A comparison of exhaled nitric oxide measurements performed using three different analysers

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KEYWORDS
Exhaled nitric oxide; Chronic obstructive pulmonary disease; Asthma

Summary
Introduction: Exhaled nitric oxide (NO) is an established technique for monitoring airway inflammation. We have compared exhaled NO measurements from 3 different analysers; 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 50 ml/s on each of the 3 analysers. Within analyser variability was determined by calculating the repeatability coefficient for each analyser. Differences between analysers were assessed by (1) the differences between group means and (2) the Bland Altman method to estimate the variability expected for an individual using the 3 analysers.
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 analysers; the Logan analyser gave the highest group mean values and Ecomedics gave the lowest group mean values. Differences between analysers were observed in all subject groups (healthy, asthma, COPD). Similar results were obtained in the 3 groups when analysed separately. Bland Altman analysis gave the following ratios [data 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).

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; NO, nitric oxide; ppb, parts per billion; ppm, parts per million
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Introduction

The measurement of nitric oxide (NO) in exhaled breath provides a non-invasive means of assessing airway inflammation. Exhaled NO levels are raised in asthma and unstable chronic obstructive pulmonary disease (COPD) patients. Studies have shown that exhaled NO may be used to diagnose asthma and to evaluate the anti-inflammatory effects of inhaled corticosteroids in patients with asthma.

Recommendations for the measurement of exhaled NO in clinical practice have enabled standardisation of the measurement procedure in different centres. However, there are now several manufacturers of NO analysers. Consequently, reports of NO measurements have often used different equipment. We hypothesised that although the procedure of exhaled NO measurement has been standardised, there may be differences due to the analysis equipment used. We therefore investigated whether there were any differences in exhaled NO measurements from healthy subjects, asthmatics and COPD patients using 3 different commercially available analysers. NO measurements were performed according to the manufacturers’ instructions. This replicated the use of the different analysers in current practice as closely as possible.

Methods

Thirty subjects participated in the study; 10 patients with asthma, 10 patients with COPD and 10 healthy volunteers (Table 1). COPD was defined according to the British Thoracic Society guidelines. All COPD patients were required to be ex-smokers and 5 were taking regular inhaled corticosteroids. All asthma patients and healthy volunteers were lifetime non-smokers, and were not taking inhaled corticosteroids. Subjects were excluded if they had experienced a respiratory tract infection in the last 4 weeks. Written informed consent was obtained and the local ethics committee approved the study. The study was undertaken according to the principles of the Declaration of Helsinki 1975.

Three NO analysers were used in this study; Ecomedics AG analyser CLD 88 (Ecomedics; Durnten, Switzerland), Niox (Aerocrine; Solna, Sweden) and Logan model LRZ149 (Logan Research; Rochester, Kent, UK). Each analyser was calibrated according to the manufacturer’s instructions. The Niox analyser was calibrated with gas containing NO at 200 ppb (Hoek Loos BV, Amsterdam, The Netherlands). The Ecomedics analyser was calibrated using source gas containing NO at 20 ppm (SIT Analytical, Sandwich, UK) which underwent dilution to a final concentration of 200 ppb. The Logan analyser was calibrated using gas containing NO at 100 ppb (BOC, Guildford, UK). After calibration, gas containing NO at 100 ppb (BOC, Guildford, UK) was passed through the sample port of each analyser at a flow rate of 50 ml/s, in order to check that there were no differences between analysers in the measurement of an externally standardised source of NO. Each analyser consistently gave a NO measurement of 100 ppb.

Subjects performed exhaled NO measurements using each of the 3 machines in random order using a computer generated randomisation sequence. These measurements were completed within 1 h on the same day. The expired flow rate for all analysers was set at 50 ml/s. The actual flow rate achieved for all 3 machines was 45–55 ml/s (within 10% of the target flow rate, as recommended in guidelines), which was verified at the end of every reading. For the Ecomedics analyser, the flow rate was calibrated daily. However, for the Niox and Logan analysers calibration of the resistors is not recommended by the manufacturers. Three acceptable readings were recorded from each subject using each machine, according to ATS criteria.

Table 1 Subject demographics (data expressed as mean (SD)).

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>COPD</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.6 (14.6)</td>
<td>65.6 (6.7)</td>
<td>32.2 (7.6)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7M/3F</td>
<td>7M/3F</td>
<td>5M/5F</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>81.4 (19.7)</td>
<td>63.5 (7.9)</td>
<td>99.5 (9.2)</td>
</tr>
</tbody>
</table>
Statistical analysis

The exhaled NO data were natural log transformed to normalise the data. The variability of measurements from the same analyser (within analyser variability) was determined by calculating the repeatability coefficient for each analyser. The exponential of this value was then calculated. The ratio of 2 readings from the same analyser will lie within this exponential repeatability coefficient for 95% of subjects.

