Offline exhaled nitric oxide in children: chemiluminescence vs. electrochemical devices

Laura Gochicoa Rangel[1], Wilmer Madrid[1], Carlos Gúzman Valderrabano[1], Luis Torre Bouscoulet[2], David Martínez Briseño [1], and David Ramirez San Juan[2]

[1]Instituto Nacional de Enfermedades Respiratorias
[2]INFIRE

August 12, 2020

Abstract

Background: Exhaled nitric oxide (eNO) is a noninvasive marker of airway inflammation that can be measured by the "online" or "offline" technique. There are few articles that measure "offline" eNO in children at tidal volume and to our knowledge there is no article that compares the concordance and correlation between 2 different technologies measured offline at tidal volume. Objective: is to report the concordance and correlation of the eNO results obtained with a chemiluminescence vs. an electrochemical device by the "offline" technique at tidal volume. Methods: A cross-sectional, observational and prospective study was conducted in the National Institute of Respiratory Diseases, Mexico City. Healthy children and those with any lung disease between 1 and 11 years of age were included. The exhaled air sample was obtained at tidal volume by attaching a mask with a connection to a Mylar® bag. Results: 36 children were studied. The average (±SD) age of the study population was 6 ± 2.6 years; 25% of subjects included were healthy, and the rest had lung disease. The concordance correlation coefficient between the two measuring devices was 0.98 (p<0.001), with an average difference of 1.46 ± 3.5 ppb and 95% limits of agreement from -5.3 ppb to 8.3 ppb. The linear regression model equation for the estimation of eNO was eNO,cl = (eNO,eq· 1.0718) – 0.1343, (r2 = 0.97) Conclusion: The measurement of eNO by the "offline" method at tidal volume can be analyzed by electrochemical devices, and the results are interchangeable with those analyzed by chemiluminescence technology.

Key message

The diagnosis of asthma in children < 5 years old is difficult because symptoms are common in other lung diseases. Additionally, in this group of patients, pulmonary function tests are difficult to perform correctly considering that they have difficulties in following the instructions to maintain the necessary constant flow for the correct measurements; therefore, the best way to measure FeNO is the collection of the exhaled air by breathing at tidal volume.

Key words: Nitric oxide; preschooler; children, infants, offline tidal breathing, exhaled nitric oxide

Introduction

Since the description of nitric oxide (NO) as an endothelial relaxation molecule and the demonstration of the presence of NO in exhaled air, the measurement of this compound has been used to determine the degree of airway eosinophilic inflammation. [1-3] According to different guidelines for the management of asthma, the measurement of NO is considered a tool for the diagnosis and treatment of patients with this disease.[4-6] In 2005, the American Thoracic Society and the European Respiratory Society (ATS/ERS) made recommendations for the "online" and "offline" measurements of exhaled nitric oxide. The most standardized technique is the online method, in which the individual should exhale with a flow of 50 mL/s while the device performs the measurement in real time. In addition, the "offline" method is described as an alternative to the "online" method because in this technique, NO is collected in a bag using one of the following procedures: 1) with a single exhalation maintaining a flow between 50 and 500 mL/s;[7] 2) breathing during constant flow against a pressure of 5 cmH_2O; or 3) breathing at tidal volume without the control of flow.[7]

Unfortunately, the diagnosis of asthma in children < 5 years old is difficult because symptoms are common to those of other lung diseases.[6] Additionally, in this group of patients, pulmonary function tests are difficult to perform correctly considering that they have difficulties in following the instructions to maintain the necessary constant flow for the correct measurements; therefore, the best way to measure FeNO is the collection of the exhaled air by breathing at tidal volume.[6,8] The standard way to measure FeNO with the "offline" method at tidal volume is chemiluminescence technology. However, equipment with this technology is expensive and difficult to access. Nonetheless, there are other devices with electrochemical technology that are portable and easy to access, but they are designed for "online" measurements.[9]

We hypothesize that the results of NO measurements collected at tidal volume can be the same whether analyzed in chemiluminescence or electrochemical devices. The objective of this study is to report the concordance and correlation of the results obtained with a chemiluminescence vs. an electrochemical device by the "offline" technique collected at tidal volume in a group of both healthy children and children with lung disease.

