Innocor for Multiple Breath Washout tests using an Open Circuit Standard Operating Procedure

“The Edinburgh Method”

Dr Alex Horsley
University of Manchester
Manchester Adult Cystic Fibrosis Centre
Manchester M23 9LT
alexander.horsley@manchester.ac.uk

Dr Nicholas Bell
Department of Respiratory Medicine
Bristol Royal Infirmary
Bristol BS2 8HW
N.J.Bell@bristol.ac.uk

Version 3.1.2, 14th October 2013
# Contents

Part I: Setting up Innocor to perform MBW  
1. Background  
   1.1. Lung Clearance Index (LCI)  
   1.2. Innocor  
2. Hardware modifications  
   2.1. Equipment required  
   2.2. Patient interface  
   2.3. Flowmeter adaptation  
   2.4. Innocor connections  
   2.5. Patient Interface  
   2.6. Flow-past circuit  
   2.7. Modification of Innocor to remove O₂ analyser  
3. Software Settings  
   3.1. Washout Test  
   3.2. Flowmeter re-zero  
   3.3. Flowmeter Linearisation  
   3.4 Gas cylinder pressure  
4. MBW test room  
   4.1. General considerations  
   4.2. Innocor Log  

Part II: Health and Safety  
5. Health and Safety  
   5.1. General considerations  
   5.2. Protective equipment  
   5.3. Spillage procedures  
   5.4. Waste disposal  
   5.5. Subject safety  

Part III: Protocol  
6. Equipment required  
7. Setting up Equipment  
   7.1. Set up equipment  
   7.2. Calibration of pneumotachograph  
8. Flow-gas delay calibration  
   8.1. Rebreathing  
   8.2. Solenoid  
   8.3. FGD data export and analysis  
9. Create / select patient in Innocor  
   9.1. Creating a new patient  
   9.2. Selecting a patient
10. Setting up the patient 27
11. Starting the test 28
12. Wash-in Phase 28
13. Disconnection of flow-past 30
   13.1. Flowmeter resets 30
   13.2. End of wash-in 30
14. Washout 30
15. Further Tests 31
16. Data Export 31
   16.1. Exit to Windows 31
   16.2. Copying to Memory stick 32
   16.3. Return to Innocor 32
   16.4. Switching off Innocor 32
   16.5. Data Analysis 33
17. Special considerations in children 33
   17.1. Standard Procedure for Older Children (5 years and over) 33
   17.2. Prior explanation of procedure 33
   17.3. Equipment 33
   17.4. Potential challenges 34
   17.5. Innocor in young children (<5yrs) 34
18. References 35

Part IV: Infection Control 36
19. Infection control policies 36
   19.1. Infection control equipment 36
   19.2. MBW apparatus 36
   19.3. Sterilisation of Mouthpieces, Masks, Noseclips & T-pieces 36
   19.4. Cleaning of Flowmeter 37
   19.5. Other Equipment 37
   19.6. Summary of recommendations 37

Appendix A 39
   Background to LCI 39

Appendix B 45
   Innocor Log 45

Appendix C 47
   Solenoid-activated Flow-Gas delay device 47
About this Manual

This manual is for the use of a modified Innocor to measure lung clearance by inert gas washout, using an open circuit (otherwise known as a “flow-past” circuit). The entire process from setting up the machine to exporting data is covered. There are separate software-specific SOP’s for data analysis. For machines already configured to perform washouts according to the earlier versions of this method, please confirm that all settings are as described here. The protocol for performing an actual test is contained within section III.
This SOP is not intended to wholly replace Innocor’s own manual which you may still need to refer to.

Please note that this manual does not refer to the latest version of Innocor (7.0), which does offer LCI measurement from both rebreathe and open circuits. This software is still undergoing validation and the use of this system is not covered here.

Authors:
Dr Alex Horsley
Senior Lecturer
Honorary Consultant
University of Manchester
Manchester Adult CF Centre
University Hospital of South Manchester
Manchester M23 9LT

Dr Nicholas Bell
Consultant Respiratory Physician
Honorary Senior Lecturer
University of Bristol
Department of Respiratory Medicine
University Hospitals NHS Foundation
Trust
Bristol BS2 8HW

The sections on infection control and measuring LCI in children were written by Dr Kenneth Macleod, and may require local validation. Dr Macleod also compiled the original description of the method to remove the Oxigraf from Innocor (section 2.7).

The text and images used are copyright the authors.
Part I

Setting up Innocor to perform MBW

1. Background

1.1. Lung Clearance Index (LCI)

LCI is a sensitive measure of airway physiology derived from washout from the lungs of an inert tracer gas over multiple breaths (Multiple Breath Washout, MBW). For a comprehensive review of LCI, please see Appendix A. In order to measure inert gas washout, a sensitive and rapid gas analyser is required. This document describes the adaptation of the Innocor™ analyser, a device originally designed for cardiac output measurements, to follow minute concentrations of the inert tracer gas sulphur hexafluoride (SF₆).

1.2. Innocor

Innocor was developed to measure cardiac output using the inert gas rebreathing technique. Cardiac output is measured as follows: the patient breathes a mixture of a blood soluble gas (N₂O) and an insoluble gas (SF₆) for a short period. The disappearance rate of the blood soluble gas (relative to the insoluble gas) is proportional to the pulmonary blood flow. This is then used to calculate cardiac output (and other derived parameters). This procedure requires rebreathing of gas mixture from a bag.

Sampled gas is drawn into the Innocor by a pump and passed through two gas analysers in series: an Oxigraf™ oxygen analyser, followed by a Photoacoustic Gas Analyser (PGA).

The PGA uses a technique known as photoacoustic mass spectroscopy to measure diatomic molecules including SF₆, N₂O, and CO₂. The gas molecules absorb energy from pulsed infrared light. This change in energy generates a sound wave which is measured by an extremely sensitive microphone. The microphone signal is then filtered to separate the three modulation frequencies that correspond to the different gas concentrations. The amplitude of each component of the sound wave is directly proportional to the corresponding gas concentration. This method is very sensitive to low concentrations of SF₆ (0-0.2%), with excellent signal to noise ratio and minimal drift.

The Oxigraf measures oxygen concentration by laser absorption spectroscopy. This analyser, which is in series with the PGA, is not necessary for measurement of LCI and increases the gas analyser response time. The removal of this component is covered in section 2.7.

The use of Innocor to measure LCI by inert gas washout is a non-standard use of the device requiring some hardware and software adaptation. These adaptations, and the process of collecting washout data, are covered in the relevant sections below. Importantly, washout data must be exported to another computer for analysis on custom software - this last process is covered in a separate SOP.
Please note that this manual does not refer to the latest version of Innocor (7.0), which does offer LCI measurement from both rebreathe and open circuits. This software is still undergoing validation and the use of this system is not covered here.

Additional background information on Innocor, and the process of validating the device for LCI measurement, can be found in Horsley et al., Thorax, 2008.

2. Hardware modifications

2.1. Equipment required
In addition to the Innocor device, the following equipment is required:

<table>
<thead>
<tr>
<th>Description</th>
<th>Manufacturer</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innocor</td>
<td>Innovision</td>
<td>Innocor</td>
</tr>
<tr>
<td>Adult flowmeter</td>
<td>Hans Rudolph, Missouri, USA</td>
<td>4700A</td>
</tr>
<tr>
<td>Paediatric flowmeter</td>
<td>DAR,Tyco Healthcare, UK</td>
<td>Barrierbac S</td>
</tr>
<tr>
<td>Adult filter</td>
<td>Air Safety, UK</td>
<td>9070/01</td>
</tr>
<tr>
<td>Paediatric Filter</td>
<td>Ferraris, UK</td>
<td>PKM0902051000000</td>
</tr>
<tr>
<td>Adult mouthpiece</td>
<td>Hans Rudolph</td>
<td>Small bite mouthpiece</td>
</tr>
<tr>
<td>T-piece for flowpast</td>
<td>Intersurgical, UK</td>
<td>Code: 1981 T-Piece 22M-22F-22M</td>
</tr>
<tr>
<td>“Flextube” elephant tubing</td>
<td>Intersurgical, UK</td>
<td><a href="http://www.intersurgical.co.uk">www.intersurgical.co.uk</a></td>
</tr>
<tr>
<td>Gas supply tubing</td>
<td>Intersurgical, UK</td>
<td><a href="http://www.intersurgical.co.uk">www.intersurgical.co.uk</a></td>
</tr>
<tr>
<td>Y-piece</td>
<td>Intersurgical, UK</td>
<td><a href="http://www.intersurgical.co.uk">www.intersurgical.co.uk</a></td>
</tr>
<tr>
<td>Bag</td>
<td>Intersurgical, UK</td>
<td><a href="http://www.intersurgical.co.uk">www.intersurgical.co.uk</a></td>
</tr>
<tr>
<td>Connector for gas tubing to elephant tubing</td>
<td>BOC special gases</td>
<td>Code: 1968</td>
</tr>
<tr>
<td>Fan to dispel expired gas</td>
<td>Any</td>
<td>Any</td>
</tr>
<tr>
<td>RVU connector</td>
<td>Innocor</td>
<td>N/A</td>
</tr>
<tr>
<td>SF₆ cylinder</td>
<td>BOC special gases</td>
<td>0.2% SF₆ in medical air Prod no: 2489639</td>
</tr>
<tr>
<td>Medical air gas regulator</td>
<td>BOC</td>
<td></td>
</tr>
<tr>
<td>3 litre flowmeter calibration syringe</td>
<td>Various</td>
<td></td>
</tr>
</tbody>
</table>

The patient will also need to be distracted during testing, and provision should be made for them to either watch TV or view an age-appropriate DVD.

2.2. Patient interface
The patient breathes air and test gas through a mouthpiece-filter-pneumotachograph assembly or “patient interface”. The patient interface that comes with Innocor (known as the rebreathing valve unit, or RVU) cannot be easily disassembled. It is designed for exercise testing and hence has both low resistance to flow and a large dead space. For the tidal breathing involved in inert gas washout, resistance to flow is much less of an issue, but minimal dead space is important. For inert gas washout, the supplied RVU should be replaced with a smaller filter and pneumotachograph (flowmeter). The recommended arrangement and component details are shown in Figure 1. The parts are listed in Table 1 and described in more detail in the following sections. Note that, in order to minimise equipment deadspace, the mouthpiece should be trimmed so that it does not extend far beyond the end of the filter attachment.