Differences between analysers were assessed by 2 methods (1) The differences between group means were assessed using paired Student’s t-tests and (2) the variability expected for the same individual using the 3 analysers was estimated using the Bland Altman method, which provided a ratio and 95% limits of agreement for each pair of analysers.

Results

Within analyser variability was relatively small; the exponential repeatability coefficients for repeated measurements performed on 30 subjects using the Ecomedics, Niox and Logan analysers were 1.12, 1.19 and 1.19, respectively.

Between analyser differences were assessed first using group mean values. Measurements in the 30 subjects were significantly higher using the Logan analyser, and lower using the Ecomedics (Fig. 1). The same pattern was observed for lower readings (<20 ppb) and for higher readings (>20 ppb)—data not shown. Differences between analysers for the 3 subject groups showed a consistent pattern (Fig. 2); the Logan analyser gave significantly higher mean values, and the Ecomedics gave significantly lower mean values in all 3 groups.

![Figure 1](image1.png)  
**Figure 1** Comparison of results obtained from the 3 analysers in all subjects (geometric mean and 95% confidence intervals shown; *P<0.0001).

![Figure 2](image2.png)  
**Figure 2** Comparison of analysers in the 3 subject groups showing individual data (horizontal bars indicate geometric mean values, *P<0.05, ^P<0.001, #P<0.0001).

The Bland Altman method was used to quantify the between analyser variability observed between individual measurements. The mean ratios (95% limits of agreement) for each pair of analysers were as follows; for Niox vs. Ecomedics 1.59 (1.02–2.50), for Logan vs. Ecomedics 1.96 (1.09–3.57) and for Logan vs. Niox 1.23 (0.72–2.13).
Discussion

This is the first report to compare readings from different exhaled NO analysers. We firstly quantified within analyser variability, which was observed to be relatively small. In contrast, significantly greater variability was observed between analysers. The group mean values provided by the Logan analyser were significantly higher, followed in order of magnitude by the Niox and then the Ecomedics analyser. Our findings indicate that exhaled NO measurements in healthy subjects and patients with airway disease differ according to the type of analyser used.

We firstly quantified within analyser variability by calculating the repeatability coefficient for each analyser. This analysis demonstrated that 95% of repeated measurements from the same individual can be expected to vary by up to 12% for the Ecomedics analyser and by up to 19% for the Niox and Logan analysers. These data can be used to interpret the between analyser differences. It is recognised that the measurement of a physiological parameter from the same subject using 2 different pieces of equipment will provide results that differ.9 The magnitude of this difference is critical in assessing the importance of this variability, as small differences may be deemed clinically insignificant. However, our results showed large differences between the 3 analysers studied, with the readings from the Logan and Niox analysers being 1.96- and 1.59-fold greater, respectively, compared to the Ecomedics analyser. Our observation that within analyser variability was smaller than differences between analysers rules out the inherent variability of repeated testing as an explanation for these findings.

The remarkably consistent results observed in asthma patients, COPD patients and healthy subjects indicates that the differences between exhaled NO analysers are independent of the presence or type of airway disease. We recruited mild asthma patients not taking inhaled corticosteroids in order to assess a homogenous group. Similarly, we recruited a homogenous group of COPD ex-smokers. The consistency of our results in 3 homogenous groups suggests that the differences between analysers would also be found in other groups, e.g. asthma patients taking inhaled corticosteroids or COPD patients who smoke.

Exhaled NO values above 100 ppb were observed in some asthma patients using the Logan machine. The accuracy of these readings is not clear, as calibration for this machine was only performed using gas containing 100 ppb NO. However, given the similar pattern of differences between analysers observed in healthy subjects and COPD, it is unlikely that errors at high values in the asthma group have significantly affected our key findings.

The reasons for our findings are unclear. The machines were calibrated and used according to the manufacturers’ instructions, using the recommended calibration gas at the suggested concentration. We deliberately used the manufacturers’ recommended calibration gases in order to replicate clinical practice as closely as possible. It has recently been reported10 that altering the calibration gas concentration used in an analyser can alter NO measurements. However, the magnitude of change observed was small compared to the differences between analysers observed in the current study. Calibration alone is therefore unlikely to be the only reason for our findings, and we validated the calibration procedure by ensuring that the analysers all correctly measured 100 ppb NO passed through the sample port. This confirmed that the each of the analysers gave identical NO measurements when gas from a cylinder is passed through a sample port at 50 ml/s. In contrast exhaled human breath does not follow the same pattern, as there were differences between analysers. Alternatively, as exhaled NO measurements are dependent on flow rate, it is possible that the differences observed could be explained by differences in achieved flow rates between the analysers. However, all 3 analysers achieved the required flow rate set at 50 ml/s, which was verified at the end of each reading. It would be interesting to investigate whether the differences we have observed are also present at other flow rates.

We have shown that 3 commonly used NO analysers give significantly different readings. Further work is needed to clarify the reasons for these differences, and to study differences between other commercially available models.

References