000249-10, December 2017
(EPS-000069)

Labeling Summary/Package Insert NIOX VERO®

(According to 21CFR 809.10(b) Labeling for In Vitro Diagnostic Devices)

Product Labeling Summary NIOX VERO®

1. Proprietary and Established Names

NIOX VERO® Airway Inflammation Monitor measures the fraction of exhaled nitric oxide (FeNO) in human exhaled breath.

2. Indications for use

NIOX VERO® measures Nitric Oxide (NO) in human breath. Nitric Oxide is frequently increased in some airway inflammatory processes such as asthma. The fractional NO concentration in expired breath (FeNO), can be measured by NIOX VERO according to guidelines for NO measurement established by the American Thoracic Society.

Measurement of FeNO by NIOX VERO is a quantitative, non-invasive, simple and safe method to measure the decrease in FeNO concentration in asthma patients that often occurs after treatment with anti-inflammatory pharmacological therapy, as an indication of the therapeutic effect in patients with elevated FeNO levels. NIOX VERO is suitable for children, 7-17 years, and adults 18 years and older.

NIOX VERO 10 second test mode is for age 7 and up
NIOX VERO 6 second test mode is for ages 7- 10 only, who cannot successfully complete a 10 second test.

FeNO measurements provide the physician with means of evaluating an asthma patient's response to anti-inflammatory therapy, as an adjunct to the established clinical and laboratory assessments in asthma. The NIOX VERO is intended for prescription use and should only be used as directed in the NIOX VERO User Manual by trained healthcare professionals. NIOX VERO cannot be used with infants or by children under the age of 7, as measurement requires patient cooperation.

NIOX VERO should not be used in critical care, emergency care or in anesthesiology.

3. Summary and Explanation

3.1 Methodology background

Nitric oxide is endogenously produced in the airways [1] and production is increased when inflammation is present [2].

Exhaled NO is elevated in asthma and correlates with generally accepted clinical markers of airway inflammation, such as sputum eosinophils [3–8] eosinophils in bronchoalveolar lavage [9], [10] and in bronchial biopsies [11], [12]. Levels of exhaled NO in patients with asthma correlate with disease activity such as frequency of beta-2 agonists use, and day and night time asthma symptoms, as measured in clinic visits and/or patient diaries [13–15].

Treatment of airway inflammation in asthma with anti-inflammatory agents such as inhaled and/or oral corticosteroids and/or anti leukotrienes reduces levels of NO in exhaled air [16–28]. Exhaled NO measurements can be used for monitoring the effect of anti-inflammatory therapy such as inhaled and/or oral corticosteroids and/or anti leukotrienes, but not to monitor the effect of bronchodilators, since these primarily relieve the bronchoconstriction and have limited effect on the inflammation.

Recommendations for standardized FeNO measurement techniques have been developed [18]. Furthermore, a guideline for the use of FeNO in clinical practice has been published by the American Thoracic Society [29].

3.2 Product characteristics

NIOX VERO® is designed as a hand-held device for measuring FeNO, a marker of airway inflammation, in exhaled breath from humans. NIOX VERO is suitable for children, approximately 7 - 17 years, and adults 18 years and older.

NIOX VERO follows in all essential aspects the recommendations from American Thoracic Society (ATS) and European Respiratory Society (ERS) for standardized measurement procedures of exhaled NO [18]. NIOX VERO uses an electrochemical sensor technology as the analytical method. One vital advantage of this technology is that

NIOX VERO requires no calibration. Built-in controls and an External Quality Control Procedure ensure reliability of measured values.
NIOX VERO can be used at hospital clinics and in a General Practitioner setting [30].

4. Training requirements

NIOX VERO should only be operated by trained healthcare professionals and only after careful reading of the NIOX VERO User Manual.

5. Clinical Limitations

NIOX VERO is a Prescription Use Device according to 21 CFR 801(D)

NIOX VERO cannot be used with infants or by children approximately under age of 7, as it requires patient cooperation. The determining factor for age limitation is based on a patient's ability to understand and execute the instructions given.

Elevated FeNO levels are also found in other inflammatory conditions aside from asthma, such as allergic rhinitis [31], systemic lupus erythematosus [32] and liver cirrhosis [33] and COPD including COPD overlap syndrome [34], [35].

Viral infections might lead to increased FeNO levels. The mechanism behind this increase is however separate from the one causing the increased levels seen in allergic inflammation. Virus related increases in FeNO may be resistant to corticosteroid treatment [36].

Recent intake of nitrate rich food, such as lettuce, can lead to increased FeNO levels [37].

Diseases associated with decreased levels of nitric oxide are for instance cystic fibrosis [38], primary ciliary dyskinesia [38] and pulmonary hypertension [39].

Smoking reduces exhaled NO levels. However, FeNO can still differentiate asthmatics from non-asthmatics among smokers. In a recent study of subjects with respiratory symptoms, those who were diagnosed with asthma and also were current smokers had an increase in FeNO of 60%, compared to current smokers with airway symptoms not diagnosed as asthma [40].

Other factors that may affect FeNO levels are reviewed in section 12, Limitations of the procedure.

In some patients, FeNO is persistently high despite anti-inflammatory treatment. This could be due to several factors, such as non-compliance, poor inhaler technique, inadequate corticosteroid dosage, or continuous allergen exposure [41]–[43]. There may also be a small number of patients, especially those with severe asthma, who are unresponsive to steroid treatment or who need additional and other treatments [44]–[46].

In a previous study performed with NIOX MINO® , 26 out of 147 of the subjects (18%) did not show a significant decrease in FeNO value despite a significant change in Asthma Control Questionnaire (ACQ). For NIOX® the figure was 28 out of 147 subjects (19%).

In a previous study performed with NIOX, 11 out of 62 patients (18%) showed no decrease in FENO after anti-inflammatory treatment, and 9 out of 62 (15%) showed no decrease in FeNO despite improvement in asthma symptoms [20].

The medical explanation behind a lack of FeNO change has not been explicitly evaluated.

6. Risks to Health

There are no known direct risks to patient health posed by use of NIOX VERO. However, failure of the test to perform as indicated or erroneous interpretation of results may lead to improper patient management. Therefore, use of FeNO measurement results to adjust a treatment regimen without consideration of other clinical factors could pose a risk.

7. Product Description and Operation

For details regarding the parts and accessories, operational and maintenance procedures, refer to NIOX VERO® User Manual.

8. Warnings

The following warnings apply in the handling and operation of NIOX VERO®:

Warning:
NIOX VERO® should only be operated by healthcare professionals.

Warning:
Use of substances containing alcohol close to the NIOX VERO instrument may cause erroneous measurement results.

Warning:
DO NOT clean the instrument or handle with alcohol or any spray or wipe containing alcohol!

Warning:
Do not use substances containing alcohol on or close to the NIOX VERO® instrument.
This includes any cleaning agents used to clean the facility, or other equipment in the area, as well as alcohol wipes or sprays used on patients.

Warning:
Operate NIOX VERO as stated in the user manual. Circassia accepts no responsibility for damaged equipment or faulty results, if the equipment is not handled according to the manual.

Warning:
When selecting an accessory for your NIOX VERO keep in mind that an accessory not recommended by Circassia may result in loss of performance, damage to your NIOX VERO, fire, electric shock, injury or damage to other property. The product warranty does not cover product failure or damage resulting from use with non-approved accessories. Circassia takes no responsibility for health and safety problems or other problems caused by the use of accessories not approved by Circassia.

Warning:
NIOX VERO should not be used adjacent to or stacked with other equipment.

Warning:
Use only the Power Supply unit provided. Pull the plug when disconnecting NIOX VERO® from the power outlet.

Warning:
Use only the breathing handle supplied by Circassia.

Warning:
No modification of NIOX VERO instrument, handle or Sensor is allowed.

Warning:
Do not drop the instrument or subject it to strong impact.

Warning:
Do not use a damaged NIOX VERO instrument or damaged components.

Warning:
Keep the Instrument and sensor out of water. Ensure that no liquid is spilled or dropped on the instrument or the sensor.

Warning:
Do not use NIOX VERO in the proximity of areas where volatile substances such as organic fluids or disinfectants are being used. Special attention should be paid to aerosols and disinfection baths (either open vessels or ultrasonic baths). Do not use the instrument in the presence of flammable anesthetic, vapors or liquids.

Warning:
Do not heat or dispose the instrument or Sensor in fire. Please refer to the “Disposal of used/ expired products” section of the User Manual.

Warning:
NIOX VERO and the NO scrubber in the breathing handle contain potassium permanganate. Used or expired instruments and breathing handles should be disposed of as hazardous waste in accordance with the local waste disposal regulations.

Warning:
Breathing handle must not be used after expiration date.

Warning:
Patient filters should be used immediately after opening.

Warning:
Do not re-use the patient filters.

Warning:
The NIOX VERO Sensor contains chemicals that could be harmful if swallowed.

Warning:
Be careful when opening the sensor can. The inside of the opening may have sharp edges.

Warning:
Do not touch or clean the white Sensor membrane.

Warning:
Do not clean the Sensor. Cleaning of the Sensor with ethanol or similar disinfectant might destabilize it for a non-predicable time period.

Warning:
After inserting a new Sensor, it is recommended to wait for three hours with the instrument switched on before performing a measurement.

Warning:
Make sure to use the correct measurement mode, otherwise incorrect FeNO results might be obtained.

9. Cautions

The following cautions apply in the handling and operation of NIOX VERO:

Caution:
Mobile phones, cordless phones and gas emitting appliances might interfere with the instrument and should therefore be kept away from the instrument. Interference could make it impossible to perform a measurement.

Caution:
The NIOX VERO instrument might produce some heat during normal operation. The temperature could increase by up to 9°F/5 ºC above the ambient temperature. Make sure that the ventilation slots are not blocked. Do not place the instrument on a bed, sofa, carpet or other soft surface.

Caution:
Normally a maximum of 10 measurements per hour can be performed during continuous use. It is possible to perform 20 measurements per hour if the instrument is paused for a minimum of 30 minutes prior to the next session of measurements. The system is not designed for continuous use, due to the risk of water condensation. Typically 30-60 measurements can be made during the course of a working day, depending on the surrounding temperature. An alert will be issued if there is a high risk of condensation due to high use frequency.

Caution:
The Sensor shall be kept in its original unopened package before installation. For transportation and storage conditions, refer to the corresponding section in the NIOX VERO User Manual.

Caution:
The Sensor is sensitive to changes in ambient temperature and humidity.
The best performance is achieved if the ambient conditions are stable. Refer to the recommended environmental conditions in the NIOX VERO User manual. Keep the unit away from windows, direct sun, radiators, stoves or open fire in order to avoid unstable conditions.

Caution:
When transporting the unit from one location to another, a prolonged stabilization period before measurement might be required. Refer to the recommended transportation conditions in the “Transport and Storage” in NIOX VERO User manual. Always use a bag or case for transportation.

Caution:
The device contains a Lithium-ion Battery which may induce an increased risk of heat, smoke or fire if handled incorrectly; do not open, crush, heat above 140°F/60°C or incinerate.

Caution:
Elevated ambient Nitrogen Dioxide (NO_2) may interfere with FeNO measurement; therefore, ensure that the patient inhales correctly according to instruction solely through the filter.

Caution:
Make sure that the gas outlet (four parallel slots to the left of the lid) on the rear side of the device is not covered.

Caution:
Keep the Sensor out of reach of children.

Caution:
Any person who connects external equipment to signal input and signal output ports of this device has formed a Medical Electrical System and is therefore responsible for the system to comply with the requirements of IEC 60601-1.

Caution:
A PC connected to the USB connector has to be certified for one of the standards IEC-60601-1, IEC 61010-1, IEC 60950 or comparable with safety extra low voltage on the USB ports.

Caution:
The connected PC should be placed out of reach from the patient. Do not, simultaneously, touch the connected PC and the patient.