Figure 1: 
Adult patient interface for inert gas washout, disassembled (top) and ready to use (bottom).

2.3. Flowmeter adaptation
The end of the flowmeter furthest from the patient needs to be drilled so that a 18G needle can be placed through the hole with the tip in the middle of the stream of air (Figure 2). The needle should be secured and sealed to ensure that there is no leak as it passes through the housing. The site of the hole should be adjacent to the pressure transducer lines and approx. 2cm from distal end of flowmeter, i.e. sufficiently far back to not interfere with the fitting of the T-piece from the flow-past circuit, but otherwise to be as far from the flowmeter membrane as possible. The gas sample line on the RVU has a Luer fitting, to allow it to be unscrewed and re-attached to the needle.
Figure 2: A: Site for sample port indicated by arrow on distal part of flowmeter housing, between main chamber and T-piece attachment. B: Flowmeter with needle hole drilled and needle in place. C: End-on view showing needle tip in centre of stream of air.

2.4. Innocor connections
Innovision will supply, on request, a connector that plugs into the RVU connection on Innocor, but which only contains patent ports for the two pressure transducer lines (Figure 3). These ports can then be connected to the appropriate ports on the flowmeter using equal lengths of plastic tubing, e.g. that provided with the device to connect the Innocor flowmeter, or a suitable alternative.

The 18G gas sample needle is connected to a small filter attached to the gas sampling port on the Innocor via a length of Nafion™ tubing with male Luer connectors on either end. The gas signal rise time may be reduced by using a shorter length of Nafion than that provided with the Innocor device. Although the minimal length of Nafion to allow equilibration of gas sample humidity with room air is 40cm, this is not long enough to allow comfortable positioning of patient interface. The tubing can however be reduced to half the supplied length, i.e. to 80cm, and still be usable. If the tubing is cut it should be careful resealed (e.g. with the same thin black tubing used to attach the flowmeter).

Figure 3: Modified pneumotachograph connector attached to RVU port on side of Innocor. Arrow indicates the gas line filter.

2.5. Patient Interface
An acceptable and comfortable method of positioning the mouthpiece for the patient needs to be employed.

When positioning the patient interface, ensure that the gas sample and flowmeter pressure transducer lines are not in a downward (6 o’clock) position. There is a theoretical risk that moisture could condense around the gas sample needle or flowmeter membrane and run down into the lines - the flowmeter should be rotated so that the lines exit between 9 and 3 o’clock (as in Figure 4).
2.6. Flow-past circuit
During the wash-in phase of the test, the patient breathes into and out from the open (flow-past) circuit. This consists of two pieces of long tubing joined to a T-piece connector as shown in the diagram in Figure 5 and in detail in Figure 6. The “upstream” limb of the tubing, which should be around 0.5m in length, is connected to a cylinder containing 0.2% SF₆ in air, with a reservoir bag placed in-line with the tubing (Figure 7) to increase the maximum fresh gas flow during inspiration and permit a lower gas flow through the circuit. The “downstream” limb of the circuit, through which exhaust gas is vented during wash-in, should be around 1m long. The PGA measures gas concentrations relative to ambient air, making it important that SF₆ does not accumulate around the Innocor. This can be achieved by maximising test room ventilation, sensible positioning of the Innocor and subject, and the use of a fan. If the test room is particularly small or poorly ventilated, it is sensible to place the exhaust limb outside of the room.

![Figure 5: Schematic of open circuit (flowpast), showing configuration of apparatus during wash-in.](image)

**Figure 4:** Patient interface for MBW measurements.

**Figure 5:** Schematic of open circuit (flowpast), showing configuration of apparatus during wash-in.
2.7. Modification of Innocor to remove O₂ analyser

Accuracy of gas volume measurement is affected by the gas analyser response time, particularly in younger subjects who tend to have more rapidly changing gas concentrations because of higher respiratory rates. The relatively slow analyser response time of Innocor can be improved by removing the Oxigraf™ oxygen analyser from the circuit. Innovision may be able to provide a machine without an Oxigraf. If one is present and you wish to remove it, this requires the Innocor machine to be opened up and the gas sampling circuit to be altered. This procedure is difficult to reverse, and cannot be done on machines for which the ability to measure oxygen concentration is important (eg if the machine is also intended to be used for cardiac output measurement). It will also mean that the flow-gas delay cannot be calculated in the standard manner and a separate device will be required (see section 8). This step may not be necessary in adults however, and importantly the oxygen analyser was present in the original publications on Innocor MBW.

**Important:** in a multicentre study all systems must be set-up identically.

Removing the Oxigraf and shortening the gas sample line will change the accuracy of the measurements, and may lead to differing results between Innocor machines configured differently. Until there are additional data to support the practice, data from differently configured machines should not be compared. Although these adaptations are unlikely to have more than a very minor effect in adults, until validated this includes use of published normal range data.

2.7.1. Innocor disassembly

1. Remove all attachments, including RVU, gas sample line, gas cylinder and power line. Tip the Innocor machine screen-down onto a soft surface (eg foam cushion), making sure that pressure is not on the touch-screen itself but on the surrounding frame.
2. Unscrew the 2 nylon screws in the handle (Figure 8a)
3. Unscrew the 8 screws in the bottom of the Innocor. Note, the 2 screws near the gas cylinder are longer than the others, and the 2 screws at the front are shorter.
4. Tip the LCD screen forward gently, and place on the foam
5. The cover can be gently eased off the base by pulling upwards (Figure 8b).

Figure 8: Disassembly of Innocor. A – indicating screws that need to be removed. B – removal of cover, with screen shown lying flat on foam cushion.

6. The Oxigraf unit is located near the top, above the photoacoustic gas analyser (PGA), as indicated in Figure 9.
7. Remove the upper of the two black tubing connections at the Oxigraf. This connects to the PGA. Feed this to the back of the Innocor device, where the gas inlet is (Figure 10), and trim to remove any excess length.
8. Disconnect the black tubing from the gas inlet (this goes to the Oxigraf).
9. Connect the tubing that leads to the PGA to the gas inlet. Take care not to damage the metal capillaries that the tubing connects to.
10. Replace the cover, taking care to ensure the screen cable is inside the cover. Slot screen back into place, gently tip forward again and replace screws.
3. Software Settings

The built-in Innocor software has changed since the original versions of this SOP.

- ✔ Instructions apply only to Innocor software versions before 7.0
- ✔ Instructions apply to Innocor software versions 7.0 and above

3.1. Washout Test Protocols
Innocor has a number of preset protocols for exercise testing, which involve incremental increases in workload and intermittent rebreathing manoeuvres to determine cardiac output. For inert gas washout, a protocol is needed which allows a prolonged period of breath by breath measurements at zero workload and without interruption for rebreathing. To set this:
• Select **Measurement**, and then **Test** from the right hand menu of the main report screen.

• Select **Measurement**, then select a patient (see section 9), any patient will do. Select **Test** from the next menu, and then **Protocol** from the menu that opens.

• If a window appears for you to enter details about patient weight, haemoglobin etc. You do not need to worry about this and can leave it blank, these data are used in cardiac output calculations. Press **OK**.

• To enter a new protocol select **New**. The following screen appears:

  ![Protocol setup screen](image)

  • Enter **Washout** as the protocol name.
  
  • The protocol appears on the top line of the yellow box. Ensure this is highlighted.
  
  • Press **RB/NIBP** repeatedly until the symbols disappear from the left hand column (the number 1 will remain).
  
  • Set the load to 0.0 watt
  
  • Set the time to 60 mins.
  
  • The columns for **Bag**, **Bolus** and **RB freq.** should have been cleared when the symbols were cleared from the left hand column.
  
  • Select **OK**.
  
  • You will be returned to the previous menu. Select **BBB graphs Setup**.
  
  • You can now select what data to display during the breath by breath manoeuvre. Bear in mind that some will be obscured by the online data graph and many of the options will not work anyway if the Oxigraf is disabled.

  • **OK** to return to protocol menu.

  • Set exercise device for your protocol to Manual Control (to prevent Innocor from looking for an exercise machine to synchronise with at the start of each test):

    o Press the 🕒 symbol near the bottom right corner of the menu until the exercise device for your protocol is selected as **Manual control**.

  • To set up the data display on the left hand side of a test, start a dummy run test. 

    o Select **Table Setup** from the bottom left corner.
    
    o Click the “?” button:
You do not need all the redundant cardiac output measurement data displayed. Suggested data to be displayed are:
- $V_t$ (tidal volume) (may not work if Oxigraf disabled)
- Resp Freq
- $V_e$ (minute ventilation)
- $VCO_2$ ($CO_2$ production)
- If using the oxygen saturation monitor, then you should also select $SpO_2$ and HR.

### 3.2. Flowmeter re-zero

A feature of the Innocor flowmeter module is that it automatically re-zeros (“resets”) itself at regular intervals to correct for any drift. Whilst doing this, the computer records a non-physiological flow rate of 100L/sec for 1 second. This helps to maintain the accuracy of the flowmeter but if a “reset” occurs early in the washout during expiration the loss of 1 second of flow data can significantly affect results. Although “resets” during washout can be corrected for during washout analysis, it is best to avoid them if possible. We try to set the interval between resets such that the reset occurs at the end of wash-in (and therefore will not happen again before washout is complete). This takes a degree of judgement and guesswork, since normal volunteers wash-in in far less time than a subject with moderately severe CF. We have found that, for adults, 5 minutes represents a reasonable compromise between an excessive wash-in time for subjects who are well, and too frequent re-zeroes affecting washout analysis. For children, 4 minutes is usually sufficient.

In order to set the time interval between flowmeter re-zero:

- Exit to Windows (see section 16.1)
- Unless you have a keyboard connected through the Innocor USB port, select On-Screen Keyboard.exe from the C:\Innocor\Shortcuts menu.
- Select directory C:\Innocor\setup and select the text file Hardware.ini
- Scroll down to the [BBB] section:
  
  ```
  [BBB]
  Installed=1
  FlowzeroAfterRB=0
  FlowzeroInterval=300
  
  (there may be additional lines in this section)
  ```
- The number after FlowzeroInterval= is the time in seconds between re-zero manoeuvres. Using the On-Screen Keyboard (or a keyboard connected through the USB port) set this to the desired time interval (in seconds) between flow “resets” (e.g. 300 for an interval of 5 minutes).
- Close the text box, and confirm that you want the changes saved when prompted.
- Open the C:\Innocor\Shortcuts folder from the left hand menu and the Restart InnoCor.
3.3. Flowmeter Linearisation
The flowmeter module within Innocor converts the pressure difference across the flowmeter screen into a voltage and thence into a flow rate measurement. The relationship between flow through the flowmeter and voltage is not completely linear and must be calibrated using same range of flow rates seen in washouts. This “linearisation” should be performed using the same type of filter used for the patient washout in place, and should be repeated after changing a flowmeter, replacing it after cleaning, or changing the type of filter used. Both filters selected have been tested and shown not to affect the flowmeter over the physiological range, providing that the output is first calibrated as described.

