

Long-term variability of exhaled nitric oxide measurements



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Clinical Implications

- Devices that measure fractional exhaled nitric oxide may produce variable results over time that could result in inappropriate changes in asthma medications. Clinicians can evaluate quality control measurements from nonasthmatic testers to determine the extent of variability and to select alternate testers, when appropriate.

TO THE EDITOR:

Measurement of fractional exhaled nitric oxide (FE_{NO}) is recommended as an adjunct in the diagnosis of eosinophilic airway inflammation, to determine the likelihood of corticosteroid responsiveness, to monitor therapy, and to assess adherence to inhaled corticosteroid therapy.¹ The American Thoracic Society Guidelines for the interpretation of FE_{NO} concentrations identify cut points in children of greater than 35 parts per billion (ppb) for eosinophilic airway inflammation and less than 20 ppb for no inflammation.¹ For adults, the guidelines recommend greater than 50 ppb and less than 25 ppb, respectively.

The NIOX MINO (Aerocrine AB, Solna, Sweden) is a commonly used portable method of analyzing FE_{NO} that does not require calibration. The accuracy and precision of this device were largely determined by extensive *in vitro* testing for US Food and Drug Administration clearance.² The same is true for its successor, the NIOX VERO.³ The clinical study in the MINO FDA submission was a single measurement before and after beginning inhaled corticosteroids, which demonstrated a decrease in FE_{NO}. Other published clinical studies are single visit comparisons with other methods or multiple technicians. Only one study compared 2 measurements on different days.⁴ Because there are no published reports on the long-term reproducibility of devices used to measure FE_{NO}, we undertook the following study.

We analyzed 451 quality control measurements obtained over a 2-year period from 5 devices. There were 5 nonasthmatic technicians (quality control subjects) who performed these measurements on each day that the device was used for a clinical study or patient care (see Table E1 in this article's Online Repository at www.jaci-inpractice.org for more details). All control subjects met the manufacturer's criteria, which included >18 years old, no ongoing cold or known airway disorder, nonsmoker, expected stable exhaled nitric oxide values between 5 and 40 ppb during the qualification period, and preferably no allergies (except seasonal) or asthma. To obtain a control FE_{NO} measurement, each technician was required to empty his or her lungs by slowly exhaling and then forming a tight seal around the mouthpiece, making sure not to let the air leak out. Then, they inhaled to total lung capacity and exhaled forcefully for approximately 10 seconds through the

mouthpiece while responding to sound and light cues on the LCD to guide the rate of exhalation.

The general linear model procedure⁵ was used to determine whether there was a device*subject interaction. Because the interaction was significant ($P < .05$) and all subjects did not perform quality control on all devices, the data were analyzed separately for devices and subjects. The Kruskal-Wallis test was used to determine the significance of differences in FE_{NO} measurements with the Tukey-Kramer adjustment for multiple comparisons. The equality of variances was tested using the *F*-test. All analyses were performed with SAS 9.3 (SAS Institute, Inc, Cary, NC).

Devices 1-4 and 5 were compared separately because the 3 operators of device 5 were different from the 2 who operated devices 1-4. The mean (SD) FE_{NO} concentrations were 19.95 (14.6), 15.75 (12.2), 13.36 (6.2), and 13.72 (4.2) ppb for devices 1-4, respectively ($P = .0008$), and 19.95 (10.8) ppb for device 5 (Figure 1). The variance of device 1 was significantly larger than variances for devices 2-4 ($P = .0001$).

The mean (SD) concentrations for subjects 1 and 2, who performed quality control of devices 1-4 were 13.09 (4.6) and 17.45 (10.2) ppb, respectively ($P > .05$). Measurements for subjects 3-5 who used device 5 were 31.79 (7.2), 11.51 (4.2), and 15.0 (4.1) ppb, respectively (Figure 2). Measurements for subject 3 were significantly higher than those for subjects 4 and 5 ($P < .0001$). Eighty-two percent of her values were above 25 ppb.

The NIOX MINO portable device employing an electrochemical methodology was cleared by FDA as "substantially equivalent" to the chemiluminescence stationary method (NIOX), largely based on extensive *in vitro* testing using certified nitric oxide in nitrogen calibration gas.² Similarly, the new NIOX VERO was cleared in the same way as "substantially

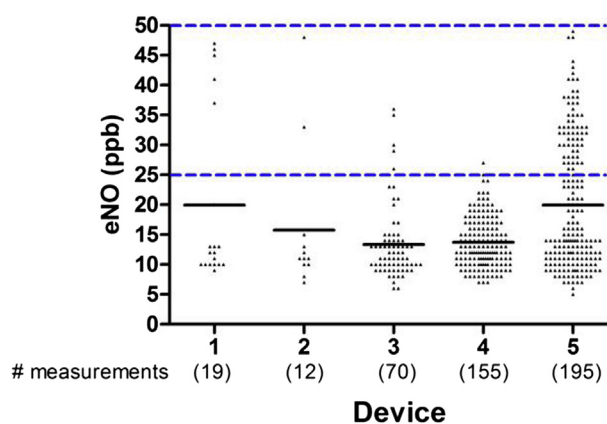


FIGURE 1. Serial measurements of FE_{NO} using 5 different NIOX MINO devices. The values within the dashed horizontal lines represent intermediate values for adults. Above 50 ppb is considered positive for eosinophilic airway inflammation, whereas less than 25 ppb is considered negative for eosinophilic airway inflammation. The bar represents the mean.