Caution:
NIOX VERO has been tested and found to comply with the limits for medical devices according to IEC 60601-1-2:2007 Safety Requirements for Medical Electrical Systems and Electromagnetic Compatibility. The test limits are designed to provide protection against harmful interference in a typical medical installation. However, because of the increased use of radio-frequency transmitting equipment and other sources of electrical noise emitters in the healthcare and home environments, such as base stations for radio, cellular/cordless telephones and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast, it is possible that high levels of such interferences due to close proximity or strength of a source, may result in a disruption of performance of the instrument. If abnormal performance is observed, it may be necessary to relocate the NIOX VERO.

Caution:
NIOX VERO can be operated with two different exhalation times, 10 seconds and 6 seconds. The 10 second test is the preferred mode. For children age 7 - 10 who are not able to perform the 10 second test, the 6 second test is an alternative. The 6 second test should not be used with patients over the age of 10.

10. Specimen collection and preparation for analysis

NIOX VERO® provides direct sampling of sequentially collected and analyzed exhaled air. No subsequent specific specimen collection, specimen preparation or reagents are required.

11. Step by step outline of recommended procedures

For details regarding the operation of NIOX VERO read the NIOX VERO User Manual.

12. Results

The FeNO results, expressed as parts per billion (ppb), are presented after approximately one minute on the display. The instrument automatically calculates the results based on the calibration settings (sensitivity) of the Sensor, expressed as nA/ppb.
For details regarding the FeNO measurement and results, please read the NIOX VERO User Manual.

13. Limitations to the procedure

Biological as well as external factors that could affect FeNO measurements have been described [25]. To assure correct results when performing FeNO measurement with NIOX VERO®, the following cautions apply, according to the ATS/ERS recommendations from 2005 [18]:

Caution:
Food and beverages
Patients should refrain from eating and drinking before NO analysis. An increase in FeNO has been found after the ingestion of nitrate or nitrate-containing foods, such as lettuce (with a maximum effect 2 hours after ingestion) and drinking water and ingestion of caffeine may lead to transiently altered FeNO levels. Until more is known, it is prudent when possible to refrain from eating and drinking for 1 hour before exhaled NO measurement, and to question patients about recent food intake. Alcohol ingestion reduces FeNO in patients with asthma and healthy subjects [18].

Caution:
Respiratory maneuvers
Because spirometric maneuvers have been shown to transiently reduce exhaled NO levels, it is recommended that NO measurement be performed before spirometry. The same stipulation applies to other taxing respiratory maneuvers, unless these can be shown to have no effect on exhaled NO. The FeNO maneuver itself and body plethysmography do not appear to affect plateau exhaled NO levels [18].

Caution:
Age/sex
In adults, there is no consistent relationship between exhaled NO level and age, but it has been reported that, in children, FeNO increases with age. In adults, there are conflicting reports regarding the effects of sex, menstrual cycle and pregnancy, so these patient characteristics should be recorded at the time of measurement [18].

Caution:
Airway caliber
It has been demonstrated that FeNO levels may vary with the degree of airway obstruction or after bronchodilatation, perhaps because of a mechanical effect on NO output. Depending on the setting, it may be prudent to record the time of last bronchodilator administration and some measure of airway caliber, such as FEV1 [18].

Caution:
Circadian rhythms
Although FeNO levels are higher in nocturnal asthma, there was no circadian rhythm in two studies, but another study did report a circadian pattern, so it is uncertain whether measurements need to be standardized for time of day. It is, however, prudent, where possible, to perform serial NO measurements in the same period of the day and to always record the time [18].

Caution:
Smoking
Chronically reduced levels of FeNO have been demonstrated in cigarette smokers in addition to acute effects immediately after cigarette smoking. Despite the depressant effect of smoking, smokers with asthma still have a raised FeNO. Subjects should not smoke in the hour before measurements, and short- and long-term active and passive smoking history should be recorded [18].

Caution:
Infection
Upper and lower respiratory tract viral infections may lead to increased levels of exhaled NO in asthma. Therefore FeNO measurements should be deferred until recovery if possible or the infection should be recorded in the chart. HIV infection may be associated with reduction in exhaled NO [18].

Caution:
Medications and exhaled NO
The potential effect of drugs on NO cannot be excluded, and so all current medication taken and time administered should be recorded. Exhaled NO falls after treatment with inhaled or oral corticosteroids in subjects with asthma and after inhaled NO synthase inhibitors. Leukotrine-axis modifiers also reduces FeNO. NO donor drugs and oral, inhaled, and intravenous L-arginine increase FeNO and nasal FeNO. Even if a certain medication does not effect NO production, it might affect the apparent level of NO through other mechanisms, such as changes in airway caliber [18].

Caution:
Other factors
The manipulation of physiologic parameters has been shown to affect FeNO. Changing pulmonary blood flow has no effect in humans, but hypoxia decreases exhaled NO and this may occur in subjects at high altitude, particularly those prone to high-altitude pulmonary oedema. The application of positive end-expiratory pressure has been shown to increase FeNO in animals, but airway pressure in humans does not affect exhaled NO plateau levels according to most reports, although one study suggests the opposite. Many studies have examined the effect of exercise on FeNO. During exercise, according to one report, FeNO falls, whereas NO output increases and this effect may last up to 1 hour. Others have reported that FeNO remains stable after exercise. It would seem prudent to avoid strenuous exercise for 1 hour before the measurement [18].

Caution:
Measurement results are to be used as an adjunct to establish clinical and laboratory assessments in asthma.

14. Expected Values

Given that physiological and environmental factors can affect FeNO, FeNO levels in clinical practice need to be established on an individual basis. However, most healthy individuals will have NO levels in the range 5-35 ppb (children slightly lower, 5-25 ppb) when measured at 50 ml/s [47–51].

The lower values reported in children indicate an age dependence of FeNO levels. This has been confirmed in a number of studies, showing that FeNO levels increase with age in children [49], [50], [52]. Furthermore, it has been shown that males have higher FeNO levels than females [48], [51], [53], [54]. There are also studies demonstrating ethnic differences in FeNO levels [55], [56].

It is established that patients with allergic asthma have higher than normal levels of FeNO [2], [3], [53], [57], [58], FeNO levels in asthma patients vary depending upon the extent of their airway inflammation. Literature data suggest that patients with asthma usually have FeNO levels in the range 25–80 ppb although higher levels may occur in some patients [3], [17], [59]. Values at the lower end of the range are usually seen in patients receiving anti-inflammatory treatment [13], [29]. FeNO levels persistently over 50 ppb in adults and over 35 ppb in children are considered to be high [29]. Allergen exposure has been shown to increase FeNO levels in asthmatics [43], [60]. Correlation between symptom improvement and decreasing FeNO has been observed [17], [41], [61], [62].

Monitoring a patient's FeNO levels before and during anti-inflammatory therapy can, therefore, be used for studying the therapeutic effect [17], [19], [23].

Note:
If FeNO levels are high despite medication, this may indicate non-compliance [42], [63], poor inhaler technique or inadequate corticosteroid dosage [41]. Continuous high levels of allergen exposure amplify the inflammatory activity. There may also be a small number of patients, especially those with severe asthma, who are unresponsive to steroid treatment [44]–[46]. Any change of anti-inflammatory therapy can affect FeNO levels and should be recorded.

Note:
Changes in airway inflammation measured as FeNO levels and lung function parameters may be non-synchronous as they have different response times to anti-inflammatory treatment [64].

15. Clinical data

NIOX VERO® is a portable device for measuring FeNO. NIOX VERO is designed to comply fully with the ATS and ERS (American Thoracic Society/European Respiratory Society) guidelines from 2005. The use of FeNO as a method of monitoring airway inflammation using NIOX VERO is comparable with NIOX MINO (predicate device).

Clinical studies were performed to support clinical validation and usability of the NIOX VERO. Tests in several institutions as well as tests within the company have demonstrated that NIOX VERO has substantially equivalent clinical performance characteristics to the NIOX MINO. Results of the clinical investigations demonstrate a clinically acceptable agreement between the NIOX MINO and the new NIOX VERO. Additionally, results have shown that the NIOX VERO provides a simple, reliable, repeatable and non-invasive method of measuring FeNO according to current ATS/ERS guidelines [18].

Section 15.1 summarizes the pooled results of two clinical investigations (AER-045 & AER-048)[1] that evaluated the agreement and repeatability of FeNO measurements using the NIOX MINO and the NIOX VERO (Method Comparison Studies). The primary objective of these studies was to evaluate the agreement between the first valid FeNO measurement obtained using the NIOX MINO and the first valid FeNO measurement obtained using the NIOX VERO. The studies also evaluated repeatability between the devices as well as the amount of agreement of FeNO measured with the NIOX MINO and FeNO measured with the NIOX VERO.

Section 15.2 summarizes a pediatric study comparing results of the 6 second mode to the 10 second mode.

Section 15.3 summarizes the pooled results of two technical validation clinical investigations (TV-014 & TV-018)[2] investigating the repeatability of FeNO measurements performed by three different operators using the NIOX VERO (Inter-Operator Variability Studies). The Inter-Operator Variability Studies demonstrated that FeNO measurements by the NIOX VERO were repeatable and consistent.

1 AER-045 Clinical Investigation Report: DCR–000049–02 and AER-048 Clinical Investigation Report: DCR–000068-00 (maintained in the Trial Master Files at Circassis Pharmaceuticals, Inc.)

2 TV-014 Clinical Investigation Report: DCR-000057-00 and TV-018 Clinical Investigation Report: DCR–000069-00 (maintained in the Trial Master Files at Circassia Pharmaceuticals, Inc.)

Section 15.4 summarizes the clinical utility of measuring FeNO in asthmatic patients. The clinical investigation evaluated the change in FeNO after two weeks of treatment with an inhaled corticosteroid. FeNO was measured with the NIOX MINO and compared to its predicate the NIOX® (using the chemiluminescence method) and, although the devices studied are different, similar results can be expected when monitoring response to treatment with the NIOX VERO.

15.1 Clinical Validation - Method Comparison Studies

Two method comparison clinical investigations were performed, comparing the NIOX VERO with the NIOX MINO. These were randomized, multi-center studies to determine the agreement, and repeatability of FeNO measurements using the NIOX MINO and the NIOX VERO. The investigations had the same endpoints and objectives hence, the data was pooled together. The pooled results for the Method Comparison Studies are summarized below.

The primary objective was to evaluate the agreement between the first valid FeNO measurement taken with the NIOX MINO vs. the first valid FeNO measurement taken with the NIOX VERO in the 10-second exhalation mode. The primary endpoint was to determine the proportion of Subjects within the tolerance limits, which was defined as the difference between the first valid NIOX MINO FeNO value and the first valid NIOX VERO FeNO value. A difference < 10 ppb for FeNO values below 50 ppb or a difference < 20% for patients with FeNO values above 50 ppb is considered to be within tolerance limits. The reference value was the NIOX MINO FeNO value.

The secondary endpoints were to evaluate repeatability between the devices as well as the amount of agreement of FeNO measured with the NIOX MINO and FeNO measured with the NIOX VERO through:

- Repeatability of duplicate FeNO measurements from each device
- Evaluation of intra-individual differences and values between the first valid NIOX MINO measurement and the first valid NIOX VERO measurement
- Evaluation of the mean differences and values between mean values of the NIOX MINO FeNO measurements and the mean values of the NIOX VERO FeNO measurements

15.1.1 Demographic Information & Disposition of Subjects

A total of 112 Subjects at 5 distinct sites were enrolled in both studies. The mean age was 28.7 + 18.56 years (range 7 – 78) and 51.8% of the Subjects were females. Half of the Subjects (50%) were randomized to the MINO:VERO device sequence.