Material and Methods

A cross-sectional, observational and prospective study was conducted in the Department of Respiratory Physiology of the National Institute of Respiratory Diseases (INER) in Mexico City and was approved by the Research and Bioethical Committee with number C14-19. Children between the ages of 1 and 11 and of both sexes were invited to participate. They were recruited by a direct invitation from the institution outpatient clinic and by relatives of hospitalized patients. The individuals could be healthy or have any pulmonary disease. However, children with facial malformations that were not able to use the mask; those who had chest, abdominal, oral, or facial pain; those with altered consciousness; and oxygen-dependent individuals were excluded from study. Finally, those who fulfilled the inclusion criteria but could not tolerate breathing through the mask at tidal volume were also eliminated from the analysis.

A respiratory health questionnaire was administered, and anthropometric measurements were taken, including weight in kilograms by a digital portable scale (Seca, model 813 and 232, Hamburg, Germany) and height in centimeters by a mechanical stadiometer (Seca, model 206 and 232, Hamburg, Germany). Subsequently, the exhaled air sample for the evaluation of offline eNO at tidal volume was taken according to the recommendations of the ATS/ERS 2005 guidelines.[7] The purpose and procedure of the test were explained to the patient/family member, and if they agreed to allow the child to participate, we proceeded with their signature in the consent letter.

Equipment Calibration

The equipment used for the study was a Sievers® 280i nitric oxide analyzer (General Electrics, Boulder, Colorado, USA), which is a high-sensitivity detector that measures exhaled nitric oxide based on a gas-phase chemiluminescence ($eNO_{,cl}$) reaction between nitric oxide and ozone. The equipment has a vacuum pump that continuously extracts gas from the analyzer at a constant pressure, which provides a vacuum of ˜ 200 ml/min. The limit for the measurement of nitric oxide in the gas phase is ˜ 0.5 parts per billion (ppb). The sensitivity is <1 ppb, range is <1 - 500,000 ppb, response time is 67 msec, and gas repeatability is 5%.

The other equipment was the electrochemical ($eNO_{,ql}$) NO-Breath® (Bedfont, Harrietsham, England), which is based on an electrochemical sensor technology that reacts to the presence of nitric oxide. The advantages are as follows: it is portable, the detection range is 5-300 ppb, the repeatability is ± 5 ppb of a measured value [?] 50 ppb and +- 10%, of measured value [?] 50 ppb,, and the response is given in <10 seconds.

It is important to mention that the chemiluminescence equipment was calibrated daily by 2 methods: 1) By zero gas calibration (NO <1 ppb), which is achieved by placing a zero gas filter (which works by obtaining NO from the environmental air through a $KMnO_4$-activated carbon filter; this process generates oxidation to

NO2, which is absorbed by activated carbon) into the sampling line connection that goes to the equipment and uncovering the distal end to allow the flow. The filter is left connected for approximately 5 minutes so that the sampling line is washed of environmental air. Once the filter is placed, the device automatically calibrates to zero by measuring ppm at 0 and making a ppb adjustment with the previous day calibration. 2) Calibration was also performed with a known gas concentration. For this procedure, a tank that contains a gas between 10-100 ppb (usually 45 ppb of NO in balance with N2) is connected to the sampling line where the analyzer provides a flow of more than 200 ml/min (generally at 300 ml/min given by the regulator). After these steps, the flow is left for approximately 5 minutes.

To ensure repeatability, 2 or 3 calibrations should be performed. In fact, the response factor measured in ppm should be within a range of 0.09768 to 0.1465, and the response factor in ppb should be within a range of 0.1953-0.293.

By contrast, electrochemical equipment is usually already calibrated according to the manufacturer's specifications, and only verification that the environmental module containing the equipment reads less than 5 ppb is performed. Therefore, it was verified that the environmental reading in both instruments was less than 5 ppb before each measurement.

Maneuver to obtain the sample

While the patient was seated with their back upright and their head slightly raised, a mask with a unidirectional inhalation and exhalation valve, connected to a filter and pressure measurement system, was placed (Fig 1). At a tidal volume, patients were asked to breathe through the mask. During the maneuver, We did not control the flow but we controlled the pressure during breathing to be above 5 cmH2O, in children that did not collaborate we collected the sample at least 2 cmH20 of pressure. After 3 respiratory cycles were taken to wash the dead space of the system, the valve was changed to obtain the sample in the 1.5-liter Mylar(r) collection bag (Sievers Instruments, Boulder, Colorado, USA). We decided to stop the process once the patient had performed 5 breaths at a tidal volume or when the bag was filled at least half of its capacity. In the end, samples were analyzed in less than 48 hours following the ATS/ERS 2005 guidelines.[7]

Equipment measurements

The analysis of the samples was carried out on both devices randomly. In regard to the chemiluminescence equipment, the sample was taken according to the manufacturer's recommendations: the Mylar(r) bag was connected to the sampling line (which has a vacuum pressure that makes a plateau with the value of NO during 5 to 10 seconds), and, subsequently, the measured values of NO were given. In the case of the electrochemical equipment, the Mylar(r) bag was connected through a filter to the device (Fig 2). Then, putting the device in ambient mode, the suction pump was automatically activated, and after approximately 20 seconds, the analysis of the sample was performed, giving the final result. Importantly, two measurements were taken in each device, ensuring a variability of less than 10%, and the final result was formulated by the average of both measurements reported in ppb. All the samples were analyzed in less than 120 minutes.

