



## NObreath® Nitric Oxide (NO) breath monitor: comparisons to other existing technologies

NObreath® is a new product. Bedfont Scientific's intention is to illustrate that it compares favourably with other commercially available products some of which have been on the market for 15 years. One such product is the Logan Research Ltd LR2000 breath NO analyzer. This uses the "gold standard" technique of chemiluminescence. Logan Research carried some brief comparison tests with their LR2000 and Bedfont's new NObreath® product. Their report is attached. It also cites two other products that use electrochemical sensor technology to detect NO on the breath.

In addition, in this package of information attached, we have tried to illustrate how existing products compare with one another. As NObreath® is so new, no clinical publications cite the product in the methodology. Bedfont has commissioned several clinical trials of NObreath®, but we are unable to publish any results pending completion and subsequent publication of the results. Looking at existing products, there appears to be a spread of agreement from very good to not too good. It is beyond the intended scope of this document to explain why, however, for convenience, there are attached, the summaries of some work carried out in this area using both chemiluminescence and electrochemical techniques. Both analytical and breath sampling flow differences are significant contributors to these disagreements. Literature references are included so that the full publication can be obtained easily.

Despite these differences, which may be regarded as analytically significant, the technique of measuring  $F_{\text{ENO}}$  is shown to be clinically useful.



## **BENCHMARK TESTING REPORT**

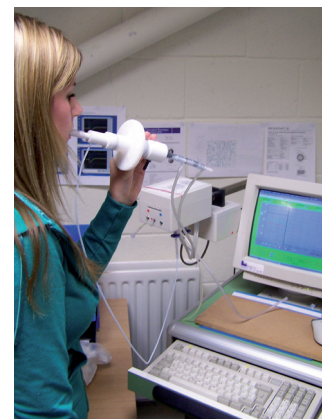
### Comparison testing carried out by Logan Research Ltd for Bedfont Scientific Ltd – Reference the NObreath Nitric Oxide monitoring product.

- 1.0 Date of testing 4/2/2009.
- 2.0 Reference gold standard system LR2000 chemiluminescence analyser (the first clinical research system to be put on the market in 1994.)
- 2.1 Relevant characteristics are High accuracy, very low noise/detection limit (+/-0.3ppb), very low cross interference to other gases, onboard CO<sub>2</sub> route mapping, mouth pressure and flow monitoring (50mls/sec), auto zeroing and patient biofeedback.
- 3.0 Products compared with the gold standard LR2000 product:-
  - 3.1 Bedfont Scientific NObreath
  - 3.2 Filt NO Vario
  - 3.3 Aerocrine Niox Mino
- 4.0 Ambient NO levels at the start/end of testing, see appendix 1.
- 5.0 Calibration:- Three units were calibrated from the same cal. Gas cylinder (51ppb NO bal. N<sub>2</sub>) The only exception was the Niox Mino which had no external calibration facility and claimed to be accurately pre-calibrated.
- 6.0 Temperature of the test area was 24deg C and remained within +/-1.5 deg C for the 4 hours of testing.
- 7.0 Inhalation scrubbing was only used/built into the Filt and Aerocrine products. Inhalation scrubbing is not generally necessary when ambient NO levels are <250ppb as inhaled NO is rapidly cleared from the lungs (in milliseconds) as a result of very rapidly combining with blood haemoglobin. Any NO remaining in the upper airway dead space is rapidly washed out within few seconds on exhalation.
- 8.0 Cross infection control is greatly improved if only exhalation into the product is required. NObreath and LR2000 only require exhalation, ie. No scrubbed inhalation through the same filter.
- 9.0 Sixteen subjects were tested – mixture of normals and asthmatics.
- 10.0 Comparison Results between LR2000 reference unit and the other products (see appendix 1 for raw data set).
  - 10.1 With the NObreath product the mean difference was minus (-) 3.81ppb (made up of -4.1ppb (24) and +2.66ppb (6)).
  - 10.2 With the NO Vario product the mean difference was +9.4ppb.
  - 10.3 With the Niox Mino product the mean difference was +13.1ppb.
- 11.0 Post testing investigation revealed that the NObreath flow calibration biofeedback marking was not in exactly the right place causing the subject to blow at too higher rate resulting in a lower NO concentration being recorded. This problem has been corrected and has resulted in a mean diff of only minus 1-2 ppb. A further response time correction factor (k=1.05) was also added narrowing the gap to +/-1ppb.