- Ensure that the Innocor is well warmed up, i.e. it has been on for at least an hour. If unavoidable, the procedure can be performed when the machine has just been turned on, but should be repeated later once warmed up.
- Connect the calibration syringe to the flowmeter using the appropriate sized filter (i.e. a paediatric filter when preparing the paediatric flowmeter, and an adult filter for the adult flowmeter).
- Exit to windows (section 16.1).
- Select the directory C:\Innocor\Shortcuts and click on Flowmertercalibration.
  - Select Stand Alone.
  - Select Calibrate.
  - Select Prepare Syringe size and ensure that correct syringe size (3L) is set.
  - For version 7.0, select Prepare. Select Syringe Size to confirm that a 3L size is selected, and then New Table to set a calibration.
  - Select Add stroke. A table appears with a flow/volume graph on the left.
  - Perform a single fill-then-empty manoeuvre with the syringe. A very large volume will be recorded. Press Accept. This very coarse calibration will at least allow you to fine tune over the following strokes.
  - Press Add Stroke again. Now perform 5 more fill-empty manoeuvres, following the instructions in the window at top left. Aim to cover a wide range of flow rates, starting at a very low flow and building up. In reality, at relaxed tidal breathing, patient flows are less typically less than 0.25L/s, so it is particularly important to ensure these low flows are covered.
  - If you make an error, that stroke can be de-selected from the right hand table by highlighting that data and pressing Use/skip.
  - Press Accept.
  - Unless the %error throughout is <3%, repeat the process. The more strokes you add, the more accurate the linearization.
  - To finish press Accept and then Cancel.
  - Select the option to save data, and select the file bbblin1.cal as the filename. You will be asked if you wish to overwrite the saved file, select Yes.
  - Select Prepare, then Save Table. Select the file BBBlin1.cal as the filename. You will be asked if you wish to overwrite the saved file, select Yes.
  - Exit takes you back to Windows.
  - Answer Yes to Save Changes?
3.4 Gas cylinder pressure
Normal function of Innocor requires a small cylinder of test gas, supplied by
Innovision, which is not needed if using this open circuit methodology. By default
Innocor software will not proceed with a washout test unless a cylinder with
sufficient pressure is present. This requirement can be circumvented by adjusting the
cylinder pressure offset such that it is higher than the default minimum pressure,
allowing you to work without an attached cylinder. To do this:

- Exit to Windows (section 16.1).
- Select GasSystem.ini
- Scroll down until you come to
  [GasPressure]
  Gain=43.75
  Offset=-22.1886
  unit =bar
- Alter the offset to 40.
- Close the window to save changes, select Setup folder to return to main
  Windows menu.

4. MBW test room

4.1. General considerations
There are a number of important considerations about the test room that need to be
taken into account when planning MBW testing. Firstly, the room should have stable
temperature and humidity and must be of sufficient size or be sufficiently well
ventilated that MBW testing will not lead to a build up of SF₆ in the room. Experience
has shown that repeated testing in a small and poorly ventilated room can cause
elevations in ambient SF₆. Although these are tiny, the sensitivity of the analyser
means that these can interfere with washout analysis. The ideal setup would be a
large test room, with air conditioning.
It is also important that there is a comfortable seat for the subject, that they will be
able to concentrate on watching a TV screen during the test and will not be
distracted by interruptions, and that there is storage space for gas cylinders.

4.2. Innocor Log
The constancy of ambient conditions is recorded on a daily log sheet, shown in
Appendix B. This is an important record, which should be stored with the Innocor
machine and completed each time a test a performed. The recording of ambient
conditions allows correction of lung volumes to BTPS. In addition, this forms an
important second record of flow gas delay, and allows daily review of gas cylinder
pressure so that it can be anticipated well in advance when this is running low and
more cylinders ordered. Record of flowmeter linearisation also allows identification
of any substantial change in linearisation parameters, which would be an indication
to review the connections and equipment and attempt re-linearisation (section 3.3).
Part II
Health and Safety

5. Health and Safety

5.1. General considerations
The main risks involved in this protocol are:

• **SF₆ (sulphur hexafluoride).** This is used at a concentration of 0.2% (2000ppm) in air. SF₆ itself is inert and non-absorbed, and there are no reported harmful effects of its use. Theoretically, use of very high concentrations may allow SF₆ to accumulate in low-lying areas (since it is much denser than air) and to displace air. The concentrations used for LCI measurement are far too low for this to occur. An important additional consideration is that SF₆ is a greenhouse gas. Although only very small amounts are used, it is nonetheless important to ensure that gas is not wasted and that cylinders are turned off after use, to prevent leakage when equipment is not being used.

• **Infection control.** This is an important consideration with all patients, and particularly so in CF where organisms can be transmitted between patients. Standard CF infection control advice applies when measuring LCI, as with any lung function assessment. Risk of cross-infection is reduced by the use of disposable filters and by ensuring all reusable equipment is either sterilised or wiped down with sterilising wipes as appropriate. Details of infection control risk management are covered in a separate section (see part IV)

• **Handling of gas cylinders.** Cylinders must be securely held within trolleys or chained to the wall to prevent falling onto personnel. Risk of explosion caused by build-up of gas pressure if cylinders heated by fire.

5.2. Protective equipment

• Wear sturdy shoes or boots if involved in moving or handling gas cylinders to protect feet from injury if a cylinder falls on them.

• Do not use PTFE tape or any grease/oil-based substances on regulators or any gas couplings – due to risk of ignition when using flammable gases. Ensure hands clean, dry and free of creams/oils when changing regulators.

• Wear gloves when handling mouthpiece, filter and noseclip to prevent transmission of any saliva-borne infections from patient to operator.

5.3. Spillage procedures
N/A
If a gas leak of SF₆ is detected, ensure ventilation of the room. This is not hazardous to health (see above), but will interfere with performance and interpretation of washouts.

5.4. Waste disposal

• All clinical waste (except sharps) must be placed in clinical waste (yellow) bags, ensuring that bags are not overfilled and are sealed and disposed of
appropriately when finished with. Clinical waste includes any wipes that have been used to clean the equipment and any tissues used by the patient.

- All sharps must be disposed of appropriately in a yellow sharps container, again ensuring all relevant guidelines about use and disposal of the sharps container are adhered to.

5.5. Subject safety
Ensure that the subject has signed the informed consent for the study and understands what the assessment involves.
Part III
Protocol

6. Equipment required

- Innocor device, modified and set up as described in part I.
- Supply of 0.2% SF₆ in air
  - BOC Ltd (tel. 0800 020800)
  - Size L tank, 2000ppm SF₆ in air, 200 bar
- Sterile single use filters
  - Adult: Bacteriobac S (see section 2.1)
  - Children: Air Safety Ltd. Bacterial/viral filter 4000/01
- Clean mouthpiece and noseclips
- 3L calibration syringe
- Electric fan
- Gloves
- Sterile wipes
- Visual feedback system if being used – computer screen visible to patient
- Television
- Innocor log to record settings (section 4.2 and Appendix B)

7. Setting up Equipment

7.1. Set up equipment

1. Switch Innocor on at least 20 minutes prior to testing to ensure that the photoacoustic gas analyser is fully warmed up. Ignore any on-screen warnings about Innocor cylinder pressure.

2. Ensure that equipment is correctly arranged and that all connections are tight and leak free. Attention should also be paid to the flowmeter pressure lines and gas sample line. These should be tightly fitted to the flowmeter and gas sample needle, and should not be kinked, twisted or occluded at any point throughout their length.

3. Ensure that the SF₆ gas cylinder is switched on by twisting the knob on the top of the cylinder. Check pressure gauge to ensure there is sufficient gas for testing.

4. Record SF₆ cylinder pressure, ambient temperature and humidity in the Innocor log (see Appendix B)
7.2. Calibration of pneumotachograph
This should be performed daily before the first washout, and repeated every four hours.
If a new flowmeter is being used (or if flowmeter has been reconnected following any alterations or dismantling), this should first be linearised, see section 3.3.

- Calibration syringe should be at room temperature
- Ensure Innocor has warmed up
- Select Setup from the menu on the opening screen.
- Select Calibration.
- From the pop-up menu select Adjust Flowmeter.
- Select Hardware and ensure Flowmeter type is set to Standard.
- Select BbB LCI.
- Connect a 3L calibration syringe to the flowmeter using the appropriate bacterial filter and set the 3L syringe size on the screen.
- Check that the syringe button displays “3 litre”. This can be adjusted using the adjacent arrows if necessary.
- Empty the syringe.
- Press Calibrate.
- When instructed, fill the syringe slowly, at a low flow rate (ie fill over 5-6 seconds), without bumping the end.
- Continue to follow the instructions on the screen. A total of five fill and empty procedures are performed: 2 slow (fill over 5-6 seconds); 2 medium (over 2-3 seconds); and 1 fast (over 1 second)
- Remember that the flowmeter is only linear for flows up to 160ml/min (approx 2.6L/s), but tidal breaths are typically in the range 0.25-0.75L/s so these faster flow rates will not generally be useful.
- After each stroke the measured volume is displayed in the table:

At the end of the calibration, the new gain is displayed (circled above). This is a factor by which the measure volume on that day needs to be adjusted by. If
linearization is correct, this should not vary much on a daily basis and should be between 0.98 and 1.02 (ie ±2%). This means that the difference between the flowmeter at this linearisation and the most recent linearisation should be less than 2%. You should aim for a range of 0.99-1.01.

- The measured syringe volume should also differ by less than ±3%. In other words, once the correct gain is applied the measured volume in each of the Fill and Empty columns should be between 2.91 and 3.09 litres. It is preferable for the accuracy to be greater than this, and we aim to achieve an error of ±2% (i.e. measured volume between 2.94 and 3.06 L once correct gain applied).