Of the 112 Subjects, 109 (97.3%) completed one FeNO measurement on each device while 107 Subjects (95.5%) completed two FeNO measurements on each device. 104 Subjects (92.9%) completed the Study (Table 15.1).

Table 15.1. Disposition of Subjects

	All Subjects
Total Subjects Enrolled	112
Subjects by Randomization Sequence ->MINO:VERO ->VERO:MINO	 56 56
Subjects with Any FeNO Completed	112
Subjects with at Least One Valid FeNO Completed on Both Devices	109
Subjects with Two Valid FeNO Completed on Both Devices	107
Did Subject Complete Study ->Yes ->No	 104 8
Primary Reason for Discontinuation Instrument NIOX VERO failed FeNO concentration was less than 5 ppb Two approved exhalations in NIOX VERO was not achieved within the stipulated maximum number of attempts allowed	 1 3 4

15.1.2 Observed FeNO Results

Table 15.2 displays a summary of the median values of the observed FeNO results for the averaged replicate. The median NIOX MINO FeNO value was 18 ppb and for the median NIOX VERO the value was 15.5 ppb. Male Subjects had higher FeNO values than female Subjects and children had slightly lower FeNO values than adults. There were no substantial differences between replicates within gender or age groups or randomization sequences.

Table 15.2. Summary of the Median Values for Averaged Replicates Observed FeNO Results overall and by gender and ages					
Observed FeNO Results for Averaged Replicate	Median FeNO Value (ppb)				
	All Subjects N=109	Males N=53	Females N=56	Children n=45	Adults n=64
Average NIOX MINO Result	18	35.5	14.8	15.5	28.3
Average NIOX VERO Result	15.5	25.5	12.8	13.5	26.3
Average NIOX MINO Result for VERO:MINO	18	43.5	15.5	15.5	30.8
Average NIOX MINO Result for MINO:VERO	18.8	32	14.5	16.3	25.5
Average NIOX VERO Result for VERO:MINO	18	32.5	14.5	11.5	28
Average NIOX VERO Result for MINO:VERO	15	22.3	10	13.5	22

15.1.3 Observed Paired Differences

Table 15.3 displays a summary of the paired difference of the first valid FeNO measurement taken with each device. The median difference was -4 ppb with differences ranging from -35 to 8 ppb. Males had higher median differences than females. Adults had higher paired difference than children. Subjects randomized to the MINO:VERO sequence had higher paired differences than those randomized to the VERO:MINO sequence. The observed paired differences for mean FeNO results showed similar results.

Table 15.3. Summary of the Observed Paired Differences for First Valid FeNO Results overall and by gender and age							
Results	Observed Difference Median Value (ppb)						
	All Subjects	MINO: VERO	VERO: MINO	Males	Females	Children	Adults
Paired Difference	-4	-4.5	-3	-5	-3.5	-3	-4
(Observed Percent Difference)	(-12.5)	(-13.3)	(-12.1)	(-12.7)	(-12.5)	(-13.3)	(-12.3)

15.1.4 Primary Endpoint

Results from this study demonstrated that the FeNO values from 99 of 109 subjects (90.8%) are within the tolerance limits (Table 15.4). Results from 8 Subjects with a FeNO value < 50 ppb and 2 Subjects with a FeNO value > 50 ppb were outside of the tolerance limits.

Table 15.4. Summary of the Number and proportion of Subjects within the tolerance limits for First Valid Measurement	
	All Subjects (n=109)
Number of Subjects with First Valid NIOX MINO FeNO < 50 ppb	81
Difference < 10 ppb	73
Difference > 10 ppb	8
Number of Subjects with First Valid NIOX MINO FeNO >= 50 ppb	28
Difference < 20% of First Valid NIOX MINO FeNO Result	26
Difference > 20% of First Valid NIOX MINO FeNO Result	2
Total Subjects within the Tolerance Limits Based on First Valid FeNO Measurements	99 / 109 (90.8%)

15.1.5 Secondary Endpoint
15.1.5.1 Repeatability

The difference between the two points for each Subject was calculated separately for the NIOX VERO and the NIOX MINO, and a Wilcoxon Signed Rank Test was used to assess the null hypothesis that the distribution of the differences was centered at 0 (Table 15.5). The results show that repeatability for the NIOX VERO was significantly better than the repeatability for the NIOX MINO ($p = 0.0112$).

Table 15.5. Repeatability Measures by Subjects with Two Valid Measurements on Each Device	
	All Subjects
Number of Subjects	107
Intra-Subject Variance for NIOX MINO N Mean (SD) Median	 107 4.87 (9.291) 1.00
Intra-Subject Variance for NIOX VERO N Mean (SD) Median	 107 4.79 (23.701) 0.25
Paired Difference in Intra-Subject Variance N Mean (SD) Median	 107 -0.08 (25.166) -0.25
Wilcoxon Signed Rank Test P-value =	0.0112

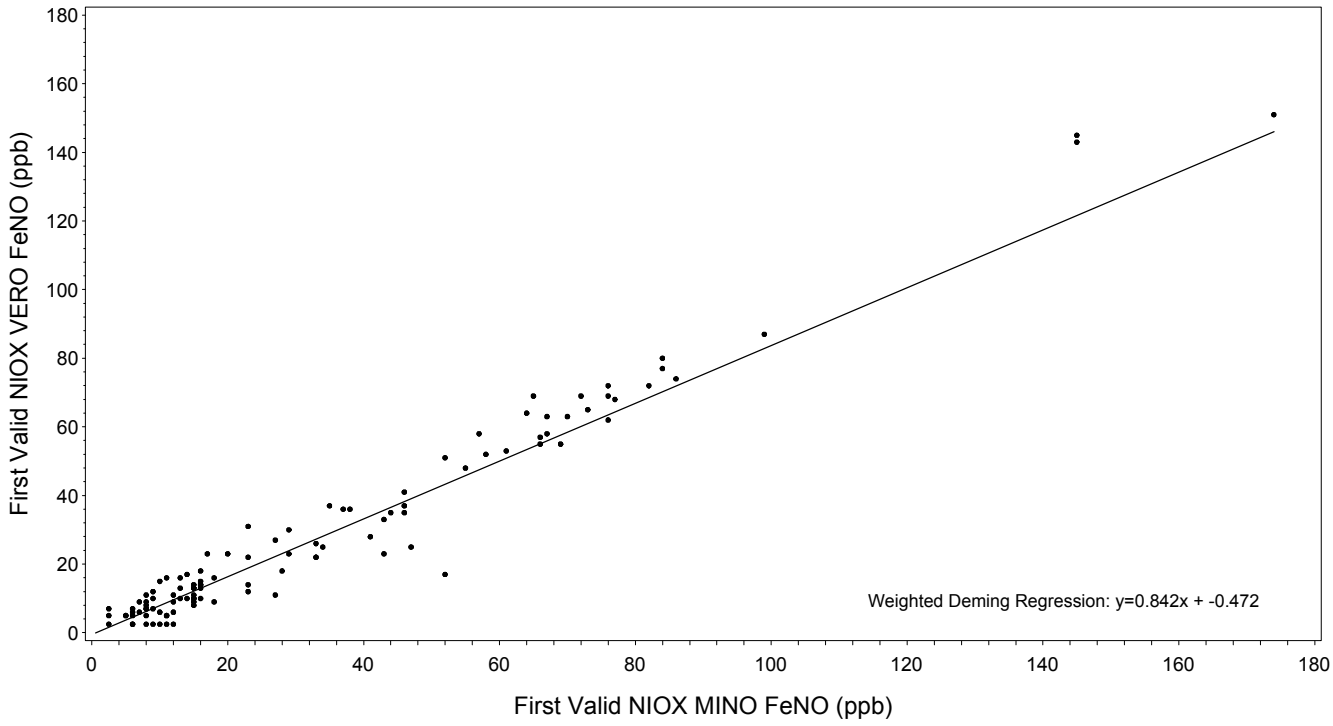
15.1.5.2 Evaluation of the Intra-Individual Difference & Values

Median paired differences of the first valid FeNO measurements on each device were not substantially different. The mean observed difference in average results (NIOX VERO - NIOX MINO) was -4.6 (confidence interval: -5.825 to -3.377; $p < 0.0001$), suggesting that FeNO measurements using the NIOX VERO on average were slightly lower than FeNO measurements using the NIOX MINO. However, this difference is within the technical specifications of the instrument, and these results provide additional evidence to support agreement between the two devices.

Table 15.6 displays the Weighted Deming Regression analysis that was performed. The confidence intervals for the slope (slope 0.842) do not include 1, which indicates a slight bias for the NIOX MINO to read at higher levels. The confidence intervals for the Y-intercept (-0.472) contain 0. This suggests good agreement between FeNO measurements taken on both devices. Figure 15.1 graphically displays the results for first valid FeNO measurements for both devices.

Table 15.6. Observed Results for First Valid FeNO Measurements Weighted Deming Regression analysis			
Parameter	Value (SE)	95% Confidence Intervals	P-value
Intercept	-0.472 (0.7793)	(-1.999, 1.055)	0.5459
Slope	0.842 (0.0434)	(0.757, 0.927)	0.0004

Figure 15.1. Observed Results for First Valid FeNO Measurements.



15.1.5.3 Evaluation of the Mean Differences & Values

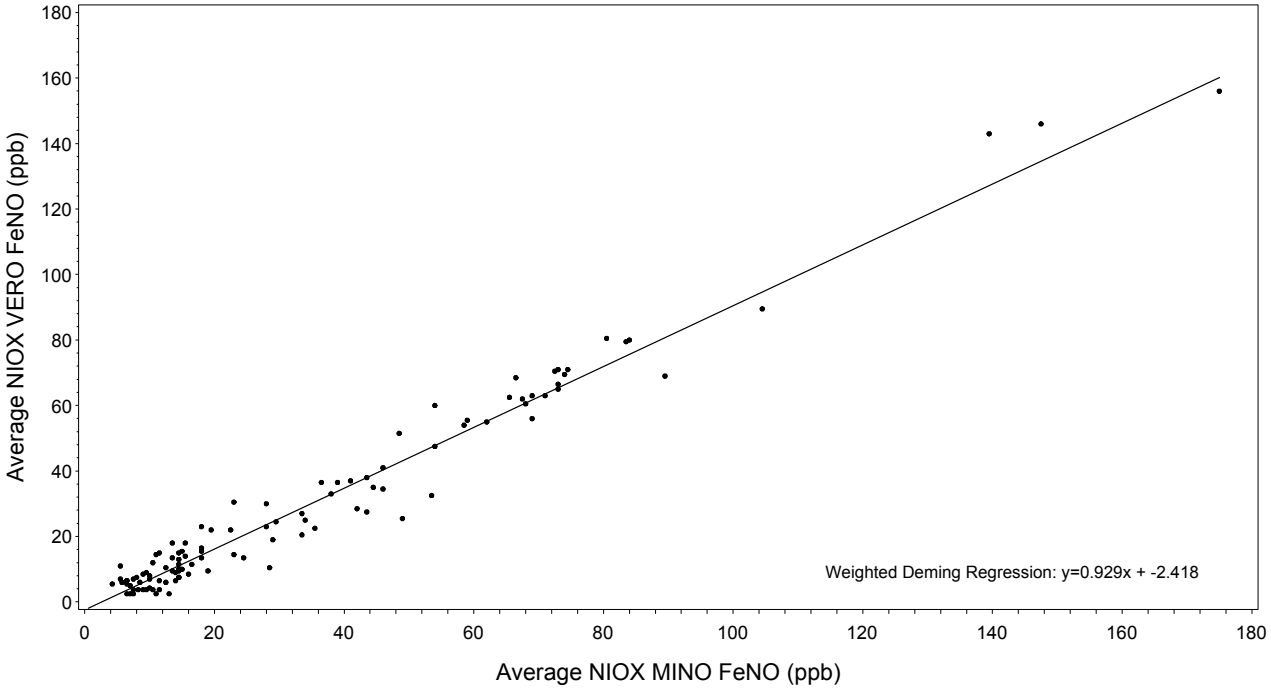
Median paired differences of the mean of both FeNO measurements on each device were not substantially different. The mean observed difference in average results (NIOX VERO - NIOX MINO) was -4.5 (confidence interval: -5.635 to -3.439; $p < 0.0001$), also suggesting that FeNO measurements using the NIOX VERO on average were slightly lower than FeNO measurements using the NIOX MINO.

