



Method Comparison Study of a NEW portable FeNO breath device: Second Generation NObreath® (V2)

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Abstract

Background: Measurement of fractional nitric oxide (NO) concentration in exhaled breath (FeNO) is a quantitative, non-invasive, simple, and safe method of measuring airway inflammation that provides a complementary tool to other ways of assessing airway diseases, including asthma. A method comparison study was conducted, comparing the NObreath® V2 to the NIOX VERO®.

Purpose: To establish that the NObreath® V2 correlates well with the NIOX VERO®.

Methods: A method comparison study was conducted in which FeNO was measured in 83 subjects; the linear relationship was estimated with Pearson's coefficient (r), absolute agreement by intra-class correlation coefficient (ICC), and bias with limits of agreement (95% of paired differences).

Results: The data was pooled together ($n=83$) and the mean readings were compared using regression analysis, which resulted in: 95% Lower CI 0.976110701117407, Upper CI of 1.03947025729795, and a slope of $y=1.0078x - 2.0855$, $R^2=0.9802$. The Bland-Altman graph result produced the mean difference (1.94), standard deviation (4.835017801), lower SD (-7.5985), and upper SD (11.46999).

Conclusions: In conclusion, the data shows strong correlation between the two devices, which is within their stated accuracy.

Keywords: nitric oxide, asthma, inflammation, airway disease, exhaled breath.

Introduction

To determine the level of airway inflammation, various markers such as bronchial hypersensitivity tests, induced sputum analysis, and fractionated exhaled nitric oxide (FeNO) have been used. Some studies have suggested that the monitoring and management of such markers will benefit the patient more and aid in control of their asthma¹.

FeNO measurement and breath analysis stands out as the easiest and most non-invasive alternative among this group of markers.

Asthma is characterized by airway inflammation and presence of airway inflammatory mediators. Its diagnosis and management are based on clinical history, physical examinations and spirometry testing, however, clinical history provided by the child and/or parent can be unreliable and spirometry may not aid in diagnosis or management, or reflect airway inflammation. Fractional exhaled nitric oxide (FeNO) has been shown to correlate with levels of eosinophils in the sputum and has been studied as a biomarker tool to help guide clinicians in the management of asthma in children⁴.

Nitric oxide (NO), signalling molecule produced by respiratory epithelial cells, is found in exhaled breath and functions as a vasodilator and bronchodilator in the lungs. NO is synthesized from L-arginine by inducible NO synthase enzymes in response to inflammatory cytokines, and is present in the exhaled breath of humans. FeNO has been found to be elevated in children with asthma and is understood to reflect eosinophilic airway inflammation resulting from the type 2 T-helper cell pathway. Given that NO is found in exhaled human breath, it was believed that the measurement of FeNO could be a non-invasive quantitative measure of airway inflammation, and that FeNO levels could provide clinicians with objective data in the treatment of children with asthma⁵.

The potential for FeNO to provide objective data is very important, considering that spirometry and specifically FEV₁ has been shown to be mostly normal in children with asthma of all severities and in children with symptoms of uncontrolled asthma. Reduced lung function in school-aged children may be present, even in the absence of respiratory symptoms, making the interpretation of spirometry results challenging⁵. FeNO testing is relatively easy to achieve in school-aged children, even in those as young as 4 years of age, and is therefore practical for clinicians to use in order to gain objective data about their patients. Levels of FeNO are increased in children with asthma compared to children without asthma. In 2011, the American Thoracic Society (ATS) published Clinical Practice Guidelines on the subject of FeNO and recommended that FeNO be used in the diagnosis of eosinophilic airway inflammation^{5,7}.

A paper recently published by Antoni Molino compared the NIOX VERO[®] to the Vivatmo-PRO and HypAIR-FENO. The results show that the NIOX VERO[®] tends to read higher than the other devices, although the devices weren't compared to the gold-standard, chemiluminescence⁶.