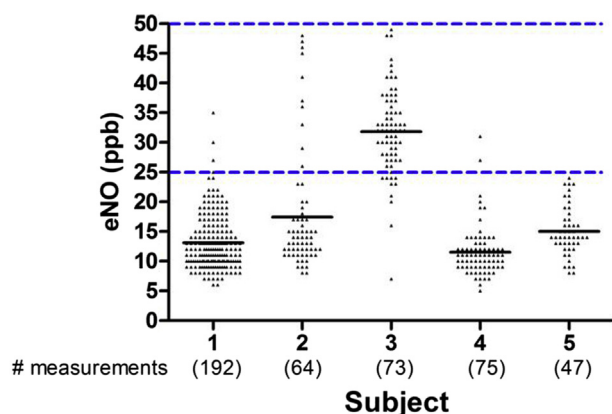


FIGURE 2. Serial measurements of FE_{NO} in 5 different subjects. See the legend to Figure 1.

equivalent” to NIOX MINO.³ Longitudinal testing of reproducibility (device precision) has not been reported for any of the 3 devices manufactured by Aerocrine. Indeed it is not feasible to test reproducibility in subjects with asthma because it would not be possible to distinguish variation of the device from variation in eosinophilic airway inflammation. Accordingly, we evaluated longitudinal variability by analyzing quality control measurements from healthy technicians who would not be expected to have elevated or fluctuating values of FE_{NO} because they were unlikely to have eosinophilic airway inflammation. Nevertheless, there was substantial long-term variability in the measurements that were related to device and subject. This is in contrast to the manufacturer specifications that claim a precision of less than 3 ppb of measured values less than 30 ppb and less than 10% of measured values greater than or equal to 30 ppb, when performed *in vitro* over 20 days.⁶ Measuring FE_{NO} on different occasions in healthy nonasthmatics with a longer interval is more relevant to clinical practice as clinic visits for patients are spread out.

The clinical relevance of our findings is that variation in machine performance could cause an inaccurate assessment of the presence of eosinophilic airway inflammation or inadvertent inhaled corticosteroid dose changes when they are not indicated. These are more likely to occur when the result is in the intermediate range.

Although there were 451 observations, the limitation to this study is the number of devices and technicians tested. Also, we

did not record the dates of each measurement so the temporal relationship is unclear.

In conclusion, the health care provider should interpret FE_{NO} values cautiously when they are in the intermediate range of 25–50 ppb for adults and 20–35 ppb for children. We suggest choosing quality control subjects who have FE_{NO} values consistently below 25 ppb. Lastly, we recommend that manufacturers of these devices evaluate the reproducibility of their devices in a similar manner to what was done in this study.

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ADDITIONAL DETAILS OF THE METHODS

The manufacturer recommends 2 external quality control procedures to ensure that the system is operating within specifications. The negative control consists of measurement of a nitric oxide free gas generated from ambient air. The positive control is a normal biological FE_{NO} sample from a qualified quality control tester, usually someone who operates the device.

The study involved 5 technicians who served as quality control testers (subjects), all of whom were Caucasian females with no respiratory conditions. Two participants (subjects 1 and 2) obtained quality control measurements with 4 NIOX-MINO devices (devices 1-4) in the Asthma Research Lab, whereas 3 subjects (subjects 3-5) obtained measurements on 1 NIOX-MINO device (device 5) in the University of Florida Pediatric Pulmonary Clinic. One subject in the Asthma Research Lab has seasonal allergic rhinitis, but measurements in her were obtained outside her allergy season.

The QC testers were qualified over the course of 3 days. They performed 3 QC measurements, 1 per day. A mean value was calculated from the 3 measurements and was within the 5-40 ppb range. Then they performed a fourth measurement within 7 days, and the result was within ± 10 ppb from the mean value. Thereafter, the QC measurement for an individual tester had to be ± 10 ppb. This means that on 2 consecutive days the QC values could be 20 ppb apart and still meet the manufacturer's

TABLE E1. Measurements by subject and device

Subject	Device					Total for each subject
	1	2	3	4	5	
1	14	10	60	108	0	192
2	5	2	10	47	0	64
3	0	0	0	0	73	73
4	0	0	0	0	75	75
5	0	0	0	0	47	47
Total for each device	19	12	70	155	195	451

recommendations. If the most recent QC measurement was older than 30 days, the tester had to repeat the qualification procedure. The negative control (ambient air) was always less than 5 ppb; otherwise, the device could not be used.

The data in our study were collected from August 2011 until June 2013 as a quality improvement project by a PharmD student. The QC measurements were obtained on any day that the device was used for a research study or during a clinic visit. Devices 1-3 were used for a research study over a 9-month period in 2014, whereas devices 4 and 5 were used throughout the 2-year period. Unfortunately, the dates of the measurements were not recorded. The number of QC measurements by subjects and devices were as shown in [Table E1](#).

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