- If the gain, or the accuracy, are outwith this range, press Calibrate and repeat the process. If the gain remains unacceptable then:
  - Ensure the correct flowmeter and correct syringe are being used.
  - Ensure that the correct filter is being used. Adding a filter changes the flow and hence pressure/flow characteristics of the flowmeter, so all calibrations and linearisation procedures must be performed with the appropriate filter connected.
  - Ensure all connections between flowmeter and tubing and tubing and RVU cable are secure and leak free.
  - Ensure the syringe is securely connected to the flowmeter.
- If the problem remains then the flowmeter linearity may need to be adjusted (see section 3.3).
- If gain and syringe volumes are acceptable, press OK and Exit.
- Always record the fill/empty gain in the log book, so that any trends in errors can be identified.

### 8. Flow-gas delay calibration

This should be performed daily, before the first washout, and recorded in the log book. It is good practice to repeat the measurement every 4 hours if performing a prolonged testing session.

It is vital that this is known accurately before lung clearance data are calculated as accurate alignment of the signals depends on being able to re-align the output signals accurately. If the Oxigraf has been removed (section 2.7) then it is not possible to measure this using Innocor’s own in-built methodology, which relies on rapid inspiration to cause changes in measured CO₂ and O₂, since we have already removed the O₂ analyser from the circuit.

We use a separate procedure and software to generate flow gas delay (FGD). There are two methods: 1) a mechanical solenoid device to release a wave of SF₆, and 2) the same rapid-inspiration technique used in the standard Innocor setup. In both cases the measurements are made as though a patient was being tested. The first time you do this, you will need to set up a patient called “FLOWGASDELAY” so these files can be easily retrieved for analysis (see section 8.3).

> Importantly, the two FGD methods are not interchangeable, and the same method should be used throughout a study. For a multi-centre study, the same method should be used at all sites. Please refer to study specific guidelines.
• From the opening menu select **Measurement** and then select the patient **FLOWGASDELAY** and press **Select**.
• Press **Test** to prepare a new measurement.
• Select **Test**, followed by **Protocol. Ignore** warning messages about gas delay calibrations expired. **OK** to accept weight data (not relevant)
• Select **“Flow Gas Delay”** from the list of possible protocols (see section 3.1 for details on how to set this. Do this the same as for “**Washout**”, but create a new protocol with the name **“Flow Gas Delay”** in order to differentiate the two assessments). Press **OK** to start.

**8.1. Rebreathing**
• Attach a bacterial filter to the flowmeter. The delay is specific to the equipment set-up so a filter and mouthpiece must be used exactly as if you were a patient undergoing a test.
• When Innocor is warmed up perform at least a slow expiration over at least 3 seconds to get a plateau of expired CO₂.
• Without pausing, follow this with a very fast inspiration for at least 2-3 seconds, so that there is a sudden transition from expired CO₂ to room air. The inspirations have to be as fast as possible to get an accurate determination of gas delay.
• Repeat this at least 10 times, giving yourself a pause of a few seconds between each manoeuvre if necessary, so that you don’t hyperventilate.
• At the end press **Stop protocol**, followed by **Yes** and follow instructions below on exporting and analysing data.

**8.2. Solenoid**
• This uses a mechanical device to open a valve that releases a puff of SF₆ gas over the gas sample needle and flowmeter. It should give more accurate and consistent measurements than the rebreathing technique, as well as being less effort for the operator. It requires the construction of the FGD insert and solenoid (see Appendix C)
• Insert the FGD-insert into the downstream port of the flowmeter, taking care that the slot for the needle aligns with the needle. Ensure this is pressed in gently but firmly so that it achieves a good seal.
• The insert should be connected to the solenoid, with the other side of the solenoid connected to gas tubing (see Figure 11)
• Connect the free end of the gas tubing to the SF₆ cylinder and turn on until the flow reads at least 2L/min. You will need a firm attachment to the cylinder to avoid sudden displacement of the gas tubing.
• You may hear hissing of gas leak from the connection. This can be minimised by ensuring a good connection between gas tubing and solenoid. You may need to use gas tape to ensure a good seal with connections.
• The solenoid should also be connected to the power supply, which should be plugged in and switched on.
• Ensure that flowmeter is horizontal and is not tipped. Since SF$_6$ is denser than air it will accumulate if flowmeter is angled with exhaust end uppermost.
• Switch on fan and direct it at exhaust of flowmeter.

![Diagram of apparatus to measure flow gas delay.]

**Figure 11:** Schematic of apparatus to measure flow gas delay.

• When Innocor is ready, press the solenoid activation button on the control box. This sends a 12v pulse to the solenoid to cause it to open, and to remain open as long as the button is pressed. You should hear a click as the solenoid is activated. Hold open for 2 seconds.
• Release the button and wait for 5-10 seconds, to allow the SF$_6$ levels within the flowmeter to fall again. You should see a sharp spike in SF$_6$ on the BBB graph, followed by a slower fall back to 0.00%.
• Repeat at least 10 times in total.
• Once complete press **Stop protocol** followed by **Yes** and follow instructions below on exporting and analysing data.

### 8.3. FGD data export and analysis
Depending on specific study arrangements these analyses may be performed centrally only. It would still be helpful to be familiar with the process described below.

• Exit to windows:
  • Touch in rapid sequence the right upper followed by the left upper corner of the opening screen
  • When given the option to exit to Windows select **OK**.
• Insert USB stick into a free port at the back of the Innocor machine and wait until this is recognised by the computer as a drive in the left hand **My Computer** panel.
• Use the mouse to select the file `C:/Innocor/RawDataBBB`
• The file you are seeking should be the most recent one, and will be labelled: `BBBFLOWGASDELAY-[Date]-[Time].ino`
• Select the FGD file for that day by clicking on or highlighting them, and click and drag them to the USB drive in the left hand menu. The files should then be copied to the USB stick.
• Once the files are saved, you can remove the USB stick.
• Select Shortcut from the list of files in the C:/Innocor/ directory followed by Restart Innocor to return to Innocor.
• Place memory stick in analysis computer and copy the file to an appropriate folder (recommend that all FGD data be stored in a separate folder).
• Open Innofileconverter.exe, load the FGD file you have just generated, and convert to a text file (ensuring that under Rawdata you have ticked the options to export Flow, SF₆, and CO₂ data).
• Open the Wavemetrics file FGDCal.pxp.
• Select Load and choose the recently generated FGD file.
• If no SF₆ concentrations over 0.05% are present, the programme will assume that FGD is to be generated from the rapid inspiration manoeuvre. Otherwise it infers that the solenoid was used. Check that the graph title indicates “Breath Cal” or “Solenoid Cal” as appropriate.
• Drag the circle next to A in the info panel below the graph window onto the trace.
• Click Single to generate FGD. The results will appear in the results window (if not visible you can retrieve using Windows → Tables → Results)
• A should be before the flow inflection, with B on the flow upstroke.
• C should be at the top of the CO₂ curve, just before it falls, with D at the bottom of the CO₂ fall (see Figure 12).
• E should be at the bottom and F at the top of the SF₆ rise.

![Flow-gas delay](image)

**Figure 12:** Flow-gas delay calculation, showing inflection in flow signal (red) and subsequent change in SF₆ concentration (purple). CO₂ concentration has not been shown for simplicity. Interval between midpoint of flow and SF₆ inflections represents the flow gas delay (indicated). Points A-F as described in the text.
• CO₂ data and graphs will be nonsensical and should be ignored for solenoid calibrations, and likewise SF₆ data should be ignored for rebreathing calibrations.
• Click **Next** to proceed to the next FGD manoeuvre. It will probably be necessary to readjust the position of point A to shortly before the flow inflection again. If you have to do this, click Single to re-analyse that manoeuvre.
• Once you have at least 6 manoeuvres analysed, inspect the results to ensure that FGD and rise time are reproducible.
• Copy and paste results to Excel, and delete any inadvertent duplicates (identified by having the same zero time).
• FGD should differ by less than 10ms from the mean. Occasional outliers may occur due to analysis errors. These should be re-inspected and if necessary excluded.
• Record FGD in the daily Log (Appendix B).
9. Create / select patient in Innocor

9.1. Creating a new patient
If this is the first testing, you will need to create a new patient ID:
- From the Measurement screen select New Patient / New Pt.
- The following screen appears:

![Patient ID Entry Screen]

- Use the keyboard to enter the relevant data and then select OK.
- ID is the patient code, and will be used in the filename of the data generated for that patient (along with the date and time).
- You should have site and study identifiers specific to the study in question, followed by a unique patient number that cannot relate back to the patient. Please refer to study specific guidelines for this.
- Also enter the other key metrics of name, DOB and gender.
- For completeness, record height and weight (if available) even though these are not directly used in our analyses
- Press OK.

9.2. Selecting a patient
- Select the patient from the list displayed on the Measurement screen (sorted by patient ID). You can use the Search facility if you cannot find the details easily.
- Press Select to select the patient.
10. Setting up the patient

It is important that regular breathing is maintained throughout the test. To achieve this, the patient needs to be comfortable, both with the position of the apparatus and with the fit of the mouthpiece (see Figure 13). They also need to be adequately distracted, preferably by watching a television. Although patients are allowed to set their own tidal volume, we are aiming for consistency. Attention should be paid to the breathing pattern to ensure that it is both regular and stable with a reasonable tidal volume (Vt), expiration to the same relaxed FRC. As a guide, you should aim for around 7ml/kg for Vt. It is also important that long inspiratory or expiratory pauses are avoided as these can complicate washout analysis.

- Ensure patient is comfortably seated and able to see the video screen.
- If seated, the patient will need to be sat upright and not slouching, as this will reduce FRC and may affect LCI.
- If lying, make sure the head is elevated a few degrees and that they can see the video screen. If using a story CD for distraction, ensure this has started before the washout commences to allow them to engage with the story without other distractions.
- Ensure a cup of water is available for the subject to drink from between tests.
- Ensure tissues are available so that mouth can be wiped at end of test (in case of accumulation of saliva).
- Demonstrate the procedure to the patient, with age appropriate language. Keep commands simple and direct.
  - Instruct patient to breathe normally during testing. They should breathe in and out regularly, without long pauses between inspiration and expiration.
  - Explain that the patient may experience a dry mouth during the test.
  - Attach noseclip to ensure this is comfortable and that there are no signs of air leakage.
  - Ensure a good mouthpiece seal can be achieved. Practice keeping the lips relaxed and the bite piece held in the mouth but not gripped tightly between the teeth. Emphasise the importance of maintaining a good seal.
  - Demonstrate the procedure for detaching the T-piece and instruct patient not to alter their breathing pattern whilst you are doing this during the test.
  - Explain that you may need to give instructions if the breathing pattern is too irregular.
  - Patients may swallow or (if absolutely necessary) cough through the device, but they should try to maintain a good seal at mouthpiece and noseclip at all times.
11. Starting the test

- From the opening screen of Innocor select Measurement and select patient (see section 9) and press Select.
- Press Test to prepare a new measurement.
- Select Protocol, Ignore error messages about gas delay or gas cylinders.
- Select “Washout test” from the list of possible protocols (see section 3.1 for details on how to set this). OK to proceed.
- For a new patient, you will be required to confirm the height & weight data before starting. You will then be required to confirm that the load is set to zero: this is for exercise testing and the instruction can be ignored but you must select OK before recording can start.
- Innocor will now start a one minute warm up phase.