- 11.1 NO Vario was investigated and found to have the flow regulating device set too low resulting in higher NO concentrations being recorded. This was corrected and did result in a reduction in mean difference in respect to the LR2000 reference unit.
- 11.2 Niox Mino was investigated. Tests could some times not be carried out due to it's self monitoring temporally disabling the unit until temperature/noise stabilisation was complete. The underlying cause was traced to poor NO sensor performance, the sensor having a very poor signal to noise ratio which would also account for the somewhat erratic values recorded. A rig was fabricated to enable calibration checks to be made using the common cal. Gas cylinder used with the other products. The result showed that this unit was out of calibration and reading high.



a) Subject being tested using the NObreath



b) Subject being tested using the LR2000

### **Conclusions**

The NObreath compared more favourably with the LR2000 than the other two electrochemical sensor based products. Additionally, other benefits were:

- a) Smaller mean difference in NO values when compared with reference unit (LR2000).
- b) Improved cross infection risk due to exhalation into unit only.
- c) Far better signal to noise ratio due to improved sensor used and lower detection limit achieved (+/-1ppb)
- d) Uniquely, the use of a combined bacterial filter and molecular sieve disposable mouth piece reduces cross interference from other interfering exhaled gases such as water vapour and CO<sub>2</sub> compared with other similar sensor technology based products.

# Appendix 1

## Benchmarking between 4 breath nitric oxide monitors 05.02.2009

### Test Results

Person	NObreath	LRL2000	NO Vario	NIOX MINO
male 1	16	18.7	32	38
male 1	17	20.2	28	n/a
male 2	13	9	19.2	24
male 2	11	10	20.2	23
male 3	5	8.5	17.1	17
male 3	n/a	8.5	n/a	n/a
male 4	4	5.8	11.1	10
male 4	5	7.4	11	n/a
male 5	12	11.9	22	25
male 5	9	13.7	22.3	23
male 6	8	12.8	22	18
male 6	12	11.6	20.3	21
male 7	19	26	37.2	49
male 7	19	26.5	37.7	38
female 1	12	15.2	20	27
female 1	20	14.1	19.7	24
female 2	3	6.2	14.9	18
female 2	12	6.9	14.2	12
female 3	6	10.5	14.7	12
female 3	8	9.3	16.6	n/a could not complete 2nd reading
female 4	12	12	17	11
female 4	17	12.6	20.1	18
male 8	27	28.6	33.9	50
male 8	20	30	38.4	44
female 5	38	50	68.6	87
female 5	42	46	69.8	n/a
male 9	34	36.3	58.1	63
male 9	38	40.6	56.2	66
female 6	10	13.1	24.1	21
female 6	13	13.8	21.9	27
male 10	2	9	15	n/a
male 10	n/a	10.1	14.9	n/a

Background NO reading before benchmarking  
LRL2000 - 21ppb  
NObreath - 20ppb

NObreath Additional readings
12ppb, 6ppb, 10ppb

Background NO reading after benchmarking  
LRL2000 - 6.2ppb  
NObreath - 8ppb  
NIOX - 11ppb  
NO Vario - 9.6ppb

## Benchmarking Papers

Stefano Pazzimenti, Massimiliano Bugiani, Pavilio Piccioni, Enrico Hefler, Aurelia Carosso, Giuseppe Guida and Giovanni Rolla. *Exhaled nitric oxide measurements: correction equation to compare hand-held device to stationary analyser*. *Respiratory Medicine* (2008) 102, 1272 – 1275.

### Summary

**Introduction:** Exhaled nitric oxide (FEno) is a reliable non-invasive marker of airway inflammation. In 2003 FEno chemiluminescence analyzer (NIOX<sup>®</sup>; Aerocrine AB, Solna, Sweden) was approved by U.S. Food and Drug Administration for monitoring asthma therapy. Recently, the same company developed a portable device using electrochemical sensors (NIOX-MINO<sup>®</sup>; Aerocrine AB). The aim of our study was to compare NIOX MINO<sup>®</sup> FEno values to those obtained by NIOX<sup>®</sup> and to calculate a correct equation.

**Methods:** Two adequate measurements obtained by NIOX<sup>®</sup> and NIOX-MINO<sup>®</sup> were recorded in 32 subjects (16 females mean age 41 years).