Table 15.7 displays the Weighted Deming Regression analysis that was performed. The confidence intervals for the slope (0.929) contains 1, which is expected for a high level of agreement between the devices. The confidence intervals for the Y-intercept (-2.418) excludes 0, which indicates slightly higher readings on the NIOX MINO than on the NIOX VERO. Figure 15.2 graphically displays the results of the mean FeNO measurements for each device.

Table 15.7. Observed Results for Average FeNO Measurements Weighted Deming Regression analysis

Parameter	Value (SE)	95% Confidence Intervals	P-value
Intercept	-2.418 (0.7366)	(-3.861, -0.974)	0.0014
Slope	0.929 (0.0380)	(0.854, 1.003)	0.0636

Figure 15.2. Observed Results for Average FeNO Measurements.



15.1.6 Discussion

There were no adverse events, serious injuries, issues or problems with use of the NIOX MINO or the NIOX VERO. The results suggest a clinically acceptable agreement exists between the NIOX MINO and the new NIOX VERO device. The same agreement was seen when comparing the first valid measurement and the mean of two measurements. While the results suggest that FeNO measurements using the NIOX VERO on average are slightly lower than FeNO measurements using the NIOX MINO, this difference is within the technical specifications of the instrument. This data provides additional evidence to support agreement between the two devices, furthermore, the NIOX VERO showed excellent intra-subject repeatability that was significantly better than in the NIOX MINO.

15.2 Study Comparing the 6 Second Exhalation Mode to the 10 Second Exhalation Mode

This is a randomized, single-center, single visit, point-of-care clinical validation study. A total of 53 subjects were enrolled, including subjects 6-10 years of age.

Results supported the agreement and repeatability of the NIOX VERO® device using the 6-second exhalation mode and the 10-second exhalation mode in subjects 6 to 10 years of age. Observed results were similar between the devices and paired differences in the average FeNO results were centered close to 0 (median = 0.50), further supporting the similarity of the results.

The weighted Deming regression analysis resulted in parameter estimates that were not significantly different from zero and one for the intercept and slope, respectively, further supporting the correspondence of FeNO values. In addition the average bias based on the predicted 6s mode results across the full range of observed 10s mode FeNO results was low (2.7%), and the absolute estimated bias at the 35 ppb cut-off value (1.43) was well below the pre-specified limit of seven ($p<0.0001$). Further, the upper bound on the estimated bias at 35 ppb was 8.7% of the cut-off value, suggesting bias well below 20%.

The Bland-Altman plot (Fig 15.3) revealed an even spread of paired differences in average FeNO results between the modes with larger differences only noted for two subjects with the largest observed FeNO results.

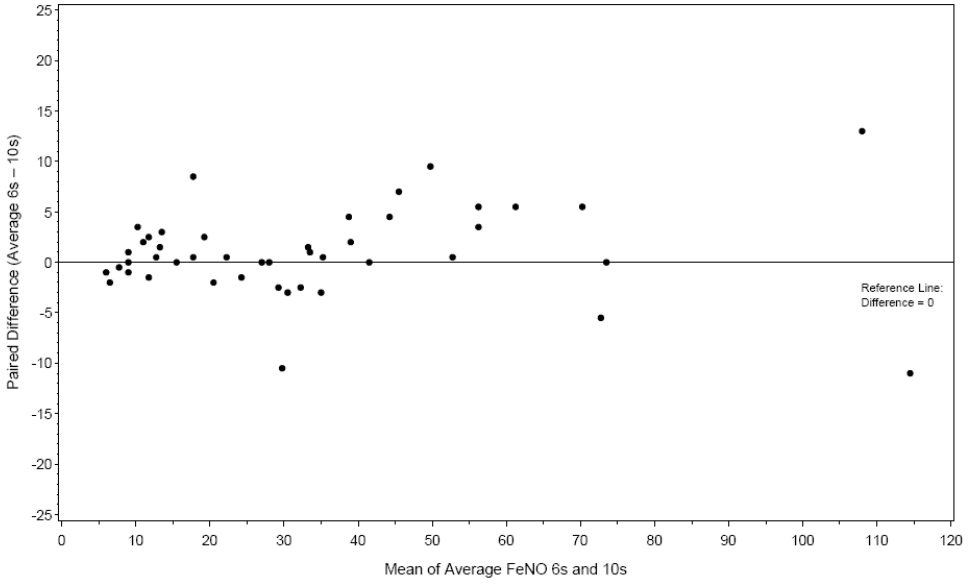


Figure 15.3. Bland-Altman Plot for Average FeNO Results for 6 s and 10 s Mode

In terms of repeatability, intra-subject standard deviations were very similar between the modes with a median paired difference of 0.0 and no statistically significant difference ($p=0.3090$). Finally, a high percentage of subjects (93.8%, 95% Exact CI: 82.8%, 98.7%) were within the tolerance limits.

The similarity of observed results between the modes, the low bias and intra-subject standard deviation, and high percentage of subjects within the tolerance limits provide evidence of a high degree of agreement between the modes and support the viability of the 6s mode as an alternative option in assessing FeNO.

15.2.1.1 Conclusions

NIOX VERO® provides a simple, reliable, repeatable and non-invasive method of measuring FeNO according to current ATS/ERS guidelines [25]. Clinical tests in several institutions and also within the manufacturing company have shown that NIOX VERO has substantially equivalent clinical performance characteristics to NIOX MINO®. In addition, clinical tests have shown that the six and ten second exhalation modes are equivalent in young children.

15.3 Clinical Technical Validation with Inter-Operator Variability Studies

Two inter-operator variability clinical investigations were performed, investigating the repeatability of FeNO measured by different operators with the NIOX VERO. These were randomized, multi-center, single visit, point-of-care, inter-operator variability studies to determine the repeatability of FeNO measured by different Operators with the NIOX VERO in Subjects with asthma using the 10 second method. The investigations had the same endpoints and objectives hence, the data was pooled together. The pooled results for the method comparison studies are summarized below.

The primary objective was to determine the repeatability of FeNO measured by different Operators with the NIOX VERO in Subjects with Asthma. The primary endpoint was the standard deviation of the intra-subject variance as assessed by the square root of the average variance.

Test subjects were defined as persons having their FeNO measured with the NIOX VERO device. Operators were defined as persons administering the FeNO measurement test using the NIOX VERO device and held professional qualifications as a physician, nurse, respiratory therapist or laboratory technician and were familiar with the NIOX MINO. The order of Operators for each Subject was randomized.

15.3.1 Demographic Information & Disposition of Subjects

A total of 122 Subjects were enrolled in both studies (Table 15.8). Three sites participated in the TV-014 study. Two of the same three sites participated in the TV-018 study. The mean age was 36.7 + 16.03 years, 39.3% were Females, 53.6% were White, and 100% had asthma. The mean age was 34.3 + 17.91 years, 56.6% were Females, 61.5% were White, and 52.5% had Asthma. All 122 Subjects (100%) completed three FeNO measurements, met inclusion and exclusion criteria, completed the study and were included in the efficacy analysis.

Table 15.8. Demographic and Baseline Information for Subjects

		All Subjects
Number of Subjects		122
Age (years)		
	N	122
	Mean (SD)	34.3 (17.91)
	Median	35.5
	Min, Max	8, 66
Sex		
	Male	53 (43.4%)
	Female	69 (56.6%)
Ethnicity		
	Hispanic or Latino	0
	Not Hispanic or Latino	122 (100.0%)
	Not Reported	0
	Unknown	0
Race		
	American Indian or Alaskan Native	2 (1.6%)
	Asian	0
	Black or African American	45 (36.9%)
	White	75 (61.5%)
	Other	0
Asthma		
	Yes	64 (52.5%)
	No	58 (47.5%)

Additionally, 54 Operators were enrolled. The mean age was 48.5 ± 13.85 years, 100% were Female, and 48 (88.9%) were White. The educational background of the Operators included 25 RNs (46.3%), 11 LPNs/ANDs (20.4%), 1 PA (1.9%), 8 HS plus Technical training (14.8%) and 9 with other backgrounds (16.7%). Eleven of the 12 Operators in TV-018 participated as Operators in TV-014.

15.3.2 Observed FeNO Results

Operator order refers only to the order in which a particular operator measured subjects; hence 1st, 2nd, and 3rd are reflective of order of time of the assessment rather than specific operators. Additionally, Subjects with a FeNO measurement < 5 ppb were considered to have a FENO measurement = 5 ppb.

A summary of overall FeNO results by Study and first, second, and third operator is displayed in Table 15.9. The mean FeNO value was 31.4 ± 32.97 ppb (range 5 – 174 ppb). A repeated measures ANOVA was completed after data collection was complete to evaluate the homogeneity of FeNO result by Operator order. The results of the ANOVA show that FeNO values were similar between all three Operators but due to the large sample size, small but statistically significant differences were noted (p = 0.0032).

FeNO results were evaluated based on mean FeNO values in order to evaluate the repeatability of the results across the potential clinical spectrum. Subjects with a mean FeNO < 50 ppb or Subjects with a mean FeNO ≥ 50 ppb were considered. A repeated measures ANOVA was completed after data collection was complete to evaluate FeNO result by Operator order (Table 15.9).
The majority of Subjects had mean FeNO values < 50 ppb (n=91). In these Subjects, mean FeNO value was 15.0 ± 10.70 ppb (range 5 – 50 ppb). In Subjects with a mean FeNO ≥ 50 ppb (n=31), the mean FeNO value was 79.4 ± 29.17 ppb (range 49 - 174 ppb). The results of the ANOVA show that the intra-subject mean FeNO values were not significantly different for either group (Subjects with FeNO < 50 ppb, p=0.9979; Subjects with FeNO ≥ 50 ppb, p=0.8006).

Table 15.9. FeNO Results by Operator Order for All Subjects & by Mean Subject FeNO Value

	Operator				
	1st Operator	2nd Operator	3rd Operator	All Observations	ANOVA P-value
All Subjects					
N	122	122	122	366	0.0032
Mean (SD)	30.6 (31.69)	31.7 (33.47)	31.8 (33.97)	31.4 (32.97)	
Median	15.0	16.0	16.0	16.0	
Range	5, 163	5, 174	5, 173	5, 174	
Subjects with FeNO < 50 ppb					
N	91	91	91	273	0.9979
Mean (SD)	15.0 (10.44)	15.1 (10.77)	15.0 (10.99)	15.0 (10.70)	
Median	12.0	11.0	10.0	11.0	
Range	5, 47	5, 47	5, 50	5, 50	
Subjects with FeNO ≥ 50 ppb					
N	31	31	31	93	0.8006
Mean (SD)	76.5 (28.35)	80.6 (29.34)	81.0 (30.52)	79.4 (29.17)	
Median	69.0	72.0	72.0	71.0	
Range	49, 163	52, 174	49, 173	49, 174	

15.3.2.1 Repeatability of FeNO Measurements

The repeatability measures for FeNO values in 122 Subjects are displayed in Table 15.10. The mean intra-subject variance was 6.61 ± 17.954 ppb (upper 95% CI = 9.41). This corresponds to an estimated SD of 2.57 (upper 95% CI = 3.07). The coefficient of variance was 0.066 ± 0.054 (upper 95% CI = 0.074).