Statistical analysis

Descriptive statistics were expressed as the mean +- standard deviation (SD) or median and interquartile range according to the distribution of variables. Comparisons between both eNO devices were made using the paired Student's t-test. The analysis of correlation was performed using the Spearman correlation coefficient (rsp), and the concordance correlation coefficient (CCC) was used to assess the degree of agreement between $eNO_{,cl}$ and $eNO_{,eq}$. Considering two measurements for each subject, an intraclass correlation coefficient of 0.8, contrasted with an expected one of 0.5, resulted in an alpha error of 0.05, a power of 0.8, and a 20% loss; therefore, the required sample size was calculated to be 35 tests. A p value <0.05 was defined to be significant, and a commercial statistical package was used to analyze the data (Stata V.13).

Results

A total of 36 children were studied, of which 24 (67%) were male participants. The average (+-SD) age of the study population was 6 +- 2.6 years old, with a minimum age of 1 year and a maximum of 11 years; 9 (25%) of the subjects included were healthy, and the rest had a history of lung disease. The most frequent lung diseases were bronchopulmonary dysplasia (12; 33.3%), asthma (11; 30.6%), suspected primary ciliary dyskinesia (3; 8.3%) and bronchiectasis not associated with cystic fibrosis (1; 2.8%). Table 1 shows the general characteristics of the population.

The CCC between the two measuring devices was 0.98, with an average difference of 1.46 +- 3.5 ppb and 95% limits of agreement from -5.3 ppb to 8.3 ppb. Fig 3 shows the Bland-Altman graphs: panel A illustrates the agreement between the two measuring devices, and panel B elucidates the Spearman's correlation coefficient: $r = 0.98$ ($p < 0.001$). The linear regression model equation for the estimation of eNO was $eNO_{,cl} = (eNO_{,eq} * 1.0718) - 0.1343$, ($r2 = 0.97$).

Discussion

This study demonstrates that the results obtained for eNO by chemiluminescence and electrochemical equipment, using the "offline" method and the tidal volume technique, are indeed interchangeable. We also described the technique to analyze the samples using electrochemical equipment, which is more accessible and inexpensive.

Asthma is the most frequent chronic disease in children, and its prevalence is higher in children under the age of 5 years.[10] The expression of the disease in these cases depends on the interaction between genetic and environmental factors, and it differs from person to person according to their phenotype, genotype and endotype.[4,6] Asthma continues to cause high morbidity and mortality around the world, mainly due to the lack of suspicion and the difficulty in making the diagnosis. As a matter of fact, this difficulty is more evident in preschoolers due to their lack of cooperation to undergo a respiratory function test, and as a consequence, the diagnosis is based mainly on predictive indices in which pulmonary function and the degree of inflammation in the respiratory tract are not taken into account.[3]

In 2001, the ATS/ERS published the clinical guidelines for the interpretation of eNO measurements in adults and children, establishing cutoff points to define when inhaled steroid treatment should be administered, as well as when it should be increased or decreased.[5] However, these guidelines and other studies considered only patients older than 4 years of age, and the method used was the "online" method, where the individual must maintain a flow of 50 mL/s.[11-16] In 2005, the ATS/ERS made some recommendations for the standardization of measurements of eNO with the "online" and "offline" methods, and the "offline" measurement of eNO at tidal volume is mentioned as an alternative to the other methods described.[7]

By the end of the 1990s, some studies were published concerning toddlers and preschoolers with wheezing in which eNO was measured with the "offline" method at tidal volume using chemiluminescence equipment, and it was shown that preschoolers presented higher values of eNO during wheezing episodes (14.1 +- 1.8 ppb), which improved after steroid treatment, reducing eNO levels up to 52%.[17]Additionally, it was shown that exhaled air could be easily collected from these children, making it possible to differentiate among asthmatic patients, those with chronic non-asthmatic cough, and healthy children.[18,8] As a result, these authors concluded that the "offline" method is easy to perform and that it can be executed on an outpatient basis.[18,17] Similar to these results, we were able to demonstrate that the sample was easy to obtain without causing any discomfort to the children.

