn}$, $p<0.0001$ and $r^2=0.45$ for $N2_{ExD}$, $p<0.0001$). The r^2 slopes are significantly different from each other (p=0.05). FEV_1 Z scores are calculated using the Global Lung Initiative reference equations (Quanjer *et al.* 2012).



OLS Figure 2 – Plot of LCI_5 (lung clearance index taken from the end point of 1/20th of the starting concentration) vs. Forced Expiratory Volume in one second (FEV_1) Z score in cystic fibrosis patients from the Innocor ($SF6_{Inn}$, open triangles) and the Exhalyzer D ($N2_{ExD}$, closed triangles). The r^2 values for each device are statistically significant ($r^2=0.3$ for $SF6_{Inn}$, $p<0.0001$ and $r^2=0.3$ for $N2_{ExD}$, $p<0.0001$) but they are not significantly different from each other (p= ns). FEV_1 Z scores are calculated using the Global Lung Initiative reference equations (Quanjer *et al.* 2012).

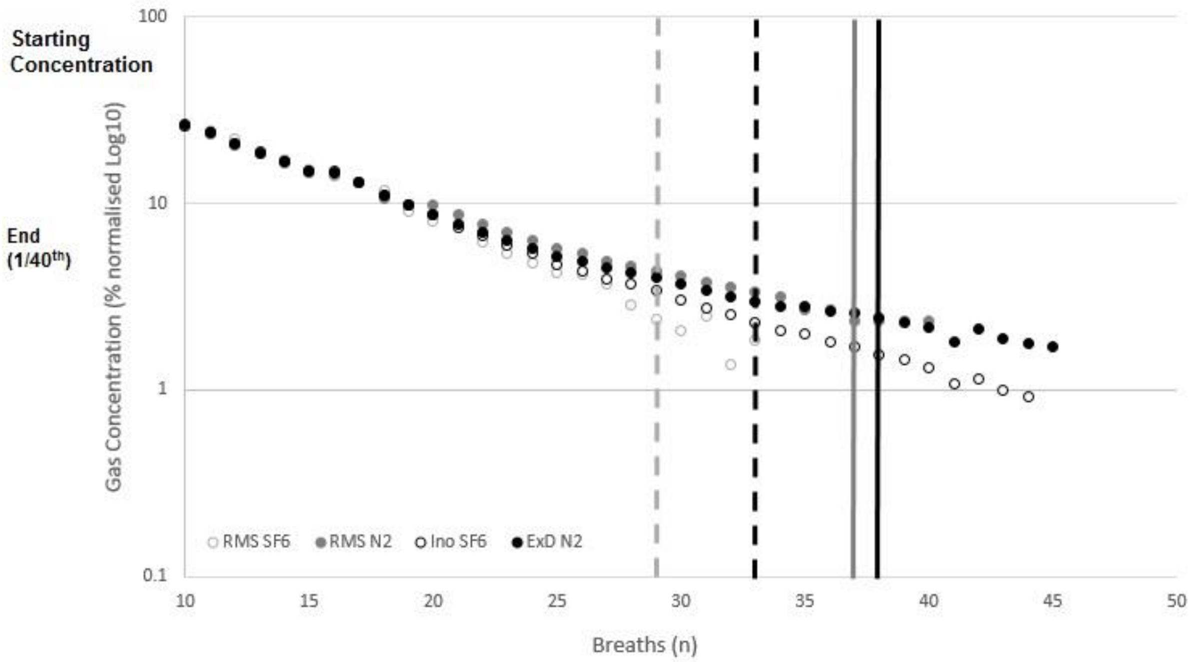
Results – RMS simultaneous washout

Breath number between the commercial devices and the RMS were compared. OLS Figure 3 shows that there were no differences between devices.



OLS Figure 3 - Simultaneous washout of SF_6 and N_2 was completed using the Innocor ($SF6_{Inn}$), and Exhalyzer D ($N2_{ExD}$) and the respiratory mass spectrometer (RMS) on 10 healthy control (HC) participants. This plot shows the difference in washout breaths for nitrogen (N_2) and sulphur hexafluoride (SF_6) (SF_6 open markers, N_2 closed markers) for 7 individuals in ExD/N_2 and $SF6_{Inn}$ (circles) vs. RMS (triangles). There were no significant differences between breath number for SF_6 (Ino and RMS) or N_2 (ExD and RMS). RMS breath results were manually calculated from the average end tidal gas concentration of gas at each breath and compared to breath number from each device.

OLS Figure 4 shows the washout decay of both N_2 (RMS and ExD) and SF_6 (RMS and Innocor). N_2 for both devices still reaches the 1/40th end point after SF_6.



OLS Figure 4 – Graph of an individual healthy control patient's gas concentration decline throughout a simultaneous washout using all devices for sulphur hexafluoride (SF_6, open circles (black=Innocor, grey= Respiratory Mass Spectrometer (RMS))) and Nitrogen (N_2, closed circles (black=Exhalyzer, grey= RMS)) vs. breath number starting from 10 breaths. The gases are displayed in a normalised log scale so that each gas concentration is shown on the same graph. The vertical lines (dashed = SF_6 (black=Innocor, grey= RMS), full line = N_2 (black=Innocor, grey= RMS)) represent the end tidal gas concentration at 1/40th of the starting concentration. In all subjects, SF_6 reached the end target first.

OLS Discussion

The inherent differences between N_2 and SF_6 washouts are further defined by the large limits of agreement in the $LCI_{2.5}$ and FEV_1 Zscore plots. This difference is less apparent in the LCI_5 showing that the discrepancy is exaggerated at the tail end of the washout and with those with worse lung disease and therefore longer washouts.

Only head to head data during the same washin and washout is likely to definitively assess differences in MBW (Vermuelen, F., et al. *Comparison of lung clearance index measured during helium washin and washout in children with cystic fibrosis.* Pediatr Pulmonol, 2013. **48**(10): p. 962-9). Therefore using the RMS to complete a simulataneous washout is in part a valid test. The simulaneous washout with both the Innocor and Exhalyzer D and the RMS attempted to directly measure both gas washouts. The results showed similar washout breath numbers and washout times i.e. N_2 reached the $1/40^{th}$ end tidal concentration after SF_6 , suggesting that, having removed some software and equipment bias, this is real observation. However as highlighted we had difficulties with signal quality of RMS testing due to the low SF_6 concentrations and the use of 100% O_2 and different characteristics of the hardware i.e. signal to rise time or sampling frequency may also have contributed to the discrepancies; therefore these results should be interpreted with caution.

In conclusion, N_2 and SF_6 washouts are inherently different, higher for N_2. A fundamental, non-linear bias in N_2 (even seen in HC individuals) should be considered when interpreting results. The variation between devices is in some cases larger than reported clinically relevant changes so are not dismissible.