The repeatability measures for FeNO values by categorical value are also displayed in Table 15.10. Subjects with a mean FeNO < 50 ppb were consistent and repeatable. The mean intra-subject variance was 1.37 ± 2.136 ppb (upper 95% CI bound = 1.77) which corresponds to an estimated SD of 1.17 (upper 95% CI bound = 1.33). The coefficient of variance was 0.072 ± 0.058 (upper 95% CI bound = 0.082).

Subjects with a mean FeNO ≥ 50 ppb had similar results. The mean intra-subject variance was 21.97 ± 30.976 ppb (upper 95% CI bound = 31.64) which corresponds to an estimated SD of 4.69 (upper 95% CI bound = 5.62). The coefficient of variance was 0.048 ± 0.036 (upper 95% CI bound = 0.060).

Table 15.10. Repeatability Measures for All Subjects & by Mean Subject FeNO			
	All Subjects	Subjects with FeNO < 50 ppb	Subjects with FeNO ≥ 50 ppb
Intra-subject Variance			
N	122	91	31
Mean (SD)	6.61 (17.954)	1.37 (2.136)	21.97 (30.976)
Median	1.00	1.00	9.00
Range	0.00, 109.00	0.00, 14.33	0.33, 109.00
Upper 95% CI	9.41	1.77	31.64
SD [2]	2.57	1.17	4.69
Upper 95% CI for SD [2]	3.07	1.33	5.62
Coefficient of Variance			
N	122	91	31
Mean (SD)	0.066 (0.054)	0.072 (0.058)	0.048 (0.036)
Median	0.054	0.069	0.041
Range	0.000, 0.286	0.000, 0.286	0.011, 0.169
Upper 95% CI	0.074	0.082	0.060

Since the studies involved precision and accuracy of the measurement devices, it was important that FeNO values acquired by Subjects covered the range of possible FeNO values observed in clinical practice (e.g., 5-200 ppb). Table 15.11 displays the pooled inter-operator clinical precision results by median FeNO measurement range. The within subject mean standard deviation (SD) for subjects in the 20 – 29 ppb range was 1.13 ppb (95% CI = 0.77, 1.53) with a CV of 4.78% while the within subject mean SD for subjects in the 40 – 49 ppb group was1.91 ppb (95% CI = 1.04, 2.90) with a CV of 4.24%. The within subject SD for subjects in the > 50 ppb range was 3.79 ppb (95% CI = 2.82, 4.83) with a CV of 4.85%.

Table 15.11. Repeatability Measures by Median Subject FeNO Value for Pooled Study Data Efficacy Subjects					
Median FeNO Value (ppb)	N	Within Subject Mean SD (ppb)	95% CI for SD	Within Subject Mean CV (%)	95% CI for CV
0 - <10	39	0.56	0.39, 0.73	7.86%	5.59%, 10.27%
10 - <20	31	1.12	0.92, 1.34	8.19%	6.89%, 9.64%
20 - <30	8	1.13	0.77, 1.53	4.78%	3.11%, 6.76%
30 - <40	9	1.32	0.89, 1.77	3.94%	2.63%, 5.38%
40 - <50	5	1.91	1.04, 2.90	4.24%	2.28%, 6.62%
≥ 50	30	3.79	2.82, 4.83	4.85%	3.63%, 6.22%

15.3.2.2 Weighted Deming Regression Analysis

Weighted Deming Regressions were completed post-hoc for the three pairs of observations (Table 15.12). The estimated percent bias was small with the estimated average bias between -1.00% and 1.20% depending on the pairs considered. These analyses help confirm the repeatability of the FeNO assessments. Figures 15.3, 15.4 and 15.5 graphically display the FeNO results for each order of Operators (dashed lines indicate 20% bias limits).

Table 15.12. Weighted Deming Regression for FeNO Results– Operator Order			
Weighted Deming Regression			
Operator Order	Parameter	Value (SE)	P-value
2nd as function of 1st	Intercept	-0.418 (0.1718)	0.0164 [3]
	Slope	1.044 (0.0140)	0.0019 [4]
3rd as function of 2nd	Intercept	-0.098 (0.1444)	0.4997 [3]
	Slope	0.997 (0.0106)	0.8077 [4]
3rd as function of 1st	Intercept	-0.516 (0.2105)	0.0157 [3]
	Slope	1.042 (0.0148)	0.0055 [4]
Bias Evaluation			
Operator Order		Average Bias	Range
2nd as function of 1st		1.20%	-3.9%, 4.2%
3rd as function of 2nd		-1.00%	-2.2%, -0.3%
3rd as function of 1st		0.20%	-6.1%, 3.9%
P-value for null hypothesis that correlation is equal to 0.0. Results for the null hypothesis that the parameter is equal to zero. Results for the null hypothesis that the parameter is equal to one.			

Figure 15.3. FeNO Results (1st Operator vs. 2nd Operator).

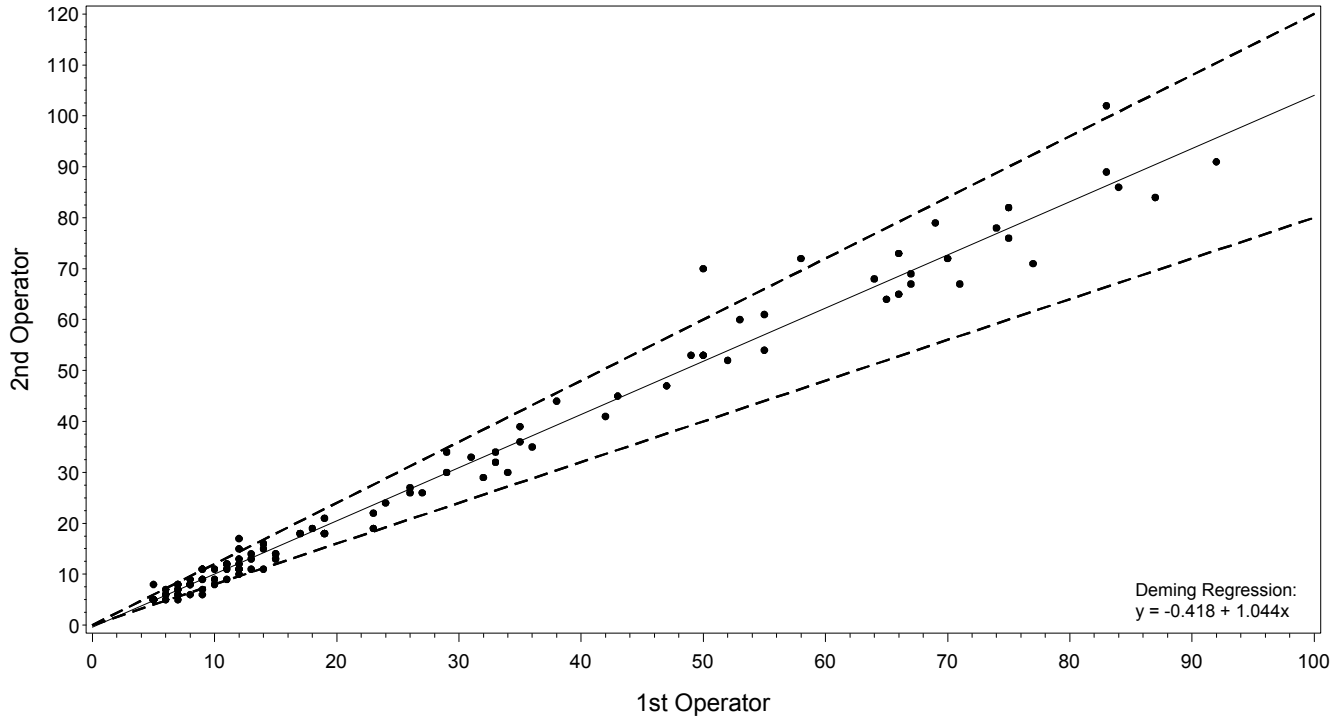


Figure 15.4. FeNO Results (2nd Operator vs. 3rd Operator).

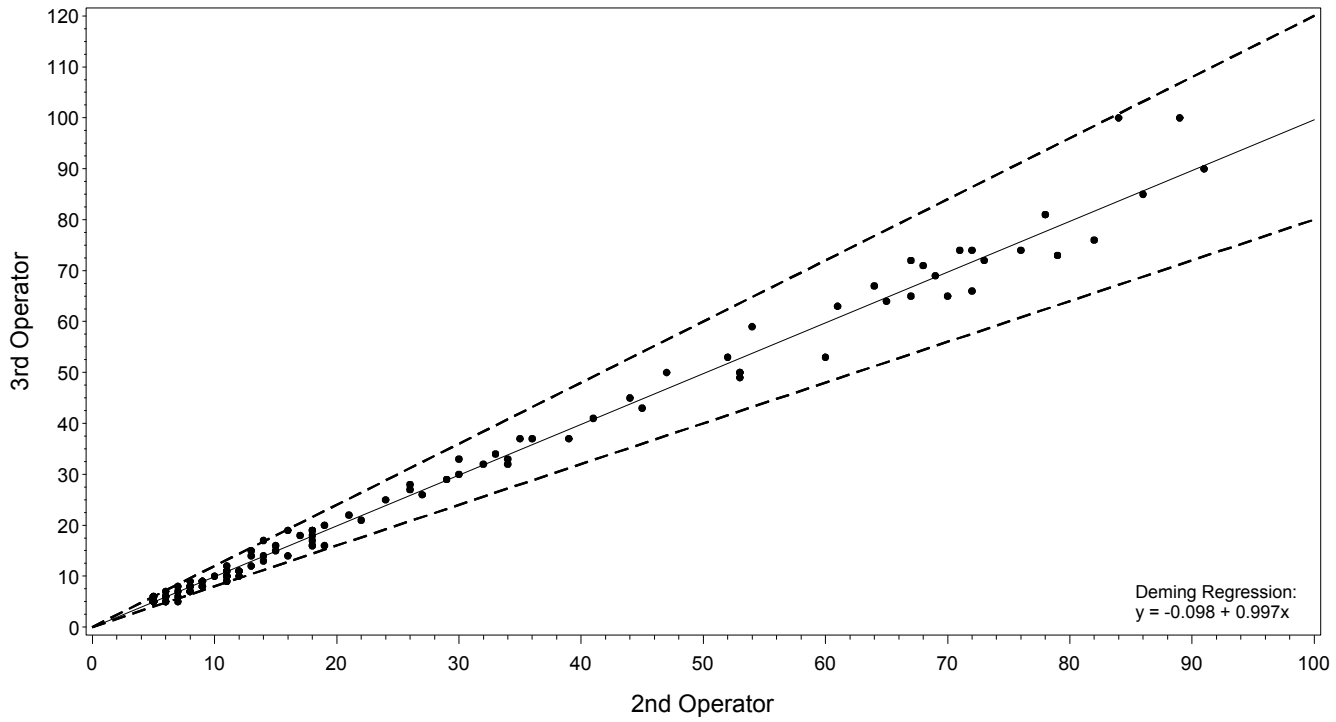
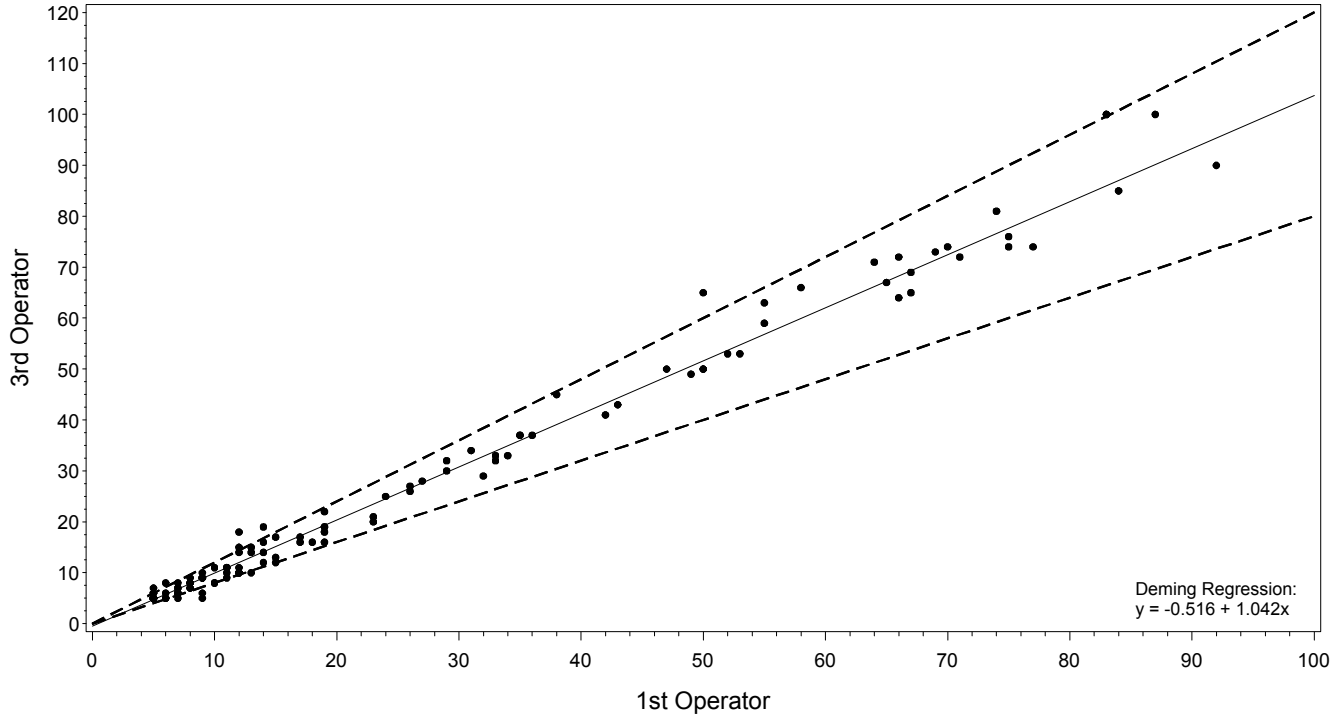