Moreover, there are several studies that have reported reference values with the "offline" method at different respiratory flows and even in children less than 2 years of age.[9,12,13,16,19-22]Even so, the main limitation to reproduce this technique is the availability of chemiluminescence equipment, which is expensive and difficult to access for most centers.[23] The present study demonstrates that the measurements of eNO obtained by the "offline" technique through breathing at tidal volume can be analyzed by electrochemical devices, and the results are interchangeable with those analyzed using the gold-standard chemiluminescence equipment. This interchangeability is proven due to high concordance and correlation coefficients ($CCC = 0.98$, $rsp = 0.97$, $p < 0.001$) and is important for the pediatric population because the "offline" method can be used in uncooperative patients. Equally important, using portable equipment that exists in the market could expand the spectrum not only in the clinical approach to patients but also as a good alternative for conducting epidemiological studies on a larger scale. Additionally, similar to other authors,[9,17,20,22] in this study, we used the bags recommended by the chemiluminescence equipment manufacturer (Mylar(r) bag), which are the typical bags made of a stretched polyester film (biaxial-oriented polyethylene terephthalate) that come with a connector and a unidirectional valve that facilitate obtaining the sample. In addition, the material of these bags is the same as that used for balloons at parties, and we consider that if it is used with another type of adapter, the cost for every measure could decrease considerably.[7]

Although our main objective in this study was to demonstrated the agreement between the two devices for exhaled nitric oxide measured "offline" at tidal volume, these same results have to be corroborated throughout the patient spectrum to be used in clinical practice (patients with diseases or conditions that decrease nitric oxide and patients with very high values).

Conclusion

The measurement of eNO by the "offline" method can be analyzed in an electrochemical device, and the results are interchangeable with those obtained in chemiluminescence equipment.

Bibliography

1. Palmer R., Ferrige A. and Moncada S., 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* , 327(6122), pp.524-526 [Accessed 20 October 2019].

2. Gustafsson L., Leone A., Persson M., Wiklund N. and Moncada S., 1991. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochemical and Biophysical Research Communications* , 181(2), pp.852-857 [Accessed 1 November 2019].

3. Cantu-Gonzalez G., Fernandez-Figueroa F., Gochicoa-Rangel L., Miguel-Reyes J., Vargas-Dominguez C., Mejia-Alfaro R. and Torre-Bouscoulet L., 2019. Fraccion exhalada de oxido nitrico. Recomendaciones clinicas y procedimiento. *NCT Neumologia y Cirugia de Torax* , 78(S2), pp.212-219.

4. Global Initiative for Asthma - GINA. 2020. *Global Initiative For Asthma - Global Initiative For Asthma - GINA* . [online] Available at: <https://ginasthma.org>.

5. Dweik R., Boggs P., Erzurum S., Irvin C., Leigh M., Lundberg J., Olin A., Plummer A. and Taylor D., 2011. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FeNO) for Clinical Applications. *American Journal of Respiratory and Critical Care Medicine* , [online] 184(5), pp.602-615. Available at: <https://www.atsjournals.org/doi/full/10.1164/rccm.9120-11ST> [Accessed 20 August 2019].

6. Larenas-Linnemann D., Salas-Hernandez J., Vazquez-Garcia J., Ortiz-Aldana F., Fernandez-Vega M., Del Rio-Navarro B., Cano-Salas M., Luna-Pech J., Ortega-Martell J., Romero-Lombard J., Lopez-Estrada E., Villaverde-Rosas J., Mayorga-Butron J., Vargas-Becerra M., Bedolla-Barajas M., Rodriguez-Perez N., Aguilar-Aranda A., Jimenez-Gonzalez C., Garcia-Bolanos C., Garrido-Galindo C., Mendoza-Hernandez D., Mendoza-Lopez E., Lopez-Perez G., Wakida-Kuzonoki G., Ruiz-Gutierrez H., Leon-Molina H., Martinez-De la Lanza H., Stone-Aguilar H., Gomez-Vera J., Olvera-Salinas J., Oyoqui-Flores J., Galvez-Romero J., Lozano-Saenz J., Salgado-Gama J., Jimenez-Chobillon M., Garcia-Aviles, M., Guinto-Balanzar M., Medina-Avalos M., Camargo-Angeles R., Garcia-Torrentera R., Toral-Freyre S., Montes-Narvaez G., Solorio-Gomez H., Rosas-Pena J., Romero-Tapia S., Reyes-Herrera A., Cuevas-Schacht F., Esquer-Flores J., Sacre-Hazouri J., Compean-Martinez L., Medina-Sanchez P., Garza-Salinas S., Baez-Loyola C., Romero-Alvarado I., Miguel-Reyes, J., Huerta-Espinosa L., Correa-Flores M. and Castro-Martinez R., 2017. Guia Mexicana del Asma: GUIMA 2017. *Revista Alergia Mexico* , 64, p.11.

