510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:
k073265

B. Purpose for Submission:
New Device

C. Measurand:
Breath Nitric Oxide

D. Type of Test:
Quantitative, biosensor

E. Applicant:
Apieron, Inc.

F. Proprietary and Established Names:
Apieron INSIGHT™ eNO System

G. Regulatory Information:
1. Regulation section:
   21 CFR § 862.3080
2. Classification:
   Class II
3. Product code:
   MXA
4. Panel:
   Toxicology (91)

H. Intended Use:
1. Intended use(s):
   Refer to indications for use below.

2. Indication(s) for use:
   The intended use of the Apieron INSIGHT™ eNO System is to quantitatively measure exhaled nitric oxide (eNO) in expired human breath as a marker of inflammation in persons with asthma. Measurement of eNO in expired human breath by the Apieron INSIGHT eNO System is a non-invasive, simple and safe method to measure a decrease in eNO in asthma patients that often occurs after treatment with anti-inflammatory pharmacological therapy as an indication of the therapeutic effects in patients with elevated eNO levels. The Apieron INSIGHT eNO System is suitable for use in children ages 8 to 17 years of age, and in adults 18 years of age and older. eNO measurements, as an adjunct to established clinical assessments, provide the physician an objective marker to evaluate the

patient's response to anti-inflammatory therapy. The Apieron INSIGHT eNO System can be used by trained operators in a physician's office laboratory setting. The Apieron INSIGHT eNO System should not be used in critical care, emergency care or in anesthesiology.

3. Special conditions for use statement(s):
   The Apieron INSIGHT eNO System can be used by trained operators in a physician's office laboratory setting.
   The Apieron INSIGHT eNO System should not be used in critical care, emergency care or in anesthesiology. Also, it cannot be used with infants or by children under the age of 8, as the measurement requires patient cooperation. Patients should refrain from eating and drinking for one hour before the breath measurement is taken. Alcohol ingestion reduces eNO in patients with asthma and healthy subjects.

4. Special instrument requirements:
   Apieron INSIGHT eNO System

I. Device Description:

The Apieron INSIGHT™ eNO System consists of a Monitor, a single-use disposable sensor cartridge and other disposable test supplies. The Monitor contains the measurement and breath sampling hardware and provides a user interface to guide the operator through the test sequence and guide the patient through the breath sampling maneuver. The disposable sensor cartridge contains a biosensor that changes its optical transmission properties when it reacts with the nitric oxide in the breath sample. The Apieron INSIGHT eNO System is transportable, operated by an AC outlet and designed for use with the disposable sensor cartridge.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   NIOX

2. Predicate 510(k) number(s):
   k021133

3. Comparison with predicate:

	Apieron INSIGHT™ eNO System	NIOX® - (Predicate Device)
Indications for Use	The intended use of the Apieron INSIGHT™ eNO System is to quantitatively measure exhaled nitric oxide (eNO) in expired human breath as a marker of inflammation in persons with asthma. Measurement of eNO in expired human breath by the Apieron INSIGHT eNO System is a non-	Measurement of FeNO by NIOX is a quantitative, noninvasive, simple and safe method to measure the decrease in FeNO that often occurs after treatment with anti-inflammatory pharmacological therapy, as an indication of the therapeutic effect in patients with elevated FeNO levels.