12. Wash-in Phase

- For the first test of a series only, allow the machine to warm up and start recording before switching on the SF₆. This serves as a zero measurement for the SF₆ analyser.
- Ensure that the T-piece connects the patient to the flow past circuit and turn on the gas. The gas in the flow-past should be set at a sufficient rate to ensure that no expired air is re-inspired. For an adult and older child this should be at least 10 L/min, but will need to be increased if the patient breathes faster or deeper than average.
- Attach the patient to the mouthpiece and ensure the noseclip is attached.
- Remind the patient to breathe normally through the system. Expiration should be to a relaxed FRC. It is important that long pauses between inspiration and expiration are avoided. It is likely that a new patient will take 1-2 minutes to get used to breathing through the device.
• In order to display the real-time SF_6 concentration, select **Show results** from the right hand menu and then select **Show online data** from the pop-up menu.

• **Select Show...** and then **Show online data**.

• This pulls up a graph that shows real-time flow and gas concentrations. To enlarge the scale and so view the SF_6 concentration, press ![enlarge icon]. If you want to close this graph press ![close icon].

• Watch the SF_6 concentrations on this graph carefully.

• **Important:** note that when observing the real-time data that the gas signals are approximately 1 second (or half a breath) behind the flow signals, and that the data are only updated once every second or so. The true value may therefore rise above or fall below the figure seen on the screen.

• With successive breaths the minimum SF_6 concentration, representing end tidal alveolar SF_6, should increase. The maximum (ie the inspiratory SF_6 concentration in air coming from the cylinder) should remain unchanged.

<table>
<thead>
<tr>
<th>Gas</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>0.154</td>
</tr>
<tr>
<td>Oxygen</td>
<td>21.066</td>
</tr>
<tr>
<td>CO2</td>
<td>0.99</td>
</tr>
<tr>
<td>SF6</td>
<td>0.001</td>
</tr>
<tr>
<td>N2O</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Important:** build-up of SF_6 around the machine (eg repeated testing in a poorly ventilated environment) will reduce the measured maximum SF_6 (since Innocor actually calculates the SF_6 signal as the difference between that in the gas sample line and the surrounding air). If this appears to be happening, review the machine positioning and ventilation.

• If washout is taking longer than anticipated, perform the following checks:
  - Check that the flow in the flow past is sufficient. If the bag in the circuit is empty at start of inspiration then this is an indicator that flow may be insufficient. This is also indicated by a sudden fall in SF_6 signal towards end of an inspiration.
  - Check that the patient is wearing a noseclip!
  - Check for leaks around the mouthpiece. Is the mouthpiece positioned comfortably? Is the patient gripping the mouthpiece with their teeth? Avoid the mouthpiece entering the mouth at an angle. Leaks are most likely to occur at the edges of the mouth.
13. Disconnection of flow-past

13.1. Flowmeter resets
Innocor flowmeter is set to re-zero every few minutes, as described and set in section 3.2. This is to maintain accuracy of the flowmeter, but has the effect of losing one second of flow data (something that has to be allowed for in the analysis). This can be particularly tricky to accommodate if the reset occurs during one of the early expirations of the washout, something that it is important to avoid. The way to avoid this is to ensure that the disconnection occurs just after a flowmeter re-zero. Re-zero’s can be anticipated by watching the time display on the Innocor washout screen to tell how long the test has been running.

13.2. End of wash-in
- Continue the wash-in phase until the inspiratory and expiratory SF₆ concentrations are equal, i.e. differ by less than 2% relative, or that the difference between the maximum and minimum concentrations you see on the display are within 0.003% of each other for three successive breaths.
- Turn on fan so that a stream of air is directed over the end of the flowmeter. Ensure that this does not blow directly into the patient’s eyes.
- Warn the patient that you are about to disconnect the T-piece. Remind them to continue to breathe in a relaxed fashion and not to pause or alter breathing pattern during or as a result of disconnection.
- Watch the patient’s chest to identify expiration. At the start of expiration (ie when the chest starts to fall) rapidly disconnect the T-piece, covering the open end of the T-piece with your hand as you do so.
- Once disconnected plug the open arm of the T-piece so that SF₆ does not escape into the test room, and then quickly switch off the gas supply.

14. Washout
- The patient now breathes room air until the peak expiratory SF₆ concentration has fallen to 0.005% for at least three breaths. This cut-off includes a safety margin to ensure that washout has completed.
- If in doubt, always continue washout for a few extra breaths. A washout terminated too soon cannot be used, and the entire procedure has been wasted.
- At the end of the test close the online data window and stop the test by pressing Stop protocol from the right hand menu.
  - If you have paused the test by pressing Hold protocol, do not press Stop protocol but restart before terminating.
- When the patient removes the mouthpiece this may cause release of saliva held behind the mouthpiece. Ensure that you have handed the patient a paper towel prior to disconnection so that they can catch any dribble.
- Offer a drink of water.
15. Further Tests

- Repeat washout testing three times. If you are concerned that one of the tests is likely to prove technically inadequate, then you should repeat the test.
- If you are concerned that a washout may have terminated too early, or that wash-in could be inadequate, it may be quicker to export the data (see section 16) and review the washout before proceeding with a further manoeuvre.
- Perform seated LCI measurements before lying the patient flat for the three supine measurements.
- Ensure that the patient has had sufficient time between washouts to feel comfortable with continuing.
- Always perform spirometry after washouts, and not before, as the forced expiration can move sputum and later ventilation heterogeneity.

16. Data Export

**Important:** Ensure that data are downloaded from Innocor before it is switched off. This is an important data back-up step and ensures that data would not be lost were anything to happen to the Innocor.

- *Use a dedicated memory stick or external hard drive*
- *Only download to a single machine, with up to date virus protection software. Innocor has no anti-virus protection of its own.*

16.1. Exit to Windows

- First exit to Windows by touching in rapid sequence the right upper followed by the left upper corner of the screen.
- When given the option to exit to Windows select **OK**.
- The following screen should appear:
16.2. Copying to Memory stick

- Insert the USB stick into a free port at the back of the Innocor machine and wait until this is recognised by the computer and displayed as a drive under My Computer in the left hand column.
- To locate the data, use the mouse to select the file C:/Innocor/RawDataBBB, this is the folder into which all the breath by breath data are saved. It is advisable to use a USB-mouse rather than to try using the touch screen for this, as it is hard to accurately select and copy the files of interest and folders may be inadvertently rearranged.
- Files are saved according to the formula: BBB[Patient ID]-[Date]-[Time].ino
  To find the most recent files, first click on the view menu ( ) and select the Details format. Then click on Date Modified to arrange data in date-generated order. You may need to click twice to get the most recent data displayed at the top of the column.
- Select the files for that day by clicking on or highlighting them.
- Right click and select Copy.
- Locate the USB drive folder in the left hand column, right click on the folder and select Paste. The files should then be copied to the USB stick.
- Once the files are saved, you can remove the USB stick.
- Ensure that the files are backed up by copying them from the USB stick to the appropriate networked folder on the analysis computer.

16.3. Return to Innocor

- Select Shortcut from the list of files in the C:/Innocor/ directory.
- Select Restart Innocor.

16.4. Switching off Innocor

- If wanting to shut the computer down, you must first return to Innocor and select Exit from the opening menu.
- Do not simply press  in the top right hand corner. This will default to a screen showing the Innovision label, which you are stuck with until you restart the computer.
16.5. Data Analysis
Data analysis is covered in a separate SOP, specific to the software, and is beyond the remit of this document.

17. Special considerations in children

17.1. Standard Procedure for Older Children (5 years and over)
The equipment used and procedure for adult patients is suitable for paediatric patients in this age-group. There are, however, some differences in approach that ensure the best quality data. For younger children you will require the use of the “speeded” Innocor with Oxigraf removed and shortened gas sample line.

17.2. Prior explanation of procedure
- It is important that the procedure is fully explained to the child in language that they will understand. Explain the purpose of the test as a whole, the gas, the mouthpiece and the tubing. Children, especially older ones, are able to understand difficult concepts if clear language is used. Allow questions and encourage them that they can come off if they feel unwell or find it difficult to breathe.
- They must have confidence in the person conducting the test or they may be unwilling to continue.
- A calm, relaxed approach means that the child will feel happy taking part and they will trust the researcher.

17.3. Equipment
- General testing environment
The room should be friendly and inviting without being too full of distractions. Make sure the chair the child sits in is comfortable for them. A cushioned chair is not necessarily the most comfortable option as it is harder to stay still in. A swivel chair or one on wheels is very hard to sit still in. One that encourages children to sit straight and not to move around is best. An example of a good chair is “q-learn chair senior” distributed by Morleys (01869320320, product code CS60281).
- Flowmeter
The recommended pneumotach for children down to 5 years is the same as that used in adults (Hans Rudolph 4700A). This is stable over a wide range of flows and has low deadspace.
- Mouthpiece
Most children down to 5 years can fit the whole mouthpiece in as it is designed. This may require some encouragement. If this is impossible, then keeping their lips on the inner rim of the mouthpiece is adequate. This method is more difficult as they require a more deliberate seal with their lips and the edges of the mouthpiece often hurt the corners of the mouth after a few minutes. Some practice before starting is a good idea to familiarise them with the procedure. Explain that the mouthpiece is like a diver’s mouthpiece and all their breath has to go through the machine, not out the edges. Most children are interested by, and understand, this explanation.
• **Breath volume feedback software (optional)**
  Some children will be able to use this to regulate tidal volume if the concept is explained. Other children may find this confusing if they are not able to change breath volume easily. The breaths may become more erratic. Also, other children may “play” with it and make huge or very fast breaths just to make the display move. If this happens it is probably advisable simply to turn the display away.