7. *American Journal of Respiratory and Critical Care Medicine* , 2005. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. 171(8), pp.912-930.

8. Canady R., Platts-Mills T., Murphy A., Johannesen R. and Gaston B., 1999. Vital Capacity Reservoir and Online Measurement of Childhood Nitrosopnea Are Linearly Related. *American Journal of Respiratory and Critical Care Medicine* , 159(1), pp.311-314.

9. Van Der Heijden H., Brouwer M., Hoekstra F., Van Der Pol P. and Merkus P., 2013. Reference values of exhaled nitric oxide in healthy children 1-5 years using off-line tidal breathing. *Pediatric Pulmonology* , 49(3), pp.291-295.

10. Lai C., Beasley R., Crane J., Foliaki S., Shah J. and Weiland, S., 2009. Global variation in the prevalence and severity of asthma symptoms: Phase Three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* , 64(6), pp.476-483.

11. Buchvald F., Baraldi E., Carraro S., Gaston B., De Jongste J., Pijnenburg M., Silkoff, P. and Bisgaard, H., 2005. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *Journal of Allergy and Clinical Immunology* , 115(6), pp.1130-1136.

12. Baraldi E., Azzolin N., Cracco A. and Zacchello F., 1999. Reference values of exhaled nitric oxide for healthy children 6-15 years old. *Pediatric Pulmonology* , 27(1), pp.54-58.

13. Wong G., Liu E., Leung T., Yung E., Ko F., Hui D., Fok T. and Lai C., 2005. High levels and gender difference of exhaled nitric oxide in Chinese schoolchildren. *Clinical & Experimental Allergy* , 35(7), pp.889-893.

14. Olivieri M., Talamini G., Corradi M., Perbellini L., Mutti A., Tantucci C. and Malerba M., 2006. Reference values for exhaled nitric oxide (reveno) study. *Respiratory Research* , 7(1).

15. Travers J., Marsh S., Aldington S., Williams M., Shirtcliffe P., Pritchard A., Weatherall M. and Beasley R., 2007. Reference Ranges for Exhaled Nitric Oxide Derived from a Random Community Survey of Adults. *American Journal of Respiratory and Critical Care Medicine* , 176(3), pp.238-242.

16. Menou A., Babeanu D., Paruit H., Ordureau A., Guillard S. and Chambellan A., 2017. Normal values of offline exhaled and nasal nitric oxide in healthy children and teens using chemiluminescence. *Journal of Breath Research* , 11(3), p.036008.

17. Baraldi E., Dario C., Ongaro R., Scollo M., Azzolin N., Panza N., Paganini N. and Aacchello F, 1999. Exhaled Nitric Oxide Concentrations during Treatment of Wheezing Exacerbation in Infants and Young Children. *American Journal of Respiratory and Critical Care Medicine* , 159(4), pp.1284-1288.

18. Avital A., Uwyyed K., Berkman N., Godfrey S., Bar-Yishay E. and Springer C., 2001. Exhaled nitric oxide and asthma in young children. *Pediatric Pulmonology* , 32(4), pp.308-313.

19. Gabriele C., Nieuwhof E., Van Der Wiel E., Hofhuis W., Moll H., Merkus P. and De Jongste J., 2006. Exhaled Nitric Oxide Differentiates Airway Diseases in the First Two Years of Life. *Pediatric Research* , 60(4), pp.461-465.

20. Meyts I., Proesman, M., Gerven V., Hoppenbrouwers K. and De Boeck K., 2003. Tidal off-line exhaled nitric oxide measurements in a pre-school population. *European Journal of Pediatrics* , 162(7-8), pp.506-510.

21. Wildhaber J. Hall G. and Stick S., 1999. Measurements of Exhaled Nitric Oxide with the Single-Breath Technique and Positive Expiratory Pressure in Infants. *American Journal of Respiratory and Critical Care Medicine* , 159(1), pp.74-78.