	invasive, and simple method to measure a decrease in eNO in asthma patients that often occurs after treatment with anti-inflammatory pharmacological therapy as an indication of the therapeutic effects in patients with elevated eNO levels. The Apieron INSIGHT eNO System is suitable for use in children ages 8 to 17 years of age, and in adults 18 years of age and older. eNO measurements, as an adjunct to established clinical assessments, provide the physician an objective marker to evaluate the patient's response to anti-inflammatory therapy. The Apieron INSIGHT eNO System can be used by trained operators in a physician's office laboratory setting. The Apieron INSIGHT eNO System should not be used in critical care, emergency care or in anesthesiology.	NIOX is suitable for children, approximately 4-17 years, and adults 18 years and older. FeNO measurements provide the physician with a means of evaluating an asthma patient's response to anti-inflammatory therapy, as an adjunct to established clinical and laboratory assessments in asthma.
Anatomical Site	Respiratory System	Respiratory System
Nitric Oxide Sensing	Biosensor: Nitric oxide from the breath sample diffuses through a glass matrix, causing a change in absorbance. The spectral change is detected with the optical measurement system that measures the ratio of light passing through the Biosensor and a reference light path.	Chemiluminescence: Nitric oxide from the breath sample reacts with ozone (O3) producing photons that are detected by a photomultiplier tube. The signal from the photomultiplier tube is processed by the instrument to create a real-time signal that is proportional to nitric oxide concentration.
Measure-ment range	10 - 200 ppb	2 - 200 ppb
Detection limit	10 ppb	2 ppb

K. Standard/Guidance Document Referenced (if applicable):

1. C24-A3 CLSI Statistical Quality Control for Quantitative Measurements: Principles and Definitions
2. EP5-A2 CLSI Evaluations of Precision Performance of Quantitative Measurement Methods
3. EP6-A CLSI Evaluation of the Linearity of Quantitative Measurement Procedures; A Statistical Approach
4. EP7-A2 CLSI Interference Testing in Clinical Chemistry Second Edition 2005
5. EP9-A2 CLSI Method Comparison and Bias Estimation Using Patient Samples
6. EP17-A CLSI Protocols for Determination of Limits of Detection and Limits of Quantitation

7. 7816-1 ISO Identification Cards - Integrated Circuit Cards with Contacts - Part 1: Physical Characteristics
8. 10993-1 ISO Biological Evaluation of Medical Devices: Part 1: Evaluation and Testing
9. 10993-5 ISO Biological Evaluation of Medical Devices: Part 5: Tests for in vitro Cytotoxicity
10. 14971 ISO Medical devices – Application of risk management to medical devices

L. Test Principle:

The nitric oxide concentration in the breath sample is measured using an optical biosensor that changes its light absorption characteristics when exposed to nitric oxide. The biosensor is contained in a single-use, disposable sensor cartridge. The measurement subsystem of the Monitor measures the change in transmission of a narrow spectrum of light through the biosensor in order to calculate the concentration of nitric oxide in the breath sample.

The sensor cartridge controls the concentration of water vapor surrounding the biosensor and conditions the incoming breath sample. Also, CO_2 is removed from the breath sample by the breath sample filter with minimal impact on the nitric oxide concentration. Solid-state thermoelectric coolers control the temperature of the sensor cartridge to prevent temperature-induced shifts in optical absorbance.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*

      Total precision was assessed analytically using simulated breath samples at NO gas concentrations of 20 ppb, 80 ppb and 150 ppb, allowing variation in INSIGHT Monitor, reference system, day, sample gas bag, operator, work session within a day, INSIGHT Sample Filter lot, and INSIGHT Sensor lot. The experimental variable for the precision testing and the sample size are shown in the table below.

Experimental Variables for Precision Testing

Parameter	Value
Replicates	2
Samples per Test Session	3
Sensors per Test Session	60
Days	5
Nitric Oxide Concentration	20, 80, 150 ppb
Biosensor Lots	3
Sensor Lots	3
Breath Sample Filter Lots	3
Operators per Monitor	3
Monitors	4
Total Sample Size	600

The results of the precision testing can be found in the table below.

Precision of the Apieron INSIGHT eNO System

NO (ppb)	Total Precision	
	(ppb)	(% CV)
20	1.50	7.5
80	2.56	3.2
150	4.22	2.3

Clinical Repeatability

Repeatability is defined as the average standard deviation of repeated measurements on a single subject. Clinical repeatability was evaluated by replicate testing on the Apieron INSIGHT eNO System at a single clinical site using 3 operators. Seventy-eight asthma subjects of varying severity were tested twice within a single measurement session. The results of this study were analyzed in clinically significant ranges of eNO concentrations and are provided in the table below.