Figure 15.5. FeNO Results (1st Operator vs. 3rd Operator).



15.3.2.3 Paired Differences Evaluation

The summary of the evaluation of paired differences by each Study is described in Table 15.13 below. A total of 366 pairs were analyzed. All 273 pairs in Subjects with a mean FeNO < 50 ppb had a difference of < 10 ppb. Ninety of 93 pairs (96.8%) in Subjects with a mean FeNO ≥ 50 ppb had a difference of < 20%.

Table 15.13. Evaluation of Paired Differences	
	All Subjects
Number of Subjects	122
Number of Subjects with Mean FeNO < 50 ppb Distribution of all Paired Differences in Subjects with mean FeNO < 50 ppb Difference ≤ 10 ppb Difference > 10 ppb Total number of pairs	91 (74.6%) 273 (100.0%) 0 273
Number of Subjects with Mean FeNO ≥ 50 ppb Distribution of all Paired Differences in Subjects with Mean FeNO ≥ 50 ppb Difference ≤ 20% of mean Difference > 20% of mean Total number of pairs	31 (25.4%) 90 (96.8%) 3 (3.2%) 93

Graphical display of FeNO values

Figures 15.6 (dashed lines represent lines of slope = 1.2 and 0.8) and 15.7 (dashed lines represent lines of slope = -0.2 and 0.2) display the repeatability of the FeNO measurements across the spectrum of results in this sample. For the majority of measurements in this study (FeNO < 50 ppb), the FeNO values were highly consistent and repeatable. While variability in intra-subject FeNO values increased with higher values, the CV remained similar.

Figure 15.6. Individual FeNO measurements vs. Subject Mean FeNO.

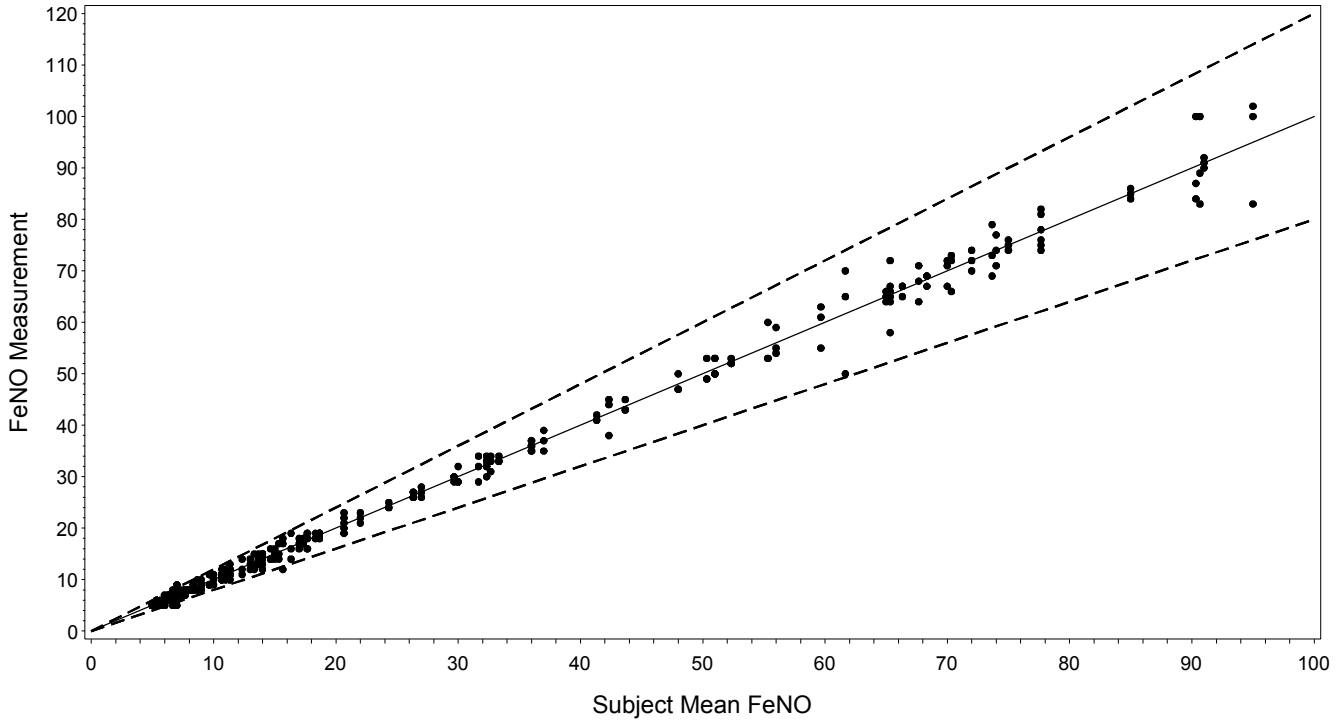
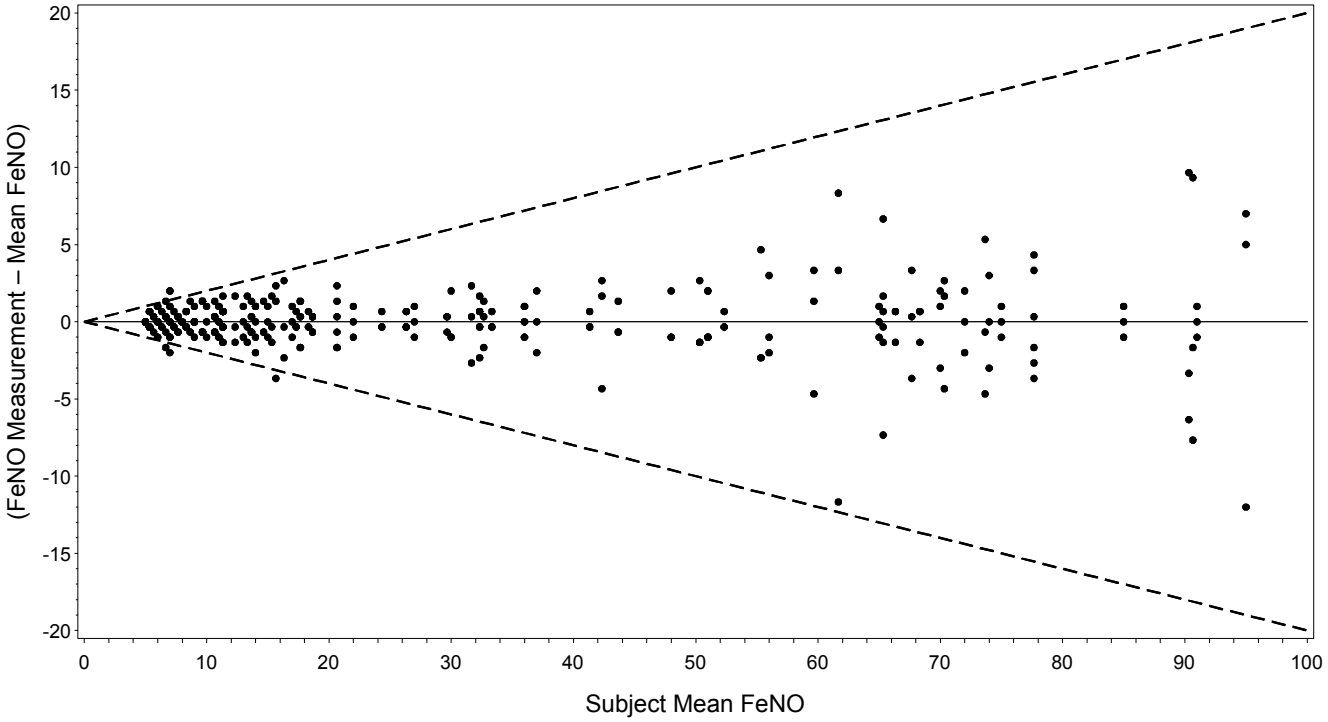


Figure 15.7. (Individual FeNO measurements – Subject Mean FeNO) vs. Subject Mean FeNO.



15.3.4 Discussion

There were no adverse events, serious injuries, issues or problems with use of the NIOX VERO in Subjects. The results of this study demonstrated that FeNO measurements by the NIOX VERO were repeatable, consistent, and well within the technical specifications of the device. Moreover the results of this study demonstrate that there is no observable pattern of a training effect or order effect on FeNO results when done three times by three different operators. The results were not affected adding additional subjects with elevated FeNO levels.

15.4 Clinical correlation and validation study, NIOX MINO (AER-036)[3]

This was a multi-center, device randomized, open-label, prospective single-cohort study aimed at demonstrating substantial equivalence between NIOX MINO® and its predicate device, NIOX® (using the chemiluminescence method). This study demonstrates the clinical utility of measuring fractional exhaled nitric oxide in patients with asthma. While this study was performed with the NIOX MINO, given the clinical performance of the two device, similar results can be expected when monitoring response to therapy with the NIOX VERO.

Change in FeNO levels, which often occurs after 2 weeks of corticosteroid therapy, was measured and compared to baseline levels. Symptomatic male and female asthma patients, from 7 years of age performed two valid FeNO measurements during each visit with NIOX MINO and NIOX. The order of the FeNO measurement on NIOX MINO versus NIOX was randomized. During each visit and for every subject, spirometry was performed and asthma symptoms were recorded with the Asthma Control Questionnaire® (ACQ) [53].

In total, 156 subjects were included (105 adults aged 18 - 70 years and 51 children aged 7 - 17 years). 147 subjects performed valid measurements at both visits and were evaluated per the protocol (see Table 15.14 for demographic data).