**Results:** FEno values measured by NIOX-MINO<sup>®</sup> were systematically higher than those obtained by NIOX<sup>®</sup> (47.1 ppb, IC 95% 35.2-59.1 and 36.9 ppb, IC 95% 25.0-49.0, respectively). There was a significant correlation ( $r = 0.998$ ,  $p < 0.001$ ) between FEno measured by the two analyzers and the following conversion equation was calculated as:  $FEno(NIOX^{\circ}) = 1.656(SE = 0.61) + 0.808(se = 0.009) \times FEno(NIOX-MINO^{\circ})$  Discussion: FEno values measured by the portable nitric oxide analyzer are reliable and strongly correlated with values obtained by the standard stationary device, with a systematic difference observed between the two instruments' values that can be described by the conversion equation we provided. This equation will help clinicians and researchers to compare data obtainable by the two analyzers. ©2008 Elsevier Ltd. All rights reserved.

B. Khalili, P. B. Boggs and S. L. Bahna. *Reliability of a new hand-held device for the measurement of exhaled nitric oxide*. *Allergy* 2007; 62: 1171 - 1174

**Background:** Given the importance of the airway inflammation in asthma, there has been an effort to incorporate inflammatory markers into its management. Measurement of fractional exhaled nitric oxide (FeNO) is a non-invasive marker of airway inflammation; however, the use of the available FeNO analyzer is limited by several factors including its cost and lack of transportability. The aim of this study was to compare the performance of a new hand-held FeNO measuring device (NIOX-MINO) to the current clinical standard- the chemiluminescence FeNO analyzer and (NIOX).

**Methods:** Subjects 6 years and older presenting to an allergy and asthma clinic underwent FeNO evaluation by NIOX and each of three NIOX MINOs. The mean of two acceptable measurements from the NIOX and the first approved measurement from each NIOX MINO were used for analysis.

**Results:** One hundred patients aged 6-86 years completed the study. Intra-subject FeNO levels obtained by each of the three NIOX MINOs revealed no significant difference between the measurements ( $P = 0.59$ ). There was a very strong correlation between FeNO measurements by NIOX and by NIOX MINO ( $r = 0.98$ ,  $P < 0.001$ ). The mean intrasubject FeNO difference between NIOX and NIOX MINO was -0.5 p.p.b. which was not statistically different from zero ( $P = 0.21$ ).

**Conclusions:** Fractional exhaled nitric oxide measurements by the NIOX MINO showed a strong correlation and a high degree of agreement with the current standard stationary device. The NIOX MINO may be reliably used in clinical practice.

J. D. Boot, L. de Ridder, M. L. de Kam, C. Calderon, M. A. Mascelli, Z. Diamant. *Comparison of exhaled nitric oxide measurements between NIOX MINO<sup>®</sup> electrochemical and Ecomedics chemiluminescence analyzer*. *Respiratory Medicine* (2008) 102, 1667 – 1671.

### Summary

**Background:** Exhaled nitric oxide (eNO) is an established, non-invasive biomarker of active airway inflammation in (atopic) asthma. Treatment with anti-inflammatory therapy, such as inhaled corticosteroids, effectively decreased eNO levels. The NIOX MINO<sup>®</sup> (MINO) is a hand-held, relatively inexpensive, electrochemical device that has been shown to yield comparable eNO measurements to the NIOX stationary unit.

**Aim:** To compare measurements of MINO with another widely used and validated stationary chemiluminescence analyzer, the Ecomedics (ECO).

**Methods:** We performed subsequent eNO measurements on ECO and MINO in 50 subjects (19 healthy volunteers, 18 healthy smokers and 13 non-smoking, atopic asthmatics, not on controller therapy) on two visits 4-10 days apart. The mean of three acceptable measurements by ECO and the first acceptable measurement with the MINO were used for analysis.

**Results:** Both devices yielded reproducible eNO values for all subjects on both visits, with an overall CV of 22.7% (ECO) and 18.3% (MINO). A significant correlation was found between both devices ( $r = 0.97$ ,  $p < 0.0001$ ). Bland-Altman plots showed a high degree of agreement for the entire population (mean difference MINO vs ECO + -10%; 95% limit of agreement were -36% and +28%) and in the three individual subgroups.