Clinical Repeatability of the Apieron INSIGHT System

eNO Level	N	Repeatability	95% CI	%CV	95% CI
All	76	3.96 ppb	2.95 to 4.98 ppb	7.28 %	5.93 to 8.62 %
≤ 30 ppb	25	1.58 ppb	0.90 to 2.27 ppb	7.67 %	4.89 to 10.45%
30 - 50	22	2.83 ppb	1.60 to 4.06 ppb	6.79 %	3.94 to 9.63 %
50 - 100	20	5.16 ppb	3.69 to 6.63 ppb	7.15 %	4.95 to 9.35 %
> 100 ppb	9	10.69 ppb	4.89 to 16.48 ppb	7.65 %	3.64 to 11.66 %

b. *Linearity/assay reportable range:*

Linearity was assessed using 24 samples at each of nine target NO gas concentrations: 0 ppb, 10 ppb, 20 ppb, 50 ppb, 80 ppb, 110 ppb, 150 ppb, 200 ppb and 240 ppb. The degree of nonlinearity was computed as the percentage deviation between the linear fit and the 3rd order nonlinear fit relative to the target concentrations. The sponsor's acceptance criterion was a percentage deviation of < 5% at each of the test concentrations within the measurement range.

A regression analysis of NO as measured by the Apieron INSIGHT eNO System versus the known concentration of NO is presented in the table entitled below.

Linearity Regression Analysis

N	216
R^2	1.00
Intercept (ppb)	-0.47
Slope	1.01

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The 1 ppm reference gas cylinders are "RATA" (Relative Accuracy Test Audit) indicating that they have been analyzed according to the method described in EPA publication (EPA-600/R-07/121). The cylinders are analyzed directly against a NIST Standard Reference Material (SRM), a NIST traceable Reference Material (NTRM), or a Netherlands Measurement Institute (NMi) Primary Reference Material (PRM). The values of the certified gas are used in the calibration of the Apieron INSIGHT™ eNO System.

The Apieron INSIGHT™ eNO System will initially be labeled with a shelf life (expiration date) of three months. Shelf life testing has been conducted through real-time aging.

d. *Detection limit:*
The Limit of Detection was tested to verify that the Apieron INSIGHT eNO System measurement technology was capable of reliably measuring eNO concentrations at the lowest reported value of 10 ppb.

The Limit of Detection (LoD) was assessed as the smallest concentration that can be reliably distinguished from the Limit of Blank (LoB) to a confidence of 95%. According to CLSI Guideline EP 17-A, the LoD is defined as the Limit of Blank (LoB) plus 1.65 times the Standard Deviation of repeated testing at a single low NO concentration. The Limit of Blank was determined by testing 60 simulated breath samples containing no Nitric Oxide (blanks) and calculating the upper 95% confidence interval of the mean. The standard deviation of a low concentration was determined by testing 60 samples of simulated breath with 10 ppb of Nitric Oxide.

The Limit of Quantitation (LoQ) was estimated by determining mean error (bias) and standard deviation of 60 simulated breath samples at 0 and 10 ppb NO. The LoQ was calculated as the absolute value of the mean error at 10 ppb plus 2 times standard deviation of error at 0 ppb. Thus, the LoQ was estimated at 2.1 ppb. Since the CLSLI Guideline EP17-A specifies that the LoQ cannot be less than the LoD, the sponsor chose the LoQ to be the same as the LoD of 6.64 ppb.

The result of this analysis is shown in the Table below.

Limit of Detection Analysis

Limit of Blank (upper 95% CI)	5.14 ppb
Standard Deviation of 10ppb Sample	0.92 ppb
Limit of Detection	6.64 ppb
Limit of Quantitation	6.64 ppb

e. Analytical specificity:

Two sets of endogenous interference experiments were conducted.
In the first set of experiments, interference testing was performed on the Apieron INSIGHT eNO System for the following common constituents of exhaled breath:

- 200 ppm H_2 (hydrogen)
- 3 ppm CO (carbon monoxide)
- 100% Relative Humidity (water vapor)
- 0.5% CO_2 (carbon dioxide)
- 0 ppb NO (nitric oxide)

Twenty-four samples containing all of the above potential interferents (balance air) and 24 samples containing only air were prepared and tested on the Apieron INSIGHT eNO System. The mean difference in response was less than 0.05 ppb. The sponsor determined that this difference was not statistically significant between the samples containing potential interferents and those containing only air.

