

Attachment 7 Nitrogen Multibreath Washout Test

NITROGEN MULTIPLE BREATH WASHOUT (N₂-MBW) TEST

Standard Operating Procedures

I. INTRODUCTION

This document addresses procedures for the performance of the nitrogen multiple breath washout test (N₂-MBW). It references the 2013 ERS/ATS Consensus Statement (Robinson et al. 2013).

II. BACKGROUND

A healthy airway tree promotes even distribution and optimal mixing of inhaled gas with resident gas. N₂-MBW can be used to assess ventilation distribution by measuring the efficiency of endogenous nitrogen (N₂) gas clearance from the lungs. In this test, 100% oxygen is inhaled and the concentration of N₂ in exhaled gas over time is monitored. N₂-MBW can detect ventilation distribution abnormalities in obstructive lung disease despite normal spirometry.

III. INDICATIONS

N₂-MBW has been used clinically and in research settings to monitor patients who have normal spirometry but are at risk for obstructive lung disease from small airways dysfunction. These patients include children with cystic fibrosis and adults who have undergone allogeneic hematopoietic stem cell transplantation, who can develop bronchiolitis obliterans syndrome.

In general, the advantage of N₂-MBW over spirometry is its sensitivity for peripheral (small airways) impairment, as can occur with certain occupational exposures. Key parameters derived from the N₂-MBW that can be abnormal in peripheral lung disease despite a normal FEV₁ are the lung clearance index (LCI), a measure of global ventilation inhomogeneity, and the acinar slope (S_{ACIN}), a measure of distal ventilation inhomogeneity.

IV. CONTRAINDICATIONS FOR TESTING

As N₂-MBW involves tidal breathing of oxygen, there are few contraindications. However, if any of the following are present, the technician should not proceed with the test:

1. Hemoptysis (coughing up blood) of unknown origin
2. Acute disorders that might affect subject performance during testing: e.g., chest, back or gastrointestinal (GI) distress/discomfort
3. Confusion or inability to follow instructions
4. Current use of supplemental oxygen, as 100% O₂ can precipitate CO₂ retention and decreased level of consciousness in some patients who require supplemental oxygen

Of note, the authors of a study of COPD patients found that 7% (4/54) were unable to complete N₂-MBW testing due to leak at the mouthpiece, dry throat, or a sensation of dyspnea (shortness of breath) when breathing high-flow O₂ (Bell et al. 2018).

V. EQUIPMENT AND SUPPLIES

ndd EasyOne Pro LAB device

mouthpiece (Spirette)

FRC-Barriette

nose clip

O₂ tank

3-Liter calibration syringe w/ calibration adapter

Tissues

Water

VI. PREPARATION AND CALIBRATION OF EQUIPMENT

1. At the beginning of each testing day the technician will perform a calibration check using a 3-Liter syringe. The calibration check should be completed using 3 different flow rates (Utilities / Check Calibration / Multi Flow).
2. Ensure that the oxygen tank has been connected to the device and open the cylinder. The regulator on the cylinder should be set to 4 bar. Ensure that the volume in the cylinder is sufficient to complete a test, tank pressure should be above 30 bars/ 400 psi. Check that there are no leaks in the gas supply connections.
3. Technician will perform Bio-QC washout procedure with at least two valid trials. While performing the procedure watch the supply pressure to verify the pressure remains above 3 bars during the inhalation of the oxygen, if the pressure falls below 3 bars check the regulator and adjust if necessary. Verify that the results are within 10% of the median values for FRC and LCI.

VII. QUALITY CONTROL

The results of QC procedures and equipment maintenance should be dated, signed, and stored in the laboratory log book.