Table 15.14. Demographics

		Adults (N=105)	Children (N=51)	Total (N=156)
Gender	**n (%)**			
Male		53 (50.5)	31 (60.8)	84 (53.8)
Female		52 (49.5)	20 (39.2)	72 (46.2)
Ethnic origin	**n (%)**			
Caucasian		100 (95.2)	42 (82.4)	142 (91.0)
African		2 (1.9)	2 (3.9)	4 (2.6)
Hispanic		-	1 (2.0)	1 (0.6)
Asian		2 (1.9)	4 (7.8)	6 (3.8)
Other		1 (1.0)	2 (3.9)	3 (1.9)
Age	**years**			
	Mean (SD)	42.9 (14.9)	12.3 (2.9)	32.9 (19.0)
	Median	42.0	13.0	30.0
	Range	18 to 70	7 to 17	7 to 70
	n	105	51	156

NIOX MINO and NIOX showed substantially similar performance in the measurement of FeNO, with minor non-significant differences between the devices (37.3% and 35.5% reduction in FeNO, respectively). The reduction in FeNO from Visit 1 to follow-up Visit 2, following corticosteroid treatment, was highly significant for both devices.

The subjects' asthma symptoms which were followed with the validated ACQ also showed a significant improvement in the same range (39.7%) as the improvement of FeNO values. The magnitude of the FeNO change and degree of improvement in ACQ are different because the scale and precision of these metrics varies.

Additionally, the subjects' spirometry results also showed a significant improvement between Visit 1 and Visit 2, although the magnitude of the improvement using this method was less obvious (+6.9%). These data (improvement in FeNO, ACQ and spirometry) were in accordance with one another. Table 15.15 below shows a summary of the primary and secondary outcome data.

Table 15.15. Mean change between visit 1 and 2 for the two devices (NIOX MINO® versus NIOX®) and change in clinical well-being and spirometry.

	Mean % change	Standard Error of Mean, %	p-value[1]	n
NIOX MINO	-37.3	2.5	<0.0001	151
NIOX	-35.5	2.7	<0.0001	151
ACQ	-39.7	3.0	<0.0001	151
FEV_1	6.9	1.2	<0.0001	149

[1] p-value for statistical significance of change vs baseline.

3 AER-036 Clinical Investigation Report: DCR–000035–01/DCR-000039 (maintained in the Trial Master Files at Circassia Pharmaceuticals, Inc.)

The relationship between the percent change in FeNO and the percent change in pre-bronchodilator Forced Expiratory Volume (FEV1), post-bronchodilator FEV1 and the total symptom scores; ACQ from Visit 1 to Visit 2 was investigated for the Intent to Treat (ITT) population.

Asthma health status is composed of several distinct components such as social, physical, clinical and occupational, mitigating the likelihood of strong statistical correlation [65]. An absolute majority of subjects that experienced a reduction of FeNO also had an improvement in asthma symptoms as measured by the ACQ.

16. Specific Performance Characteristics

Table 16.1	
Performance Parameter	**NIOX VERO® Limits**
Measurement range	5 - 300 ppb
Lowest Detection Limit	5 ppb
Linearity	Squared correlation coefficient r^2 > 0.998, slope 0.95 – 1.05, intercept ±3ppb Determination based on pooled regression analysis from 10 instruments using standard gas reference samples at 7 different concentration levels covering the operating measurement range.
Precision	< 3ppb of measured value for values < 30 ppb < 10% of measured value for values ≥ 30 ppb Expressed as one standard deviation for replicate measurements with the same instrument, using a certified gas concentration of Nitric Oxide reference standard
Accuracy	±5 ppb for measured values ≤ 50 ppb or 10% of measured values > 50 ppb. Expressed as the upper 95% confidence limit, based on absolute mean of differences from certified gas concentration of Nitric Oxide
Method Comparison	< 10 ppb for values ≤ 50 ppb, < 20 % for values > 50 ppb Expressed as the difference between a NIOX MINO® FeNO value and the corresponding FeNO value measured with NIOX VERO instrument from Circassia.
Inhalation parameters	Inhale to TLC (Total Lung Capacity) before start of exhalation. Inhalation in instrument is triggered by a pressure of -3 cm H_20.
Exhalation parameters	Exhalation time: Standard mode: 10 s (clinical use) All exhalations are to be performed at an exhalation pressure of 10 - 20 cm H_2O, to maintain a fixed flow rate of 50 ±5 ml/s. The instrument stops the measurement at pressures outside the interval. Warning alerts sounds at 10 - 12 and 18-20 cm H_2O.

17. Evaluation Methods, Performance Data

The instrument is verified to fulfill the specified performance under the temperature range within 50 to 95°F/+10 to +35 °C, relative humidity range of 20- 80% and pressure range of 700-1060 hPa. The following provides a summary of performed tests with protocol design, data, results and conclusion.

FeNO measurement time

The time from end of exhalation until the result is presented on the screen was measured.
The FeNO measurement time was determined to be 55 seconds.

Temperature stabilization time:

The time from power up until the system is ready for use was measured.
The temperature stabilization time were determined to be <30 min., typically < 1 minute.

Measurement range

The measurement range was determined in a laboratory setting using mixtures of standard reference NO gas. Certified NO in N_2 calibration gas of 200 ppb and 2000 ppb was mixed with nitrogen gas in a gas mixer, connected in-line with the NIOX VERO instrument, (with mounted NIOX VERO sensors), to obtain 7 NO concentration levels (3, 5, 25, 100, 200, 300 and 330 ppb). Five replicate determinations of the concentrations at 3 and 5 ppb, and three replicate determinations on the other intervals were made.
5 ppb was the lowest detectable level, and 300 ppb the highest detectable level.

Lowest detection limit

Lowest detection limit was determined in a laboratory setting, using mixtures of standard reference NO gas and N_2 gas below and above the detection limit, at 3 and 5 ppb. Five replicate determinations of each concentration were made at each occasion. 10 NIOX VERO sensors, continually mounted in 10 NIOX VERO instruments, respectively, were used in these tests. Measured data at 3 ppb and 5 ppb are presented in table 17.1.

Table 17.1: Measured data at nominal 3 ppb and 5 ppb for 10 NIOX VERO instruments

NIOX MINO No		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Nominal	Replicate											
3	1	3.6	4.5	3.8	4.3	3.9	3.1	3.3	3.5	4.3	3.0	
	2	3.9	4.2	3.9	4.0	3.5	4.1	3.8	3.6	3.8	3.1	
	3	3.5	4.3	2.9	3.8	3.5	3.4	2.7	3.2	3.4	3.5	
	4	2.9	3.7	2.5	4.3	3.3	3.6	3.7	3.5	3.8	1.8	
	5	2.5	3.8	3.4	4.4	3.4	2.8	3.6	3.1	3.5	3.0	
	Average	3.3	4.1	3.3	4.2	3.5	3.4	3.4	3.4	3.8	2.9	**3.5**
	Stdev	0.5	0.3	0.5	0.2	0.2	0.4	0.4	0.2	0.3	0.5	**0.4**
Nominal	Replicate											
5	1	7.3	8.9	8.1	9.3	8.2	8.0	7.1	7.8	8.6	6.8	
	2	7.3	9.2	8.2	8.7	7.9	8.3	7.4	8.4	8.7	6.9	
	3	7.5	8.9	7.6	8.4	8.4	7.9	7.8	8.4	8.7	7.5	
	4	7.7	8.9	7.7	8.9	8.2	8.5	7.9	8.1	8.6	7.2	
	5	8.5	8.8	8.8	8.7	8.7	8.1	8.1	8.2	8.4	7.8	
	Average	7.7	8.9	8.1	8.8	8.3	8.2	7.7	8.2	8.6	7.2	**8.2**
	Stdev	0.4	0.1	0.4	0.3	0.2	0.2	0.3	0.2	0.1	0.3	**0.5**

At nominal value of 3 ppb, the overall mean measured value was 3.5 ppb (95%CI 3.3, 3.7)
At nominal value of 5 ppb, the overall mean measured value was 8.2 ppb (95%CI 7.9, 8.5)
Thus it can be concluded that the lowest detectable level for NIOX VERO is reached at around 3 ppb, which is why the specification limit for lowest detectable level is set at 5 ppb.

Linearity

Certified NO in N_2 calibration gas of 200 ppb and 2000 ppb was mixed with nitrogen gas in a gas mixer, connected in-line with the NIOX VERO instrument, (with mounted NIOX VERO sensors), to obtain 7 NO concentration levels (3, 5, 25, 100, 200, 300 and 330 ppb). Five replicate determinations of the concentrations at 3 and 5 ppb, and three replicate determinations on the other intervals were made.
The regression analysis gave an average slope of 1.047 and average intercept of 0.5ppb. The squared correlation coefficient r^2 was > 0.999 for all the 10 devices tested. The results conclude that NIOX VERO® linearity is within the specification of r^2 > 0.998.

Table 17.2 Linearity, squared correlation coefficient r^2 , slope and intercept for individual instruments

Test instrument #	squared correlation coefficient r^2	Slope	Intercept [ppb]
NV_1	0.9995	1.054	-0.3
NV_2	0.9998	1.083	1.3
NV_3	0.9997	1.034	0.5
NV_4	0.9998	1.071	1.5
NV_5	0.9997	1.055	0.7
NV_6	0.9996	1.021	0.5
NV_7	0.9996	1.057	0.0
NV_8	0.9997	1.069	0.3
NV_9	0.9997	1.038	1.1
NV_10	0.9995	0.990	-0.4

Analytical Precision

Analytical precision was determined in-house. Certified NO in N_2 calibration gas of 200 ppb was mixed with nitrogen gas in a gas mixer, connected in-line with the NIOX VERO instrument, to obtain four NO concentration levels (5, 25, 75, and 200 ppb). Two replicate determinations of each concentration were made twice a day (more than 2 hours apart) for 20 days.
Repeatability is an estimate of variation within one test run in one day. Within-device precision is an estimate of variation between test runs and days. The repeatability and within-device precision were calculated for the 5 instruments. The results at 5 and 25 ppb are expressed as absolute values in ppb. The results at the 75 and 200 ppb levels are expressed as percentage of the measured NO concentration. Both standard deviation estimates met the precision claim at all three concentration levels.
The results are presented in figure 17.1 and table 17.3.