In the second set of experiments seven additional compounds were tested at physiologically relevant concentrations by comparing the response of the Apieron INSIGHT eNO System to the test gas (balance nitrogen) to nitrogen alone. The test gas contained 0 ppb NO (nitric oxide). A compound was deemed non-interfering by the sponsor if, at the tested concentration, the response was within ±5 ppb NO equivalent when compared to the nitrogen control. The table below summarizes the results of this testing.

Endogenous Interference Testing

Compound	Measured Concentration	N	Mean Response	t-test p-value
Isoprene	411 ppb	10	-0.30 ppb	0.319
Ethanol	154 ppm	11	0.60 ppb	0.038*
Acetone	403 ppm	11	0.14 ppb	0.624
Ammonia	1.0 ppm	10	0.10 ppb	0.742
Acetaldehyde	4.04 ppm	10	0.16 ppb	0.590
Methanethiol	2.03 ppm	10	0.75 ppb	0.014*
Methane	4.97 ppm	10	-0.35 ppb	0.251

*****Significant with $p < 0.05$

None of the tested compounds exhibited interference greater than ±5 ppb equivalent NO when compared to a nitrogen control.

Two compounds (ethanol and methanethiol) showed a statistically significant ($p<0.05$) response at the test concentrations; however, the mean response from both of these compounds was less than 1 ppb.

Clinical Interference Testing (Exogenous Compounds)
The influence of mouthwash containing alcohol, alcohol-free mouthwash, toothpaste, breath mints, throat lozenges, carbonated beverage with caffeine and caffeine-free carbonated beverage on eNO was assessed in a clinical study. Twelve healthy adult subjects (9 males and 3 females; mean age 36 ± 11.8 yrs; range 20-62 yrs) participated in the clinical study. The eNO measurements for adults ranged from 5 ppb to 99 ppb. The endpoint was the difference in eNO before as compared to one hour after exposure to each compound. None of the tested exogenous compounds were found to interfere (at ± 5 ppb) with the eNO measurement at one hour post-exposure.

In pediatric subjects, the influence of alcohol-free mouthwash, toothpaste, breath mints, and caffeine-free carbonated beverage on eNO was assessed in a clinical study. Seven healthy children (6 males and 1 female; ages 5-17 yrs) participated in the clinical study. The eNO measurements for children ranged from 3 ppb to 27 ppb. The endpoint was the difference in eNO before as compared to one hour after exposure to each compound. None of the exogenous compounds tested were found to interfere (at ± 5 ppb) with the eNO measurement at one hour post-exposure.

f. Temperature:
Testing is to be conducted at an ambient temperature between 15 – 34 º C.

g. Assay cutoff:
Not Applicable

2. Comparison studies:
   a. Method comparison with predicate device:
   See clinical studies below.
   b. Matrix comparison:
   Not applicable.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable.
   b. Clinical specificity:
   Not applicable.

   c. Other clinical supportive data (when a. and b. are not applicable):
   Monitoring the effect of anti-inflammatory therapy in patients with asthma
   A multi-center study enrolled 73 steroid naïve subjects at 12 sites with uncontrolled asthma and elevated exhaled nitric oxide (eNO) to assess the effect of corticosteroid therapy on eNO. The demographic data for this study are presented below. Testing was performed by 45 trained technicians, including: nurses, respiratory therapists, medical assistants, nurse aides, nurse practitioners and asthma educators. Using the Apieron INSIGHT eNO System, eNO was measured at baseline and after 2 weeks of corticosteroid therapy (follow-up). At study entry, the adult asthmatics all had eNO levels ≥ 30 ppb and the children asthmatics all had eNO levels ≥ 25 ppb. The objective of the study was to show that a change in eNO after therapy was related to a change in asthma symptoms indicating a therapeutic effect of corticosteroid treatment.
   Subject Demographics