17.4. **Potential challenges**
The issues that can make multiple breath washout difficult in children are:
• Not understanding the procedure – make sure they know to keep a good seal round the mouthpiece with their lips. They may need to be reminded.
• Playing with the equipment. Make sure hands are kept away from the pneumotach, especially the open port.
• Becoming easily distracted with other things going on in the room - keep conversation and movement to a minimum.
• The feeling that they don’t know what’s going on – make sure there is a member of staff visible to the child all the time. If this is not possible tell the child where you are going, even if it is just behind them.
• Talking or moving during the test – it is important that they don’t talk during the test as this obviously upsets the natural breathing pattern and introduces leaks. Make sure any verbal instructions are phrased to discourage a reply. Children can forget they are not to talk. Generally keep verbal communication to a minimum. It is frustrating to be talked to and not be able to reply.
• When explaining the test make sure the change-over from washin to washout is explained before the test starts. It is better not to say anything before removing the T-piece during the test as this often interrupts the breathing pattern or the child may think the test is over and detach themselves from the mouthpiece.
• Suitable distraction is vital. Children are generally easily distracted and often forget instructions they have been given. Therefore a cartoon that keeps their attention will ensure they don’t start talking or moving around too much. We find that one of the best cartoons is the Simpsons as it is liked by all age groups. Have an alternative e.g. a movie or another cartoon. Try to avoid videos that have singing or too much excitement as the child may join in. Make sure the screen is easily visible (i.e. straight ahead of them) and the lights are dimmed if possible. Make sure the sound is loud enough so that the child is unlikely to be distracted by other noise or movement.

17.5. **Innocor in young children (<5yrs)**
The concern about using Innocor in this age group has been the speed of the gas analyser. To be able to integrate a gas concentration signal with a flow signal and calculate volume of gas expired the signal rise has to reflect the true rise in gas concentration with each breath. This is conventionally documented as the time taken for the gas concentration to rise from 10% to 90% of the concentration after a step-change. On analysis of artificial step-changes in gas concentration, a mass spectrometer has a documented 10-90% rise time of 80milliseconds. This is when
measuring both the rise in expired gas concentration and fall in inspired concentration. For this reason, ATS/ERS guidelines on performing multiple breath washout tests in those less than 5yrs old have recommended a gas analyser response time of <100ms². For adults and children >5yrs, the resting respiratory rates are slower, and any error caused by the slower rise time is minimal. Innocor has a documented rise time of 120ms but was found to be 150ms in practice. This may therefore introduce inaccuracies in the integrated gas volumes at faster respiratory rates (e.g. in younger children). The current system, by removing the Oxigraf, should have a rise time closer to 100ms (range 90-120ms). At this point we cannot recommend using the current set-up in children under 5yrs old. We are however actively looking at methods to enhance the gas analyser response in Innocor. More details on this research can be obtained from the authors.

18. References

Part IV
Infection Control

19. Infection control policies

To ensure safety of patients during studies we need to adhere to strict infection control guidelines that ensure minimal risk of contamination of re-usable parts that can infect other patients.

All CF patients are colonised with infectious organisms which can be passed to other susceptible patients via aerosol spread. Although we specifically avoid testing patients with MRSA or B. cepacia organisms, all patients should be treated as possible carriers of these organisms. Guidelines apply to all patients at all visits.

After discussion with infection control team at the Royal Hospital for Sick Children (RHSC) in Edinburgh, it was agreed that it is reasonable to have more than one patient in one day if local policy can be adhered to regarding separation of patients and adequate cleaning of equipment between patients. Two patients will not be tested or be in the test room at the same time.

Special attention has to be paid to cleaning and disinfecting parts in direct contact with exhaled breath or mucous membranes. It may be necessary to obtain similar approval from your own site.

19.1. Infection control equipment

Standard procedures recommended for cleaning and disinfecting equipment are:

(may be adapted according to local hospital policy)

- **Hard surface wipes** – Soap-based cleaning cloths (not alcohol-based).
- **Disinfection tablets** – Disifin or locally agreed alternative (e.g. Tristal). Adherence to protocol is required.
- **Disposal** – clinical waste.
- **Hand washing** – soap/ alcohol gel.

19.2. MBW apparatus

Non-disposable equipment in contact with patients during run-in studies:

- Multiple breath washout apparatus
- Other equipment: furniture etc.

See Table 1 for summary of how to disinfect these apparatus.

19.3. Sterilisation of Mouthpieces, Masks, Noseclips & T-pieces

These items can be reused and should be sterilised as below.

- Dissolve 1 tablets of Disifin (RMP UK Ltd, London) in 0.5L of hot water.
- Clean mouthpieces or masks in warm soapy water to remove any deposits.
- Soak in Disifin solution for a minimum of 30minutes. Can be left overnight.
- Rinse in cold running water for a minimum of 60minutes.
- Allow to dry.
- Disifin solution should be discarded after 24hrs.
19.4. Cleaning of Flowmeter
Because we are using a filter, this is not necessary after every use but should be performed if there is reason to expect that the flowmeter has become contaminated. It is also good practice to routinely inspect the flowmeter and clean it if there appears to be debris on the mesh.
Cleaning is according to the manufacturer’s instructions. We recommend the following steps:

- Carefully disassemble the flowmeter.
- Wash in Disfin solution for 30 minutes.
- Rinse in cold running water for 60 minutes.
- Allow to dry, preferably overnight.
- Visual inspection to confirm apparatus is clean and dry and reassembly.
- You will need to repeat the flowmeter linearisation after cleaning (see section 3.3)

19.5. Other Equipment
Chairs, trolleys and other hard surfaces should be wiped clean after every patient with sterile wipes. This should include all surfaces that come into contact with the patient, including door handles etc.
This step is not necessary when testing only healthy controls, but still needs to be performed before and after testing any CF patients.

19.6. Summary of recommendations
- See also Table 1.
- Use a new disposable filter for each patient.
- Wash hands between patients. Either use gloves to handle filters, mouthpieces and noseclips or wash hands after doing so.
- After each patient use, wipe the machine, swing arm and seat with detergent wipes.
- Mouthpieces and noseclips should be sterilised as above.
- Although there is no evidence that any potentially harmful organisms will be missed by these procedures, it is advisable to change the flowpast circuit periodically (i.e. dispose of and replace the tubing, the T-pieces can be sterilised as per mouthpieces). This should be done every ten patients, and the date of this recorded in the Innocor log (under Notes)
<table>
<thead>
<tr>
<th>Multiple Breath Washout Equipment</th>
<th>Disinfection procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innocor</td>
<td>Not in contact with patient. Can be wiped with hard surface wipes (not touchscreen).</td>
</tr>
<tr>
<td>Pressure lines</td>
<td>Protected from patient by in-line filter. Should be wiped thoroughly after each patient (hard surface wipes).</td>
</tr>
<tr>
<td>Flow-meter stand (if applicable)</td>
<td>Should be wiped (hard surface wipes) after each patient</td>
</tr>
<tr>
<td>Bias flow elephant tubing and T-piece</td>
<td>Protected from patient contamination by filter but attention should be made to any evidence of condensation inside tubing. Given that patient’s breath is exhaled through exhaust end, tubing should be changed periodically – every 10-15 patients. T-piece should be disinfected</td>
</tr>
<tr>
<td>Rebreath bag and gas tubing</td>
<td>As per bias flow system</td>
</tr>
<tr>
<td>Gas tank and flow tap</td>
<td>Not in contact with patient.</td>
</tr>
<tr>
<td>Mouthpiece</td>
<td>Disinfected after every patient. Strict separation of clean and used.</td>
</tr>
<tr>
<td>Noseclip</td>
<td>Disinfected after every patient. Strict separation of clean and used.</td>
</tr>
<tr>
<td>In-line filter</td>
<td>Single use. Disposed of after testing. New filter for each patient and visit</td>
</tr>
<tr>
<td>Furniture etc.</td>
<td>Chairs, trolleys, other hard surfaces should be wiped after every patient. This should include all surfaces in contact with patient e.g. door handles etc.</td>
</tr>
</tbody>
</table>

**Table 1**: Summary of cleaning procedures to be applied to MBW apparatus
Appendix A
Background to LCI

REVIEW

Lung clearance index in the assessment of airways disease

Alex Horsley a,b,*

a Department of Respiratory Medicine, Edinburgh Royal Infirmary, 51 Little France Crescent, Edinburgh EH4 2XU, United Kingdom
b UK Cystic Fibrosis Gene Therapy Consortium, United Kingdom

Received 17 October 2008; accepted 29 January 2009

KEYWORDS
Lung clearance Index; Multiple breath washout; Lung physiology; Small airways; Cystic fibrosis

Summary
In the last few years there has been a growing interest in lung clearance index (LCI), a measure of lung physiology derived from multiple breath washout tests. This resurgence of interest was initially driven by the recognition that such assessments were capable of detecting early airways disease in children, and are more sensitive and easier to perform in this population than conventional lung function tests [Aurora P, Kozlowska W, Stocks J. Gas mixing efficiency from birth to adulthood measured by multiple-breath washout. Respir Physiol Neurobiol. 2005;148(1-2):125–39]. With an appreciation of the importance of earlier identification of airways dysfunction, and prevention of irreversible structural airway changes, methods of following airways disease in these “silent years” are especially important. LCI has now been reported in studies involving all age groups, from infants to adults [Lum S, Gustafsson P, Ljungberg H, Hultskamp G, Bush A, Carr SB, et al. Early detection of cystic fibrosis lung disease: multiple-breath washout versus raised volume tests. Thorax. 2007;62(4):341–7; Horsley AR, Gustafsson PM, MacLeod K, Saunders CJ, Greening AP, Porteous D, et al. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. Thorax. 2008;63:135–40], and has a narrow range of normal over this wide age range, making it especially suitable for long-term follow-up studies. In cystic fibrosis (CF) particularly, there is a pressing need for sensitive and repeatable clinical endpoints for therapeutic interventions [Rosenfield M. An overview of endpoints for cystic fibrosis clinical trials: one size does
In the last few years there has been a growing interest in lung clearance index (LCI), a measure of lung physiology derived from multiple breath washout tests. This resurgence of interest was initially driven by the recognition that such assessments were capable of detecting early airways disease in children, and are more sensitive and easier to perform in this population than conventional lung function tests.\(^1\) With an appreciation of the importance of earlier identification of airways dysfunction, and prevention of irreversible structural airway changes, methods of following airways disease in these “silent years” are especially important. LCI has now been reported in studies involving all age groups, from infants to adults,\(^2,3\) and has a narrow range of normal over this wide age range, making it especially suitable for long-term follow-up studies. In cystic fibrosis (CF) particularly, there is a pressing need for sensitive and repeatable clinical endpoints for therapeutic interventions,\(^4\) and LCI has been proposed as an outcome measure in future CF gene therapy studies.\(^5\)

This review will consider how LCI is derived, how it differs from conventional lung function testing, and its applications and limitations.