The aim of this study was to conduct a method comparison between the NIOX VERO[®] and NObreath[®] V2, to evaluate the agreement of FeNO measured with both these devices at 4 different sites^{1,2}. As the NObreath[®] V2 is a new device, there is no data comparing this device to other FeNO portable devices, although the sensor in the NObreath[®] V2 is the same its predecessor (NObreath[®])⁷.

Subjects and Methods

The method comparison followed the FDA guidelines and was conducted at 4 different sites.

83 subjects were tested and the subject's characteristics were recorded. The diagnosis was followed by the protocol provided and following the ATS guidelines.

Each site received a performance pack which contained:

- A NObreath® V2
- Mouthpieces
- Clinical Protocol (listing method)
- Manual
- Usability Surveys
- AZO wipes
- USB stick (with all printed information)
- Patients, (7 years of age and older) with and without asthma, were tested according to the international guidelines regarding eligibility to take part in the study and were prospectively recruited over a 2-month period from 4 different clinics.

FeNO breath measurements

FeNO readings were measured according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines, using the hand-held NObreath® V2 device and a stationary NIOX VERO®⁶.

The order of the measurements was random and for both types of measurements, patients were seated in the upright position without a nose clip. In a subgroup of patients, the reproducibility of the NObreath® V2 and the NIOX VERO® was assessed by repeating of up to 3 measurements (if possible).

In detail, FeNO results using the NObreath® V2 were obtained by asking subjects to exhale, guided by a visual and auditory cue, through the mouthpiece, whilst keeping the visual incentive (i.e. car, fish or flowmeter) central to maintain a constant flow rate of 50 ml/s (following ATS guidelines). The required exhalation time is approximately 12s. To ensure a breath sample was taken at the correct flow rate, the monitor was held upright at all times during the test.

NIOX VERO® tests were performed by asking the subjects to deeply inhale NO-free air to total lung capacity through a filter connected to the device, and then to exhale for 10s at a constant pressure guided by a visual cue to stabilize flow rate to maintain a fixed flow rate of 50 ml/s (following ATS guidelines). Measurements were repeated after a brief rest period until two acceptable values (± 5 ppb for measurements < 50 ppb and $\pm 10\%$ for measurements ≥ 50 ppb) were performed (maximum three attempts). The mean of two adequate values for each subject was recorded for analysis. For NIOX VERO®, the system calibration was performed every 14 days.

After using both the NObreath® V2 and NIOX VERO®, patients were asked to complete a usability study on the NObreath® V2

Results

Statistical analysis was conducted with the data; numerical variables were expressed as mean \pm SD, unless otherwise specified. The relationship between the measurements was estimated by Pearson's correlation coefficient (r) and linear regression analysis. Spearman's correlation coefficient (r_s) was used to identify any potential tendency for the separation of agreement at higher or lower values. The repeatability of measurements was expressed as intraclass correlation coefficient (rl).

Graph 1: A scatter plot graph comparing the mean of FeNO measurements from the NObreath® V2 against the NIOX VERO® and the associated regression line