N		73
Mean Age		25.5 +/- 14.3
Sex	Males	67.12% (42)
	Females	32.88% (24)
Age Groups	≥18	52% (38)
	<18	48% (35)
Race	Caucasian	64% (47)
	African American	13% (10)
	Asian	4% (3)
	Hispanic	7% (5)
	Other	11% (8)

In the study population (n=73) eNO decreased significantly ($p<0.0001$) in all subjects combined (-48 ± 25%) and in both the adults (-50 ± 26%) and children (-46 ± 25%) Of these, there were 5 subjects who did not show a decrease in eNO following 2 weeks of therapy which represents 7% of the study population. Exhaled nitric oxide values obtained at baseline and follow-up were representative across the measurement range of the Apieron INSIGHT eNO System (10 ppb to 200 ppb). The results are presented in the table below.

Changes in eNO (mean ± SD)

eNO (ppb)	Baseline	Follow-up	Change	% Change	p-value
All subjects (n=73)	73 ± 43	35 ± 25	-38 ± 34	-48 ± 25%	<0.0001
Adults (n=38)	78 ± 49	36 ± 25	-43 ± 40	-50 ± 26%	<0.0001
Children (n=35)	66 ± 36	35 ± 26	-32 ± 25	-46 ± 25%	<0.0001

In the entire study population, a greater decrease in eNO was associated with a greater improvement in asthma symptoms over time ($p<0.039$) following corticosteroid therapy. Higher eNO levels at baseline were significantly associated with greater improvement at follow-up in the spirometry measurement pre-bronchodilator forced expiratory volume in 1 second (FEV1). The relationship between In(eNO) and FEV1 in all subjects (n=73) were analyzed and resulted in a coefficient of 0.2376 with a p-value of 0.040 with 95% confidence interval (0.0113 – 0.4638). The results are presented in the table below.

eNO / Symptoms / FEV1 Comparison Table

Measurement Variables	% Change Visit 1 to Visit 2
eNO	-48%
Symptoms	-36%
FEV1	+14%

A method comparison was conducted using Exhaled Nitric Oxide measurements taken on both the Apieron INSIGHT eNO System and the NIOX System. A study was conducted on 82 non-randomized asthmatic subjects at a single site comparing eNO measurements taken with both the Apieron INSIGHT eNO System and Aerocrine's NIOX System. The demographic data for this study are presented below. A total of 58 adults (ages ≥18 years) and 20 children (ages <18 years) completed the study resulting in n=78 subjects eligible for analysis. Each subject performed two breath maneuvers on each system. The testing was performed by 3 trained technicians. Results were analyzed to evaluate the performance of the Apieron INSIGHT eNO System as it compares to the NIOX System in a clinical setting.

Subject Demographics

N		82
Mean Age		35 +/- 19
Sex	Males	56% (46)
	Females	44% (36)
Age Groups	≥18	72% (59)
	<18	28% (23)
Race	Caucasian	87% (71)
	African American	2% (2)
	Asian	5% (4)
	Hispanic	2% (2)
	Other	4% (3)

Agreement: Regression analysis performed on the first measurement from each system. The linear regression equation of Y = 0.95X + 1.3 ppb with an R^2 of 0.95 between the Apieron INSIGHT eNO System and the NIOX System was obtained. The values ranged from I0 to 197 ppb.

The mean difference between the Apieron INSIGHT eNO System and the NIOX System was -1.14 ppb. The mean absolute difference between the Apieron INSIGHT eNO System and the NIOX System was 5.86 ppb.

Clinical Agreement: Using a clinically relevant decision point (30 ppb), an analysis was performed that compared the results of measurements taken on the Apieron INSIGHT eNO System as they compared to the NIOX System results from the same subject. The positive percent agreement was (51/51) 100% and negative percent agreement was (25/27) 93%.The results of the evaluated performance characteristics are found in the table below.