**Multiple breath washout tests**

LCI is derived from Multiple Breath Washout (MBW) tests. The basic principles behind MBW are relatively simple, and were first described more than 50 years ago.\(^6\) The test involves following the washout of an inert tracer gas from the lungs during normal tidal breathing. The tracer gas can be nitrogen that is normally resident in the lungs, washed out when the subject is switched to breathing 100% oxygen. Alternatively, it can be an exogenous tracer gas that must first be washed into the lungs to equilibrium. Each approach has its own advantages and challenges, but the principle is the same: namely that the tracer gas should be inert and neither absorbed nor excreted by the body to any significant degree. With each successive breath of the washout, there is a fall in the peak concentration of exhaled tracer (Fig. 1).

As shown by hyperpolarised helium MRI studies, airways disease tends to be patchy.\(^7\) Airway narrowing due to factors such as mucus retention, inflammation and airway wall structural damage causes unevenness of ventilation. This unevenness, or ventilation heterogeneity, affects the overall gas mixing efficiency of the lung, and can be measured by following the washout of a tracer gas during tidal breathing. In disease, the washout will take longer to complete, requiring a greater number of breaths.

A number of different indices of deranged ventilation can be calculated from the washout tracings, but one of the most robust and sensitive, and hence one of the most widely reported, of these is the lung clearance index (LCI).

**Derivation of lung clearance index**

For calculation of LCI, the washout is deemed completed when the end-tidal tracer gas concentration has fallen to 1/40th of the starting concentration. The reason for using 1/40th is largely historical, as this represents the limits of the linear operating range (2–80%) of the early nitrogen analysers. However, it has stood the test of time and represents a workable compromise between ending a washout too soon (and therefore losing sensitivity) and an excessively protracted procedure.

Functional residual capacity (FRC) is calculated from multiple breath washouts from the starting end-tidal fraction of tracer gas (Cₘᵢₙᵣₙ), the final fraction of tracer (Cₜₐₐₙₐ₅), and the total volume of tracer gas exhaled up to the end of the washout (Vₜₐₐₙₐ₅):

\[
FRC = \frac{V_{\text{tracing}}}{C_{\text{Ex}} - C_{\text{End}}}
\]

LCI is then defined as the cumulative expired volume (CEV), divided by the FRC:

```
Please cite this article in press as: Horsley A, Lung clearance index in the assessment of airways disease, Respiratory Medicine (2009), doi:10.1016/j.rmed.2009.01.025
```
Innocor for inert gas washout: Instruction manual v3.1.

Figure 1 Typical washout tracing of a healthy adult subject. Flow is shown in the upper trace (expiration upwards), with the scale on the left hand y-axis. Tracer gas (in this case, 0.2% SF₆) concentration is shown in the lower trace — with each successive breath, there is a fall in peak expiratory SF₆ concentration.

LCI = CEV/FRC

In other words, LCI represents a measure of the number of times the volume of gas in the lung at the start of the washout (the FRC) must be turned over in order to wash out the tracer to the pre-defined endpoint. With increasing disease severity, LCI increases.

Clinical use of LCI

Because no complex respiratory manoeuvres are required, MBW tests are especially useful in children, and the majority of recent studies reflect this. The earliest work on lung gas mixing was performed using nitrogen washout apparatus. A number of studies, comparing small numbers of groups of subjects with different respiratory diseases, were performed in the 1970s–1980s. The gas analyser and washout analysis technology used in these studies was relatively crude and constructed in-house. Although abnormalities in gas mixing indices were demonstrated in disease, there was little suggestion that these would be useful clinical assays. A combination of improved analyser technology and data analysis software, as well as a general increased interest in the need for robust infant lung function techniques, has been a major driving force behind the recent resurgence of interest in LCI.

In 2003, a Swedish group described the use of a mass spectrometer to perform washouts using 4% sulphur hexafluoride (SF₆) as the inert tracer gas. They reported that LCI was elevated in 43 children with cystic fibrosis (CF) (aged 3–18 years) compared to 28 healthy controls. More importantly, they showed that LCI was more sensitive than spirometry, being elevated in 22 of the 33 CF patients with normal spirometry. A similar system, using the same technique and analysis software, was subsequently established at Great Ormond Street Children's Hospital in London. Using this apparatus, Aurora et al. confirmed the Swedish findings in school age children, and then went on to use the technique in younger age groups. In pre-school children LCI was higher in those patients infected with Pseudomonas aeruginosa, an important lung pathogen in CF and one that is known to be associated with a poorer prognosis. More recently, the same group have measured LCI in infants as young as 10 weeks old and showed that LCI was elevated in those with CF (mean age 41 weeks) compared to age matched healthy controls. Using a different analyser technology altogether, a third group have reported on LCI in adults and children with CF. The normal range of LCI in the healthy volunteers in all of these studies was almost identical, despite differences in subject age, location, and technology. Cumulatively, these studies have demonstrated that multiple breath washouts can be performed in large numbers of subjects in clinical studies, and that LCI can be reliably and reproducibly measured, even in young subjects. The group mean coefficient of variation of intra-visit repeat LCI measurements, a simple measure of reproducibility, ranged from 7.8% in pre-school children with CF (mean age 4.4 years), to 3.2% in healthy adults (mean age 33 years). Although no association between age and reproducibility has been described in children, our own observations suggest that reproducibility declines with deteriorating LCI.

Longitudinal studies of LCI are particularly important in establishing how LCI tracks disease progression. A large Swiss cohort has been followed between the ages of 6 and 20 years. 142 children with CF have had at least 4 serial annual evaluations of conventional lung function (spirometry, specific airway resistance and FRC at plethysmography), P. aeruginosa infection status, and LCI (performed using a nitrogen washout apparatus). They demonstrated that LCI was the earliest measurement to deteriorate, followed by FEV₁, FVC and finally FEV₁. LCI was elevated in more than half of those with FEVs within the normal range. Furthermore LCI continued to increase, along with pulmonary hyperinflation and trapped gas volume, beyond the age of 12 years, whereas FEVs z-scores stabilised. In subsequent papers derived from the same dataset they
also showed that LCI was more elevated in those with ABPA, and that the slope of longitudinal progression of LCI was greatest in those chronically infected with P. aeruginosa. LCI was also the most sensitive discriminator between groups divided on the basis of chronic and intermittent P. aeruginosa colonization of the lower airway.

It is not possible to know exactly what LCI represents at a histological level, since airway histopathology in subjects with mild disease is not available. Most of the work published in LCI so far has looked at CF, and our understanding of this disease is that it affects primarily the small airways, at least initially. This, and other evidence, leads us to believe that LCI is particularly sensitive to small airways dysfunction. Further support for this came from a study of CT appearances, spirometry and LCI in 44 children (age 5–19 years) with CF. Sensitivity of FEV1 to structural abnormalities on CT was poor. LCI was the most sensitive measure of structural lung abnormalities, particularly air trapping, for which it had a sensitivity of 94%. In addition, LCI was also elevated in one third of those with a normal CT score, which may represent the presence of physiological abnormalities due to disease that is below the limit of resolution of the CT scanner. Normal LCI in a patient with CF almost excluded the presence of structural abnormalities on HRCT.

LCI also has a potential role in asthma and wheeze. It is known that asthma has a number of different phenotypes in childhood, some of which may be associated with structural airway wall changes (airway remodelling). There is also evidence from bronchial biopsy studies of structural airway changes in children with wheeze as young as 3 years old. This has clinical implications as, in many cases, asthma symptoms diminish in late childhood but inflammation and airway remodelling may be progressive in adulthood. There is now a recognized need for robust and repeatable surrogate measures to detect and track early lung function abnormalities in the presence of progressive airway remodelling.

Two studies have measured LCI in asthmatic children with a view to investigating evidence of ventilation heterogeneity and its response to acute treatment. In the first of these, Gustafsson reported on LCI and spirometry in children with asthma and CF. He showed that LCI was elevated in asthma, and fell in response to nebulised bronchodilator. The CF patients had a similar degree of impairment in FEV1 to the asthmatics (groups mean FEV1 72% and 77% predicted, respectively), but had significantly higher LCI, which did not respond to bronchodilators. More recently, Macleod et al. reported that LCI in a cohort of children with well-controlled asthma (mean FEV1 z-score of -1.26) was significantly elevated compared to age matched healthy controls, although the absolute elevation in LCI was modest (mean LCI 6.59 in the asthmatics versus 6.24 in controls). Despite adequate preventative treatment and clinical stability, FEV1 improved significantly following inhaled salbutamol. LCI did not improve, but remained significantly higher than controls, indicating evidence of non-bronchodilator responsive residual airways disease that was not detected by spirometry. LCI may offer an alternative method of assessing airway physiology in this and similar populations.

Advantages of LCI

It is the sensitivity to small airways dysfunction that makes LCI such a valuable measure of airway physiology. Spirometry, essentially a measure of airway resistance at flow limitation, has long been known to be an insensitive measure of small airways disease. The small airways (those less than 2 mm diameter) have a large combined cross-sectional surface area and therefore have low mean flow rates and combined resistance. In healthy adults, they contribute less than 10% of the total airways resistance. Considerable structural damage to these airways can therefore occur before there is any impairment of FEV1. Using hyperpolarised helium MRI to image the distribution of inhaled helium. It has also been shown that FEV1 is insensitive to disturbances in ventilation distribution. LCI therefore fills an important gap in our ability to follow airways disease non-invasively — the so called "silent zone" between onset of pathology and detection of this with standard lung function tests.

Several studies have now shown LCI to be considerably more sensitive to disease than spirometry. The particular sensitivity of LCI to CF lung disease may reflect the underlying lung pathology, which is one of uneven small airway inflammation and obstruction. There is growing interest in the early identification and treatment of lung disease, and this is particularly true in CF where untreated disease leads to a progressive and irreversible decline. Because only tidal breathing is required, LCI is ideal for use in children. No complex or forced respiratory manoeuvres are required. The test can therefore be successfully performed in the majority of those down to pre-school years, though very young children may require sedation. In infants, the challenges are greater because of the technical demands on the apparatus, but the demonstration that this can be done successfully is an exciting development in this field.