**Conclusions:** Exhaled NO Values measured with the MINO are reproducible and in agreement with the ECO. Our results add further evidence to the reliability of the MINO and warrant its applicability in research in clinical practice. ©2008 Elsevier Ltd. All right reserved.

Zoe Borrill, David Clough, Nick Truman, Julie Morris, Stephen Langley and Dave Singh. *A comparison of exhaled nitric oxide measurements performed using three different analysers*. *Respiratory Medicine* (2006) 100, 1392 – 1396.

### Summary

**Introduction:** Exhaled nitric oxide (NO) is an established technique for monitoring airway inflammation. We have compared exhaled NO measurements from 3 different analyzers; Ecomedics (E), Niox (N) and Logan (L).

**Methods:** Thirty subjects (10 non-smoking healthy subjects, 10 non-smoking patients with asthma and 10 ex-smoking COPD patients) performed 3 repeated measurements of exhaled NO at a flow rate of 50ml/s on each of the 3 analyzers. Within analyzer variability was determined by calculating the repeatability coefficient for each analyzer. Differences between analyzers were assessed by (1) differences between group means and (2) the Bland Altman method to estimate the variability expected for an individual using the 3 analyzers.

**Results:** The repeatability coefficients (expressed as ratios) were 1.12, 1.19 and 1.19 for N, E and L, respectively. There were significant differences ( $P < 0.05$ ) between analyzers; the Logan analyzer gave the highest group mean values and Ecomedics gave the lowest group mean values. Differences between analyzers were observed in all subject groups (healthy, asthma and COPD). Similar results were obtained in the 3 groups when analyzed separately. Bland Altman analysis gave the following ratios [date are mean ratio (95% limits of agreement)]; N:E 1.59 (1.02-2.50), L:N 1.23 (0.72-2.13), L:E 1.96 (1.09-3.57).

Sergei A. Kharitonov. *Exhaled markers of inflammatory lung diseases: ready for routine monitoring?* *Swiss Med Weekly* 2004; 134: 175 – 192.

### Summary

Assessing airway inflammation is important for investigating underlying mechanisms of many lung diseases, including asthma and chronic obstructive pulmonary disease (COPD). Yet these are not measured directly in routine clinical practice because of the difficulties in monitoring inflammation. The presence and type of airway inflammation can be difficult to detect clinically, and may result in delays in initiating appropriate therapy. Non-invasive monitoring may assist in differential diagnosis of lung diseases, assessment of their severity and response to treatment. There is increasing evidence that breath analysis may have an important place in the diagnosis and clinical management of asthma, COPD, primary ciliary dyskinesia (PCD) and other major lung disease. The article reviews whether current non-invasive measurements of exhaled gases, such as nitric oxide (NO), hydrocarbons, inflammatory markers exhaled breath condensate (EBC) are ready for routine use in clinical practice.

Andrew D. Smith, Jan O.Cowan, Sue Filsell, Chris McLachlan, Gabrielle Monti-Sheehan, Pamela Jackson, and D. Robin Taylor. *Diagnosing Asthma: Comparisons between Exhaled Nitric Oxide Measurements and Conventional Tests*. *Am J Respir Crit Care Med* Vol 169. pp 473-478, 2004.

### Summary

International guidelines recommend range of clinical tests to confirm the diagnosis of asthma. These focus largely on identifying variable airflow obstruction and responses to bronchodilator or corticosteroid. More recently, exhaled nitric oxide (FEno) measurements and induced sputum analysis to assess airway inflammation have been highlighted. However, to date, no systematic comparisons to confirm diagnostic utility of each of these methods have been performed. To do so, we investigated 47 consecutive patients with symptoms suggestive of asthma, using a comprehensive fixed-sequence series of diagnostic tests. Sensitivities and specificities were obtained for peak flow measurements, spirometry, and changes in these parameters after a trial of steroid. Comparisons were made against FEno and sputum cell counts. Sensitivities for each of the conventional tests (0-47%) were lower than for FEno(88%) and sputum eosinophils (86%). Overall, the diagnostic accuracy when using FEno and sputum eosinophils was significantly greater. Results for conventional tests were not improved, using a trial of steroid. We conclude that FEno measurements and induced sputum analysis are superior to conventional approaches, with exhaled nitric oxide being most advantageous because the test is quick and easy to perform.