VIII. PREPARATION OF THE SUBJECT

1. Show the equipment to the subject with focus on the mouth piece and the nose clip.
2. Demonstrate what sounds are to be expected during the test procedure. For this purpose the valve can manually be closed and opened (Utilities / Configuration/ Device / DLCO Valve).
3. Explain to the subject that he/she should not feel a difference between breathing 100% oxygen and normal air. The oxygen might feel more 'dry' which can lead to increased saliva production and, in rare cases, coughing.
4. Tell the subject that he/she should breathe normally, continuously and steadily. The mouth must be closed and the lips must seal tightly around the Spirette. The subject must not laugh, speak or yawn during the test.
5. The teeth must be placed on-top of the Spirette, not biting down on the Spirette.
6. Swallowing is OK if necessary.
7. Sighing during the washout phase should lead to exclusion of the test (as it may significantly elevate FRC).
8. Each trial takes about three to five minutes.
9. The time between two trials must be twice as long as the trial time. If shorter interval is used, confirm N₂ gas concentration has returned to baseline. During this time the subject should remove the mouth piece and may remove the nose clip as well. The subject can drink between tests, but he/she should not drink carbonated drinks (this can cause artifacts due to CO₂).

IX. TESTING PROCEDURE

To prepare the MBW test:

1. Partly unwrap the spirette.
 - a. For hygiene reasons, grip the partly unwrapped spirette only with the wrapper at the mouthpiece. Do not touch the spirette.
2. Fully insert the spirette into the spirette holder, but keep the partly unwrapped wrapper on the mouthpiece of the spirette.
 - a. The shape of the spirette and the spirette holder guide the orientation of the spirette. You can only insert the spirette fully if it is in the correct orientation.
3. Place a new FRC barriette into the valve unit, making sure to align the arrows on the side of the barriette with the mark on the valve unit.
4. On the main menu, choose Patients.
5. Select the patient that you want to test or add a new patient and complete the demographics information on the first screen.
6. At the bottom of the screen, choose Test.
7. Choose FRC (MBW).
8. If the environment conditions screen is displayed, verify or adapt the current values and choose Confirm >>.

The temperature and humidity sensor measures the required data automatically. However, check these values for plausibility. For example, if the temperature and humidity sensor is too close to the fan on the back of EasyOne Pro/LAB, the

- measured temperature and humidity can be wrong.
9. To confirm that you have inserted the FRC barriette, choose OK.
 - a. The motor and valve unit blocks the spirette. The baseline is set automatically and other preparations are performed automatically.
 - b. Hold the flow sensor away from the patient because gas is flushed from the patient tube. If the patient inhales the gas that is flushed, this can influence the later measurement.
 - c. After all preparations are finished, Tidal Breathing is displayed in the colored status bar.
 10. Remove the wrapper from the spirette, but keep the wrapper.
 11. Have the patient place the nose clip on their nose.
 12. Hand the flow sensor to the patient.

To perform MBW with linked SVC maneuver:

1. Tell the patient to take the spirette into the mouth and to seal the lips around the spirette.
 - a. The patient must not block the opening with the tongue or teeth or bite down excessively on the spirette.
2. Tell the patient to breathe at rest.
 - a. The patient will breath at rest for approximately 4-5 breaths or unit the machine has determined the patient has reached a stable breathing pattern.
 - b. The machine will then display a message 'Inhale fully', instruct the patient slowly inhale to maximal capacity and then to slowly and steadily exhale fully. Encourage the patient to keep exhaling until the flow-volume curve shows a distinct plateau.
 - c. Tell the patient to return to normal relaxed breathing, once the patient's breathing has returned to a stable pattern click the 'Active MBW' button.
 - d. After clicking the button the machine will wait for 5 more breaths before activating the washout phase. The valve will close automatically and the patient inhales the O₂ gas. Let the patient know there will be a noise from the valve/tank and that they may feel cold, dry air in their throat. Remind them to maintain normal tidal breathing while the oxygen is flowing.
 - e. During the washout maneuver the patient only needs to breathe at rest. The patient must not breathe particularly deeply or forcefully.
 - f. Tell the patient to watch the "inspiratory target" graphic on the far right middle of the screen. They should stay in the white area, hence causing them to take breaths of a preferred depth, between 0.9 and 1.5 liters. Instruct the patient to try to inhale to the same level with each breath.
 - g. If the patient has to cough, you must abort and repeat the trial. If at any time during the washout the patient comes off the spirette the trial must be aborted. This will usually appear as a rapid increase in the N₂ concentration shown in the bar chart on the display.
 - h. During the washout also watch the flow volume plot making sure the flow-volume curves do not get narrower as this may indicate the patient is beginning to hyperventilate. If you see this remind the patient to breath slower and relaxed.