A particular problem with other measures of small airway function, such as mid-expiratory flows and single breath washout tests, is that they are poorly reproducible. LCI however has good intra-subject reproducibility (with a coefficient of variation of repeat measurements of around 3-8%), which is as good, or better, than most lung physiology assessments in the lab. Inter-subject reproducibility is also good in healthy volunteers.

The range of LCI in normal subjects is remarkably narrow across a wide age range, and consistent throughout the various studies. Unlike spirometry, it is also unaffected by height or gender. Because it is derived using the FRC, differences due to physical size are already accounted for, leaving only the effects of gas mixing. This is especially important for longitudinal studies, particularly in children. Since spirometry changes with age, height and gender, it is normally expressed as a percent predicted. But this means accepting a wide range of FEV1, which would be considered "normal" for any individual, and the equations most commonly used to determine normal range change in late teens. Use of different prediction equations for "normality" can have profound effects on the measured rate of decline in "% predicted" values for spirometry. These problems are particularly acute when assessing lung
function during the adolescent growth spurt, which itself may be affected by disease processes (e.g. CF). Normal LCI however remains unchanged, allowing any deviation to be easily identified. Although LCI was slightly higher in the infants, this may be due to differences in protocol (the test was performed supine) or due to the effects of serial deadspace.  

Disadvantages of LCI

The test takes much longer to perform than simple spirometry, and (for an exogenous tracer) requires both a wash-in and a washout phase. In normal children or those with mild disease, the entire process takes little more than 5 min, but is conventionally repeated in triplets and therefore takes around 15 min to complete. In adults, a single wash-in and washout may take twice as long. A good mouthpiece seal is required, and this can be difficult to sustain for prolonged periods.

The sensitivity of LCI means that, whilst it is a simple and useful test in those with early airways disease, it is much less informative and more protracted in those with significant airflow obstruction. In addition, particularly in those with severe CF, interventions that reduce the burden of airway infection and inflammation may open up previously poorly ventilated lung regions. This may actually increase the heterogeneity between well and poorly ventilated units, paradoxically worsening LCI. In these subjects, spirometry is probably a more useful indicator of the state of their airways.

Practicalities of measuring LCI

Although well established in a research setting, the technology required for multiple breath washout assessment is considerably more complex than it at first appears. Until recently there have been no commercial apparatus available, and no universal standards for performing the tests. Studies so far have therefore relied upon apparatus and protocols developed in-house. During a nitrogen washout, the fractional nitrogen and oxygen concentrations alter during the course of both individual breaths and the washout as a whole. This alters the viscosity of the expired air, and hence the measured flow, by up to 12%. In order to accommodate this, continuous adjustment of flowmeter output is required according to the measured nitrogen concentration. Although this can be done by computer, in the absence of an off-the-shelf commercial system it requires individual programming by the user.

In addition, with nitrogen washout systems the contribution of additional body nitrogen excreted during the lungs may become significant with prolonged washouts, though is not felt to be significant in normal subjects. Sufficient time must be left between washouts for additional oxygen to be expired or absorbed, and the resting gas concentrations return to baseline—this is recommended to be at least 15 min. The alternative approach of using an exogenous inert marker gas requires the subject to first wash in the tracer until inspiratory and expiratory marker gas concentrations are equal. The supply of gas is then disconnected and as the subject breathes room air the marker gas is washed out from the lungs in the same way as nitrogen is during the nitrogen washout. This approach relies on the availability of an inert marker gas, and the two gases that have been used in previous studies are helium and SF₆, although only data from SF₆ washouts have been reported. The advantages of using a mass spectrometer to follow SF₆ concentrations are that it offers a stable gas signal, with a rapid analyser response time. This has been crucial in the development of this technology in the assessment of very young subjects, including infants. The mass spectrometer also offers the possibility of measuring more than one gas, so that simultaneous washouts of gas species with different diffusion coefficients (helium and SF₆) can be performed in order to explore the effects of diffuson on gas mixing.

Mass spectrometers however are costly and temperamental devices, and a separate supply of tracer gas is required (unlike the nitrogen washout system which can use the hospitals piped oxygen). An alternative photoacoustic gas analyser, known as Innocor®32 has been employed to measure LCI in washouts from 0.2% SF₆. This has the advantages of being considerably more compact and robust than the mass spectrometer, but cannot measure the same range of different gases and has a slower analyser response time which may limit its use to school age children and older. Innocor also requires modification and custom-built software in order to use it in this way (see Fig. 2).

A third system has also been used, based upon an ultrasonic flowmeter to measure both flow and molar mass (a measure of gas density) (ndd medizintechnik, Berne, Switzerland). This is used in the mainstream position, which reduces apparatus deadspace and response time (both important for use in very young subjects with low tidal volumes and high respiratory rates). However the gas density is also affected by heat, moisture and CO₂ content of the gas sample, so complex experimentally derived algorithms are required to correct for this, and dynamic

Figure 2 Subject performing a washout. The supply of wash-in gas (0.2% SF₆ in air) is provided by the cylinder in the background. An Innocor® gas analyser is used to measure flow and SF₆ concentration and expiratory volume is displayed to the subject on a separate screen.

Please cite this article in press as: Horsley A, Lung clearance index in the assessment of airways disease, Respiratory Medicine (2009), doi:10.1016/j.rmed.2009.01.025
change in these variables remains difficult to correct for. At the moment, MBW tests remain restricted to a small number of laboratories, where they are used primarily as research tools. However, the simplicity and reproducibility of the technique make it especially useful for long-term follow-up in children and a few units have successfully integrated these measurements into their annual assessments of CF lung function. In addition, LCI is now also being used for work as a possible endpoint in trials of CF gene therapy. There is now considerable interest in using MBW to assess airways function, and progression, in a range of other diseases.

Recognising the importance of clear guidelines in assisting development of this technology, the European Respiratory Society and American Thoracic Society are currently preparing guidelines on the performance and analysis of multiple breath washouts. It is hoped that this will standardize the procedures used in different units. Clear guidance should also permit and encourage manufacturers to develop commercial apparatus for the measurement of LCI. If this happens, it will allow units without specialist technical and computing support to access the technology, and should further facilitate progress in this emergent field.

Acknowledgements

The contribution of Dr Alastair Innes and Dr Kenny Macleod, for their ongoing input and discussions, as well as valuable feedback on this review, is gratefully acknowledged.

Competing Interests

None.

Funding

The author’s research has been funded by the Cystic Fibrosis Trust Gene Therapy Consortium.

References

Appendix B
Innocor Log

The Innocor log is intended to be a daily record of the machine use and outputs. By recording flow gas delay and flowmeter gain daily, any large or sudden variations in these variables can be detected early. Similarly a log of cylinder pressure allows the operator to identify when replacements are required. Ambient conditions and flow-gas delay are necessary for the correct interpretation of the raw data, and even if stored on the patient data sheet the presence of a copy of these data on the daily log is a useful back-up.
A full sheet log is presented on the next page for printing off.
### Innocor Log

<table>
<thead>
<tr>
<th>Date</th>
<th>Operator</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Flowmeter Cal. Gain

<table>
<thead>
<tr>
<th>Flow Gas Delay</th>
<th>SF₆ cylinder press (start)</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ msecs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Atmospheric pressure

<table>
<thead>
<tr>
<th>Relative Humidity</th>
<th>Room Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bar</td>
<td>%</td>
</tr>
<tr>
<td>°C</td>
<td></td>
</tr>
</tbody>
</table>

#### Empty

- Atmospheric pressure
- Relative Humidity
- Room Temperature

<table>
<thead>
<tr>
<th>Bar</th>
<th>%</th>
<th>°C</th>
</tr>
</thead>
</table>

#### Fill

- Atmospheric pressure
- Relative Humidity
- Room Temperature

<table>
<thead>
<tr>
<th>Bar</th>
<th>%</th>
<th>°C</th>
</tr>
</thead>
</table>

Remember to close cylinder at end.
Appendix C
Solenoid-activated Flow-Gas delay device

This involves three new components, one of which can be easily purchased and 2 of which need to be custom made. The principle is simple: an electronic valve is activated which rapidly opens to allow a stream of gas to pass over the gas sample line and the flowmeter mesh to generate a square wave of flow and SF₆. The interval between detection of these components is the flow gas delay. In practice, the gas flow needs to be directed over the needle using a specially constructed insert, which will need to be custom made by your local medical physics department. The exhaust for this is adjacent to the flowmeter mesh. In this way, the gas wave is contained within a narrow passage in the insert, and maintains a square wave. Without the insert the gas would disperse before sampling at the gas samples needle. The electronic valve is operated by a solenoid, and can be bought of the shelf, but needs a custom built 12V DC supply and switch to activate it.

Figure C1: FGD apparatus, inserted into end of flowmeter, with key components labelled

C.1 Solenoid valve
The item used is an EV-2-12 2 way electric valve, 12V DC, from the West Group (www.westgroup.co.uk). You will also need brass gas tube connection nipples: 12842-PKG 10-32 to 1/8 ID hose fitting.

C2. Activation of valve
You will need a 12V DC supply with a button to activate the valve. The valve comes with bare wires and will need a suitable connector to plug this into the electricity supply. Unless you have the skills to construct this yourself, you should seek help from medical physics. Suggested parts (all with codes from Maplin.co.uk):

- MG81C - 12V DC supply
- KC90X - ABS box
- JK09K - 2.1mm socket x 2
- HH60Q - 2.1mm plug
- FF96E - Push button
C3. Insert
This is the key component, and needs custom machined from nylon by medical physics. It must fit snugly into the exhaust port of the flowmeter, with a central hole for the passage of tracer gas. It also has a slot cut to accommodate the gas sample line (Figures C2 and C3). The hole for the gas should pass the end of the gas sample line.

![Figure C2: illustration of FGD insert.](image)

The hole at the solenoid end must fit to the brass gas nipple purchased with the solenoid. The other port of the solenoid connects to the SF₆ supply. You should be able to set the gas flow to around 4L/min. If there is leaking from the connectors, gas tape will help seal these. You may also need to use zip-ties to secure the gas line to the gas supply once pressure has built up.

![Figure C3: photo of solenoid with plug attached. This inserts into the exhaust port of the flowmeter.](image)