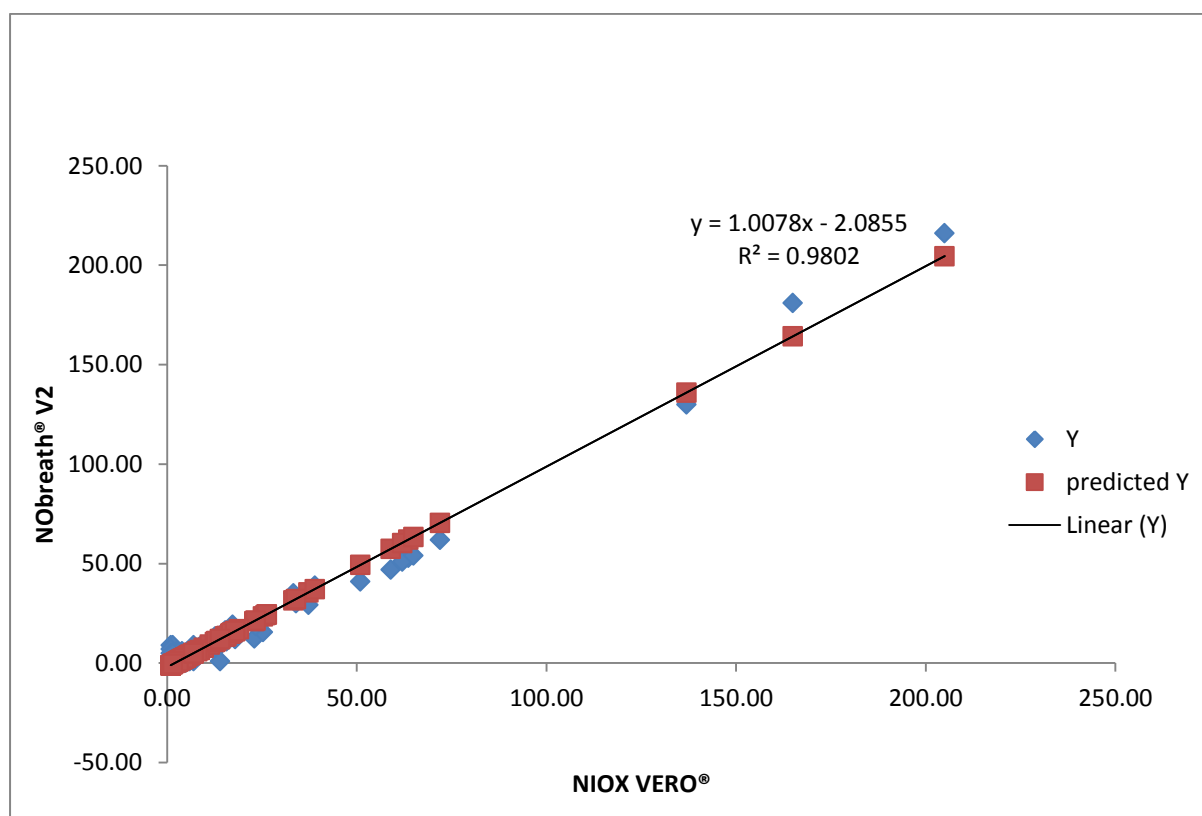


Table 1: Regression analysis of the NObreath® V2 and NIOX® VERO method comparison study of FeNO measurements

	Coefficients	Standard Error	P-value	Lower 95%	Upper 95%
Intercept	-2.085514152	0.617621079	*0.001129191	-3.314386366	-0.856641939
X Variable 1	1.007790479	0.015921996	*9.57156E-71	0.976110701	1.039470257