Figure 17.1

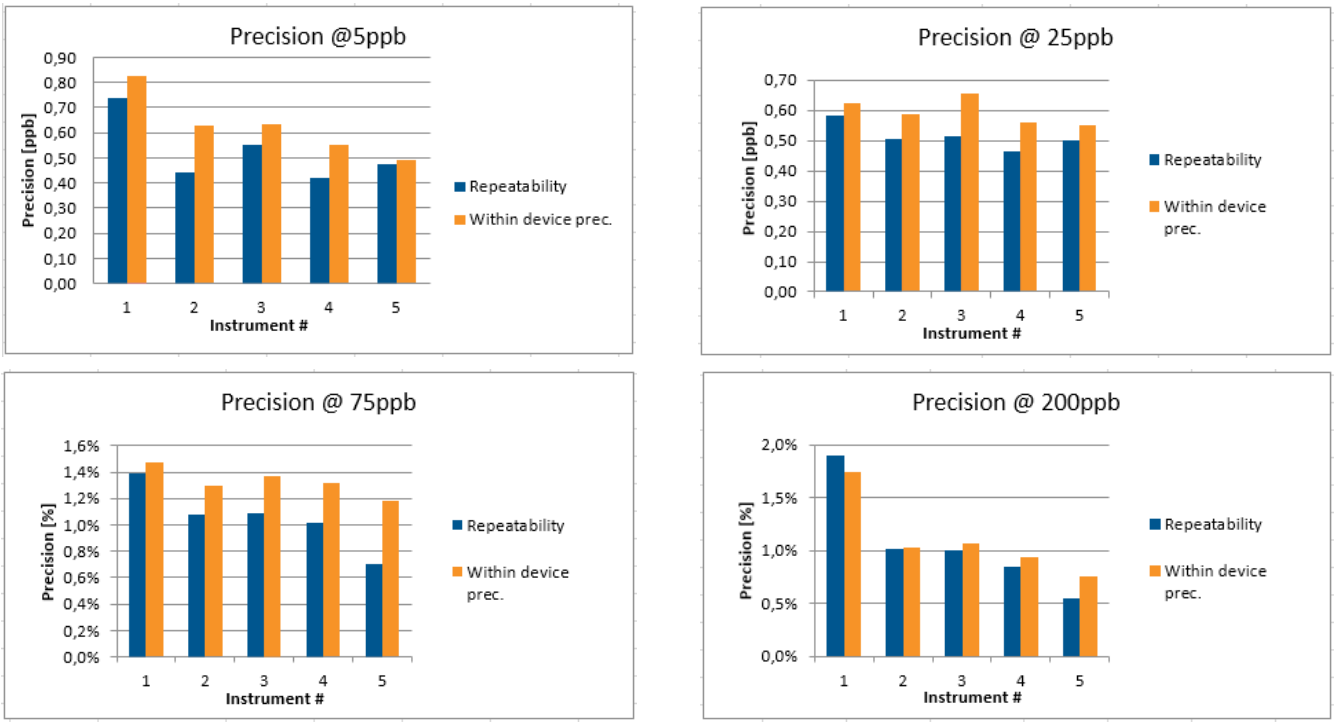


Table 17.3 Precision								
		Repetability			Within device precision			
NO concentration, ppb	5 ppb	25 ppb	75 ppb	200 ppb	5 ppb	25 ppb	75 ppb	200 ppb
Claim	=< 3 ppb	=< 3 ppb	=< 10 %	=< 10%	=< 3 ppb	=< 3 ppb	=< 10%	=< 10%
Serial No. NIOX VERO /Sensor	[ppb]	[ppb]	[%]	[%]	[ppb]	[ppb]	[%]	[%]
735 / 4847	0.74	0.58	1.4%	1.9%	0.83	0.63	1.5%	1.7%
741 / 4848	0.44	0.51	1.1%	1.0%	0.63	0.59	1.3%	1.0%
748 / 4849	0.55	0.51	1.1%	1.0%	0.63	0.65	1.4%	1.1%
755 / 4850	0.42	0.46	1.0%	0.8%	0.55	0.56	1.3%	0.9%
762 / 4859	0.47	0.50	0.7%	0.5%	0.49	0.55	1.2%	0.8%

The results conclude that the repeatability and within-device precision are well within specification limit: < 3 ppb of measured value < 30 ppb, < 10 % of measured value > 30 ppb. Both standard deviation estimates for repeatability and within-device precision met the precision claim in the labeling at all four concentration levels.

Accuracy

Analytical accuracy is the deviation of the measured value from a known nominal value, i.e. the certified concentration of a nitric oxide reference standard. Mixtures of a certified calibration gas of 200 ppb NO in N_2 were used, yielding concentrations 5 ppb, 25 ppb, 75 ppb and 200 ppb. The gas mixer was connected in-line with the NIOX VERO instrument. A total of 5 sensors mounted in 5 NIOX VERO instruments were used for accuracy evaluation. Two replicate determinations were made at each occasion.
The temperature and relative humidity were within the claimed operational range for the entire test period.
For each test occasion the mean NO concentration (M) for each set of replicates, and the absolute deviation of each replicate mean (D) from the nominal gas concentration (G), were calculated. The mean D, the standard deviation, and the 95% confidence interval for all instruments were calculated. At > 50 ppb concentration levels the deviation D is expressed as percentage of the nominal NO concentration.
The results for the 5 NIOX VERO systems are presented in table 17.4. The accuracy was in all test occasions within the technical specification, i.e. ±5 ppb ≤ 50 ppb or max 10% at > 50 ppb

Table 17.4: Accuracy

Nominal		n	Mean deviation [ppb]	SEM	95% UL of M
5 ppb	Device 1	40	-1.62	0.63	-1.79
	Device 2	40	-0.97	0.54	-1.12
	Device 3	40	-1.30	0.49	-1.43
	Device 4	40	-1.19	0.46	-1.31
	Device 5	40	-0.87	0.35	-0.97
25 ppb	Device 1	40	-3.88	0.46	-4.00
	Device 2	40	-2.88	0.46	-3.00
	Device 3	40	-3.54	0.53	-3.68
	Device 4	40	-3.07	0.45	-3.19
	Device 5	40	-1.87	0.42	-1.98
75 ppb	Device 1	40	-5.92	0.75	-8.2%
	Device 2	40	-4.23	0.73	-5.9%
	Device 3	40	-6.14	0.77	-8.5%
	Device 4	40	-4.96	0.76	-6.9%
	Device 5	40	-2.01	0.76	-3.0%
200 ppb	Device 1	40	-6.70	2.10	-3.6%
	Device 2	40	-5.17	1.40	-2.8%
	Device 3	40	-9.68	1.49	-5.0%
	Device 4	40	-7.26	1.34	-3.8%
	Device 5	40	-1.16	1.25	-0.7%

Climate effects

The combined effects of temperature and relative humidity (RH) were measured at 10°C, 25°C and 35°C (50°F, 77°F and 95°F) and 20%, 50% 80% RH. This covers all the conditions within the low and high temperature and humidity ranges. Measures were taken at 15 ppb, 75 ppb and at 200 ppb, with standard reference gas mixtures of NO in N_2. (see table 17.5 and table 17.6).

The mean absolute difference from nominal NO concentration obtained at each test occasion is shown in Table 17.5 and 17.6. The results at NO concentrations below 50 ppb are presented in ppb, and results above 50 ppb are presented as percentage of nominal NO concentration.
The deviations are within the technical specification, i.e. ±5 ppb for the level 15 ppb and max 10 % for the levels 75 and 200 ppb at 95% confidence interval.

Table 17.5: absolute difference from nominal concentration 15 ppb							
Test Number	1	2	3	4	5	6	7
Temp. [C]	10	10	25	25	25	35	35
RH%	20	50	20	50	80	50	80
Nom. Conc. [ppb]	15	15	15	15	15	15	15
Average Dev. [ppb]	0.9	0.4	0.3	0.0	0.4	0.9	1.4
Stdv [ppb]	0.8	1.4	0.9	1.2	1.9	2.0	2.1
Conf. Int. [ppb]	0.3	0.6	0.4	0.5	0.7	0.8	0.8
UCL [ppb]	1.2	0.9	0.6	0.5	1.2	1.7	2.2

Table 17.6: relative difference from nominal concentrations 75 and 200 ppb														
Test Number	1		2		3		4		5		6		7	
Temp. [C]		10		10		25		25		25		35		35
RH%		20		50		20		50		80		50		80
Nom. Conc. [ppb]	75	200	75	200	75	200	75	200	75	200	75	200	75	200
Average Dev. [ppb]	8.1	2.1	6.8	1.3	5.4	0.7	4.1	1.4	2.8	1.0	1.4	0.4	0.1	0.1
Stdv [ppb]	4.6	3.6	6.0	3.4	7.3	3.3	8.6	3.4	9.8	3.4	11.0	4.3	12.2	4.5
Conf. Int. [ppb]	1.4	1.2	1.9	1.1	2.4	1.1	2.8	1.2	3.3	1.1	3.7	1.5	4.2	1.5
UCL [ppb]	9.5	3.3	8.7	2.4	7.8	1.8	6.9	2.6	6.0	2.1	5.1	1.9	4.3	1.6

Interference of analytically determined interfering substances

Sensor interference levels were tested in a laboratory setting, by generating the applicable concentrations of each substance and measuring the sensor signal. Substances were selected based on their oxidizing potential, which could interfere with the electrochemical signal for NO detection. The concentrations were in the same range or higher than expected concentration of each substance in exhaled breath [57, 58]. The interference is calculated in relation to the highest NO level in the measurement range, i.e. 300 ppb. The applicable concentration of each substance was generated, the gas stream was fed to the sensor by a gas-mixer, and the sensor signal was measured. All tests were performed at normal ambient conditions; Temperature between 68 and 75° F/ 20 and 24°C, relative humidity between 45 and 55%.

Nitrogen dioxide and hydrogen sulfide were the only detected interferents, according to table 17.7 below. When using NIOX VERO, the patient first inhales through a mouthpiece connected to a scrubber that eliminates Nitric Oxide and Nitrogen Dioxide and also other contaminants from the ambient air.

Table 17.7 Interfering substances			
Substance	**Concentration tested**	**Expected concentrations in exhaled breath of healthy subjects**	**Sensor Interference, equivalent to ppb NO**
Acetaldehyde	1000 ppm	100 ppb	Non detectable
Acetone	100 ppm	10 ppm	Non detectable
Acetonitrile	500 ppm	100 ppb	Non detectable
Ammonia	100 ppm, balance air	0.5 ppm	Non detectable
Carbon dioxide (CO_2)	5% Vol, balance air	8%	Non detectable
Carbon monoxide (CO)	250 ppm, balance air	50 ppm	Non detectable
Ethanol	1000 ppm, balance air	165 ppm	Non detectable
Hydrogen (H2)	500 ppm, balance nitrogen	50 ppm	Non detectable
Hydrogen peroxide (H_2O_2)	500 ppm, balance air	1 ppm	Non detectable
Hydrogen sulfide (H_2S)	1 ppm, balance nitrogen	1 ppm	2.0
Isoprene	1000 ppm, balance air	1 ppm	Non detectable
Nitrogen dioxide (NO_2)	9.2 ppm, balance nitrogen	200 ppb	2.5
Oxygen (O_2)	100% Volume	21%	Non detectable

Interference of exogenous substances

A clinical validation study was performed to assess the influence of exogenous substances (chewing gum, carbonated beverage and mouthwash) on FeNO measured with NIOX VERO [AER-049]. The subjects were healthy volunteers between 20 and 65 years of age, (12 planned, 12 analyzed).

The primary endpoint was the difference between baseline FeNO and FeNO measured directly after exposure, in addition to measurements taken at one and two hours after exposure to each exogenous substance.

The results of this study show that there is little or no effect of exogenous substances on the measurement of exhaled nitric oxide. The differences that were seen were all within the performance characteristics of the NIOX VERO.

18. Operating Conditions

Ensure stable operating conditions by avoiding placement of the instrument in direct sunlight, near sources radiating heat, or ventilation. NIOX VERO® operates during the following conditions:
NO in ambient air up to 300 ppb
To verify NO in ambient air, perform an ambient measurement, see User Manual.
Temperature range of 50 to 95° F
An atmospheric pressure range of 700 hPa to 1060 hPa
A relative humidity range of 20 to 80 %, non-condensing
Performance shall be sustained when measuring continuously at a rate of up to 10 measurements / hour.
Measurement cycle groups of 20 measurements / hour with sustained performance for one hour, with a minimum of 30 minutes in between each measurement cycle group of 20 measurements / hour.

18.1 Calibration

The manufacturer performs calibration for each NIOX VERO® Sensor. No additional calibration is needed during the lifetime of the sensor.

18.2 Quality Control

Built-in quality control functions continuously monitor functionality and detect any potential drift from zero baseline.

19. Routine Maintenance

Please refer to NIOX VERO® User Manual.

20. Periodic Service

No periodic service is performed.

21. Manufacturer Information

Representative in North America:
Circassia Pharmaceuticals Inc.
5151 McCrimmon Parkway, Suite 260 Morrisville, NC 27560
Phone: 866-275-6469
Fax: 877-630-6469

All questions regarding technical and application support in North America region:
Phone: 866-275-6469
E-mail: service.us@circassia.com

Responsible Manufacturer:
Address:
Circassia AB
Hansellisgatan 13
SE-754 50 Uppsala
Sweden

www.circassia.com
www.niox.com

NIOX VERO is CE marked according to In Vitro Device Directive IVDD 98/79/EC.
NIOX VERO® is RoHS compliant.

22. Bibliogaphy

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