22. Daniel P., Klug B. and Valerius N., 2007. Exhaled nitric oxide in healthy young children during tidal breathing through a facemask. *Pediatric Allergy and Immunology* , 18(1), pp.42-46.

23. Kim S., Moon J., Kwak H., Kim S., Park D., Kim J., Kim T., Sohn J., Shin D., Park S. and Yoon H., 2012. Comparison of two exhaled nitric oxide analyzers: The NIOX MINO hand-held electrochemical analyzer and the NOA280i stationary chemiluminescence analyzer. *Respirology* , 17(5), pp.830-834.

Table 1. General characteristics of participants. Data are presented as median (percentile 25-75)

Age, years
Weight, kg
Height, cm
$eNO_{,cl}$ (ppb)
$eNO_{,eq}$ (ppb)

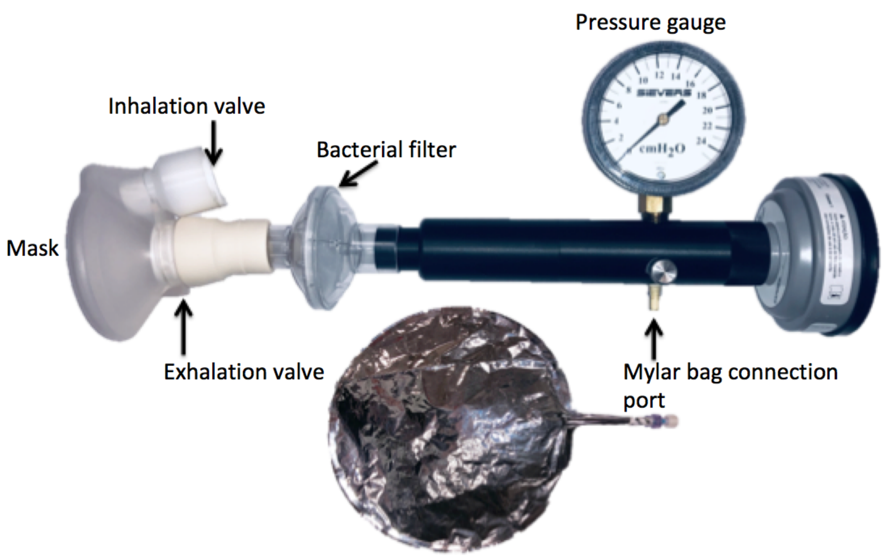
$eNO_{,cl}$ exhaled nitric oxide measured by the chemiluminescence device; $eNO_{,eq}$ exhaled nitric oxide measured by the electro

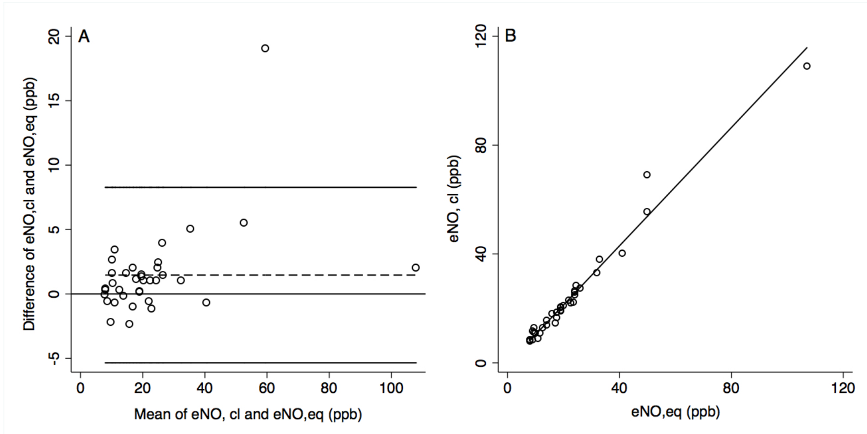
Figure legends

Fig 1 Mask with a unidirectional inhalation and an exhalation valve connected to a filter and a pressure measurement system.

Fig 2 (A) Mylar® bag connected to the sampling line. (B) Sampling line connected to the filter and to the electrochemical device.

Fig 3 (A) Bland-Altman graph showing the agreement between both devices: the Sievers® ($eNO_{,cl}$) and the Bedfont® ($eNO_{,eq}$). CCC=0.98. (B) Spearman correlation between both devices, rsp=0.98.




A
Difference of eNO,cl and eNO,eq (ppb)
Mean of eNO, cl and eNO,eq (ppb)
B
eNO, cl (ppb)
eNO,eq (ppb)