* Statistically significant if $p < 0.05$

** An R^2 of 0.9802 indicates that the regression predictions fit the data very closely.

Aerocrine method comparison using the NIOX MINO®

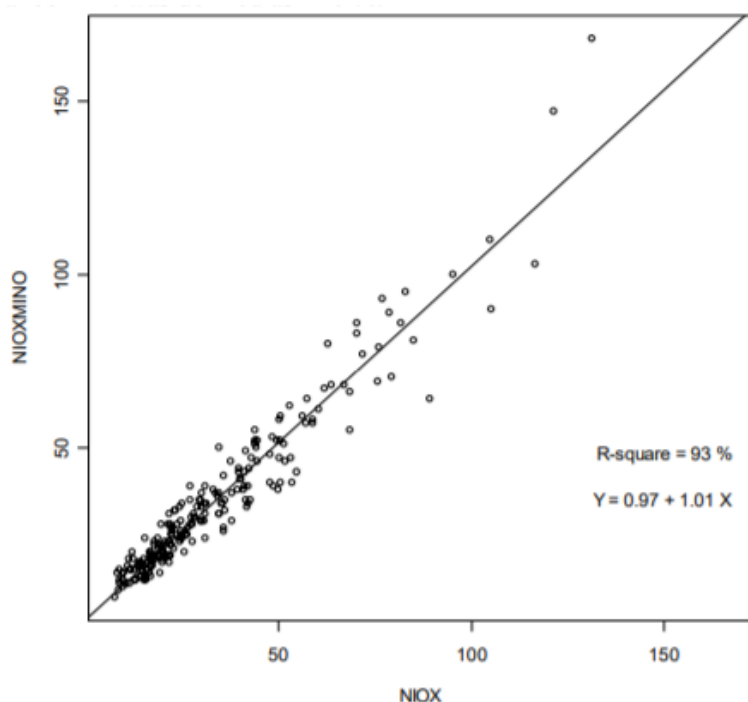
A method comparison in FeNO values between the NIOX MINO® and the predicated device, NIOX®, using the same subjects, was performed. Data from the clinical study and the published study by Alving et. Al. (2006) was analysed. The first reading from NIOX MINO® was compared with the mean of readings from NIOX® for each subject. Pooling the data yielded an N of 208 subjects and a regression analysis resulted in an intercept of 0.97 (95% CI - 0.65; 2.59) and a slope of 1.01 (95% CI 0.98; 1.05)⁹. The results are presented in the following table and graph:

Table 2: Results and regression analysis of the NIOX MINO® and NIOX® method comparison study of FeNO measurements⁹

Percentage of patients within sponsor's tolerance limits			
	First NIOXMINO		
	Total	Subset <30 ppb	Subset >30ppb
	N=209	N=111	N=98
Proportion of patients within limits	94.2%	100%	87.7%
Lower limit of 95 % CI	91.6%	100%	82.3%

* For subjects with a FE_{NO} value below 50 ppb (mean of NIOX and NIOX MINO) the sponsor's tolerance limit was defined as ±10 ppb. For subjects with a FE_{NO} above 50 ppb the tolerance limit was defined as ±20 %.

Graph 2: A scatter plot comparing the mean of two measurements with the predicate, NIOX®, with the first measurement in NIOX MINO® and the associated regression line⁹



FENOM comparison results

FENOM also conducted a clinical trial. The trial was conducted at 10 different sites with 43 operators. 84 patients were tested with the FENOM both pre and post treatment.

Table 3: Correlation between change in FeNO and change in FEV1 and ACQ in the ITT population (adults and children combined)

		FeNO
Change in FEV ₁	R-sqaure correlation	-0.14
	P-Value	0.2161
Change in asthma	R-sqaure correlation	0.21
Symptom score (ACQ)	P-Value	0.0637

Discussion

A method comparison study in FeNO values between the NObreath® V2 and the predicated NIOX VERO®, using the same subjects, was conducted at 4 different sites. This data was then analysed using statistical software.

The data was pooled together (n=83) and the mean readings were compared using regression analysis, which resulted in: 95% Lower CI 0.976110701, Upper CI of 1.039470257, and a slope of $y=1.0078x - 2.0855$, $R^2=0.9802$.

These results were then depicted on a scatter plot graph (Graph 1).

The NIOX VERO® results were generally, slightly higher than that the NObreath® V2, but showed good agreement. The P- value is <0.05 (see Graph 1), thus being a statistically significant result.

Measurements were analysed using regression and the Bland-Altman method to determine the limits of agreement. Comparing the NObreath® V2 to the NIOX VERO®, we found the mean readings from the NIOX VERO® to be slightly higher, although, from both a clinical and statistical point of view, this difference is minor as the regression analysis showed good agreement between the two devices. The results in Graph 1 show great correlation at the lower levels of ppb (near the cut off levels of 50ppb), which bodes well for the accuracy of the NObreath® V2.