Performance of the Apieron INSIGHT eNO System vs. the NIOX System

Parameter	Analysis	Results [95% CI]
Agreement	Ordinary Least Squares Regression	$R^2 = 0.95$
	Slope	0.95 [0.90 to 1.00]
	Intercept	1.3 ppb [-1.9 to 4.5]
Bias	Mean Difference	-1.14 ppb [-3.08 to 0.80]
Precision	Mean Absolute Difference	5.86 ppb
INSIGHT Repeatability	Within-Subject Standard Deviation	3.96 ppb [2.95 to 4.98]
Clinical Agreement @ 30 ppb	Positive Percent Agreement	100%
	Negative Percent Agreement	93%

4. Clinical cut-off**:**
   Not applicable.

5. Expected values/Reference range:
   The labeling includes the information from American Thoracic Society (ATS) and the European Respiratory Society ERS as follows: "Guidelines for measuring exhaled nitric oxide (eNO) were established in 2005 through the recommendation of the American Thoracic Society (ATS) and the European Respiratory Society ERS as studies have demonstrated that a number of factors can affect eNO levels. Reference eNO values for healthy children and adults without asthma, obtained using the standard exhalation rate of 50 ml/s, range from 1.6 to 28.8 ppb. Exhaled nitric oxide levels are higher in patients with asthma (25 to 80 ppb), are increased during an asthma exacerbation and are reduced with corticosteroid therapy. Exhaled nitric oxide is also reported to show a dose-dependent response to corticosteroid treatment and the response of eNO to corticosteroids has clinical utility."

N. Instrument Name:

Apieron INSIGHT eNO System

O. System Descriptions:

1. Modes of Operation:
   The Apieron INSIGHT eNO System measures eNO via an optical biosensor that undergoes a change in its optical absorbance when binding with nitric oxide. The change in optical properties is measured with a ratiometric, two-channel optical system that measures the change in light absorbance through the Biosensor, which is contained inside the INSIGHT Sensor. The breath sampling system of the INSIGHT Monitor follows the flow rate and exhalation pressure recommended by the 2005 American Thoracic Society Recommendations.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this product:

   Yes X or No _____

3. Specimen Identification:

   There is no mechanism to identify the specimen

4. Specimen Sampling and Handling:

   The user obtains a breath sample by exhaling into the device.

5. Calibration:

Each sensor is calibrated at the factory and requires no calibration by the user. The monitor electronics are calibrated at the factory and are designed to adjust automatically to minor changes. If there is drift out of range, an error message appears on the screen. The system will not take eNO measurements while the monitor is out of calibration range.

The chemistry of the sensor is completely reversible (i.e., when exposed to an NO-free environment the sensor returns to its pre-exposed state). The response of each Sensor Cartridge to NO is characterized, stored with the Sensor Cartridge, and used to calculate the exhaled nitric oxide (eNO) concentration during a patient test.

6. Quality Control:

The Apieron INSIGHT eNO System is designed to include a QC system into the design and manufacturing process of the Monitor and supplies as follows:

• Daily Field System QC Test with controlled test gases at 0 (±6 ppb) and 150 ppb (±30 ppb)
• Continuous on-board parameter checking
• Breath maneuver monitoring
• Factory calibration of sensor cartridges.

The Field QC Test is designed to detect system malfunctions that could result in significant measurement inaccuracy. The 'false positive' rate for the Field QC Test is estimated at approximately 1%. The daily Field QC Test measures the response of the Apieron INSIGHT eNO System to 0 ppb NO blank and 150 ppb NO gas samples and automatically determines if the response is within acceptable operating parameters. During the breath maneuver, exhalation pressure and flow are continuously checked to ensure compliance with the American Thoracic Society 2005 recommendations. During every test, key operating parameters in the pneumatic, temperature control, and optical systems are automatically checked by the Monitor to ensure proper operation.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.