- i. In the corner on the top right, the progress of the N2 washout is indicated in percentages. When the N2 concentration is falls below 2% for at least 3 consecutive breaths the test will end automatically.
3. Tell the patient to take the spirette out of the mouth and to breathe normally again.
 - a. If the trial is acceptable, the green quality message is displayed.
 - b. If the trial is not acceptable, a yellow or red quality message is displayed suggesting how to improve the breathing maneuver.
4. Let the patient take a break.
 - a. The length of the break varies according to the length of the previous trial. In any case, a countdown for the length of the break is displayed on the results screen.
 - b. When the countdown is up, a Start button is displayed.

To add trials:

1. Choose Start.
 - a. Again, preparations are performed automatically.
 - b. Hold the flow sensor away from the patient because gas is flushed from the patient tube. If the patient inhales the gas that is flushed, this can influence the later measurement.
 - c. After all preparations are finished, Tidal Breathing is displayed in the colored status bar.
2. Repeat the previous procedure “To perform the MBW breathing maneuver” and this procedure “To add trials” until the green Session Complete! message is displayed.
 - a. In the Test Information area the quality grade for the test is displayed.
3. Do the following:
 - a. Review the test quality that is displayed in the Test Information area.
 - b. Decide whether the test quality is sufficient or not.
 - c. If necessary, add a trial and repeat the procedure “To perform the MBW breathing maneuver.”

Conduct a minimum of three acceptable trials. FRC and LCI variability should be within 10%. Tests where FRC differs by 25% from the median FRC value across the three tests should be automatically rejected.

To end the MBW test:

1. Grip the spirette with the wrapper around again and pull the spirette out of the spirette holder. Do not touch the spirette.
2. Dispose of the spirette together with the wrapper.
3. CAUTION! Always wear disposable gloves. Between patients, always exchange disposable gloves, clean the flow sensor, and disinfect hands. Make sure that no fluid penetrates the flow sensor or the inside of EasyOne Pro/LAB while cleaning. To clean the flow sensor, do not immerse the flow sensor into any fluid. To clean the flow sensor of EasyOne Pro/LAB and to disinfect your hands after each patient, do the following:
 - a. Clean the outside of the flow sensor.
 - b. Put down the flow sensor of EasyOne Pro/LAB.
 - c. Take off or change the disposable gloves and disinfect your hands before you put

on new disposable gloves.

X. SAFETY

1. To prevent electrical shock, all equipment will be plugged into a grounded electrical outlet.
2. Infection control measures:
 - a. Only disposable mouthpieces will be used.
 - b. Equipment will be cleaned and disinfected daily.
3. Subjects in wheelchairs will perform the test while seated in their wheelchairs. Wheelchair wheels will be locked prior to testing.
4. Subjects will never be left unattended during the test procedure.
5. An emergency instruction card will be present at the test site. This card will include:
 - a. Instruction to call 911.
 - b. Arrangement information for transportation to nearest hospital.
 - c. Address and telephone number for the nearest hospital.

XI. TEST SEQUENCE WITH OTHER TESTS

The recommended testing order is:
FeNO, MBW_N2, iOS, spirometry, and DLCO.

XII. REFERENCES

Bell AS, Lawrence PJ, Singh D, Horsley A. Feasibility and challenges of using multiple breath washout in COPD. *Int J Chron Obstruct Pulmon Dis*. 2018;13:2113-2119.

ndd. Application Note. Multi-breath washout FAQ. 2018.

ndd. EasyOne Pro/Lab Operator's Manual V5.0.

Nyilas S, Baumeler L, Tamm M, Halter JP, Savic S, Korten I, Meyer A, Singer F, Passweg JR, Latzin P, Stolz D. Inert gas washout in bronchiolitis obliterans following hematopoietic cell transplantation. *Chest*. 2018;154(1):157-168.

Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *Eur Respir J*. 2013;41(3):507-22.