Graph 3: Bland-Altman plot graph showing the difference between each subject's FeNO measurements

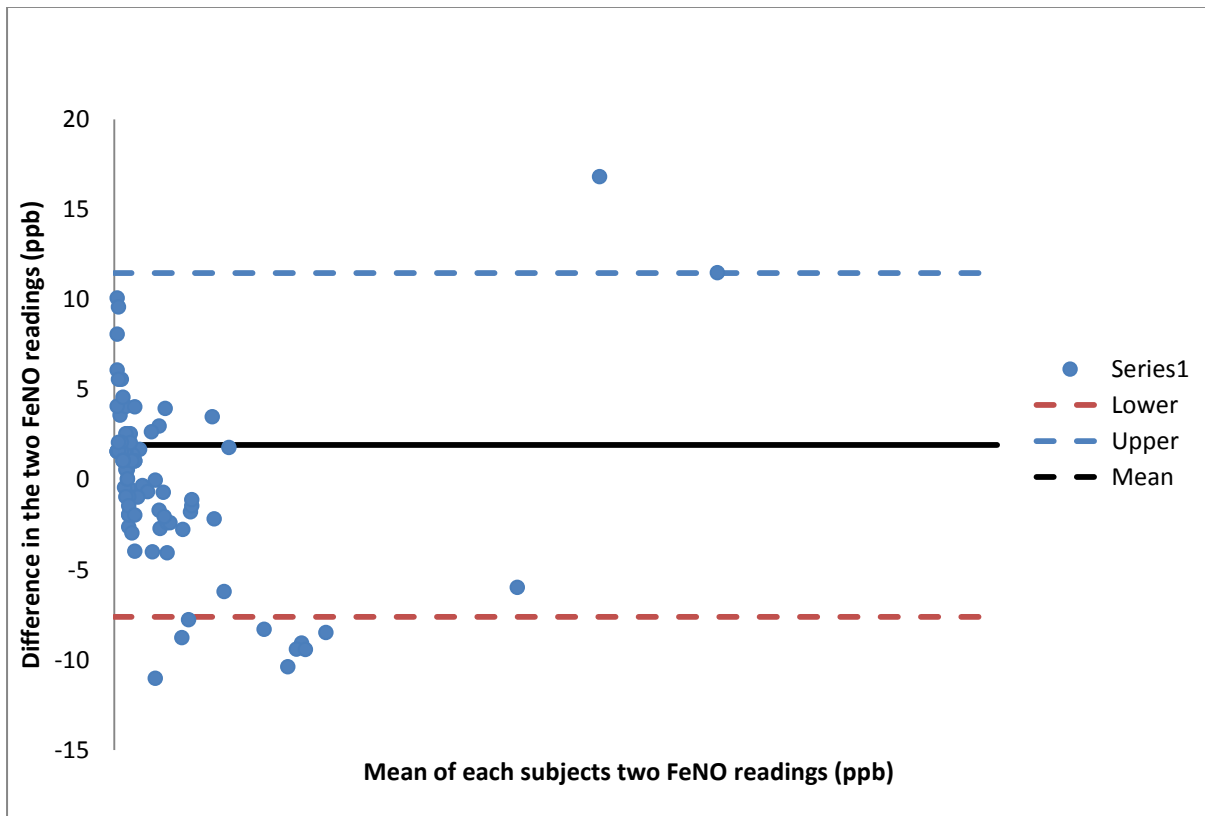


Table 4:Result table

Mean	1.94
Standard deviation	4.864
Lower SD	-7.5985
Upper SD	11.4699

Each point represents the absolute difference between the first and second FeNO measurements for each participant versus the mean of these two measurements (n = 83). Reference lines correspond to the mean difference in two FeNO measurements taken in one individual and the 95% Confidence Interval⁸. The results from this graph show that both devices have good correlation between each other, especially at lower levels of ppb. The mean inter-device difference in FeNO level was 1.94 ppb and the limits of agreement were -7.60 and 11.47 ppb.

In conclusion, the NObreath[®] V2 shows good agreement against the NIOX VERO[®], the values generated were statistically significant (P<0.05 – see Graph 1) and within the stated accuracy of both devices.

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