

ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005

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HISTORY OF THIS DOCUMENT

The field of exhaled nitric oxide (NO) and nasal NO measurement has developed remarkably over the last 15 years, with more than 1,000 publications in the field. The understanding is increasing of how the measurement of inflammation may contribute to the management of lung disease, and the incorporation of this measurement into clinical practice, has commenced.

Despite numerous publications, the field of exhaled and nasal NO measurement has been characterized from its onset by a marked variation in published fractional exhaled NO (F_{ENO}) levels in health and disease, much of which is still attributable to variable techniques of measurement.

A taskforce of the European Respiratory Society (ERS) published European recommendations in 1997 (1), and the American Thoracic Society (ATS) published a statement in 1999, which is updated in this document (2). Recently, a separate statement on pediatric exhaled NO measurement was approved by the boards of the ATS and ERS (3).

The recommendations in the ATS document from 1999 were updated by international investigators in the field of exhaled and nasal NO, at a workshop sponsored by the ATS (December 2002, Toronto, Canada). Also attending as committee members to provide expertise in the technical recommendations were scientists from NO analyzer manufacturers: Aerocrine, Eco Physics, Eco Medics, Ionics Instruments, and Ekips Technologies. The workshop reviewed the following areas: adult online exhaled NO measurement, offline exhaled NO measurement, pediatric online exhaled NO measurement, nasal NO measure-

This document supersedes the 1999 ATS statement on exhaled and nasal NO measurement with updates that were proposed at an ATS workshop held in Toronto, Ontario, Canada, in 2002.

Members of the Ad Hoc Statement Committee have disclosed any direct commercial associations (financial relationships or legal obligations) related to the preparation of this statement. For this document, the information is available in the online conflict of interest section, which is accessible from this issue's table of contents at www.atsjournals.org

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ment, and technical recommendations. New areas were identified for mention in the revised document—namely, technologic advances, NO in ventilated patients, and physiologic models of NO excretion from the lung.

The standardization of techniques has opened the way for the collection of comparable data in numerous centers in normal subjects and in those with disease states. As in the previous ATS statement from 1999, the document is divided into a background section, which deals with aspects common to all sections, followed by sections dealing with adult online/offline measurement, pediatric measurement, nasal NO measurement, new developments, and technical aspects of NO analysis. Wherever possible, the recommendations are based on published material, including abstracts, as referenced; in the absence of clear data, the document relied on the experience of participants in the workshop. Where aspects concerning exhaled NO measurement are undetermined, this has been clearly stated in the text.

Although these recommendations encourage uniformity of measurement techniques in future studies, this document does not intend to invalidate previous or ongoing studies that have used other techniques. Wherever practical, investigators are encouraged to include the recommended method in addition to the measurement techniques with which they are familiar, so that the knowledge concerning the recommended methods will increase. This will allow future modifications to these recommendations to be made on scientific grounds.

SECTION 1: BACKGROUND

Assessment of Airway Inflammation

Airway inflammation is a central process in asthma and other lung diseases (4), but monitoring inflammation is not included in current asthma guidelines despite recent evidence that this may improve control (5, 6). The direct sampling of airway cells and mediators can be achieved by invasive techniques, such as bronchoscopy with lavage and biopsy, or by the analysis of induced sputum. However, exhaled breath contains volatile mediators, such as NO (7), carbon monoxide (CO) (8–10), ethane and pentane (11–13), and nonvolatile substances in the liquid phase of exhalate, termed breath condensate (e.g., hydrogen peroxide) (14–17). The noninvasive measurement of exhaled mediators makes them ideally suited for the serial monitoring of patients.

Exhaled NO

The presence of endogenous NO in exhaled breath of animals and humans was first described in 1991 (7). Soon after, several publications reported high fractional concentrations of orally exhaled NO (F_{ENO}) in subjects with asthma as compared with unaffected subjects (18–23) and a fall after treatment with corticosteroids (24–26). Similar findings have been described in the pediatric age group (27–30). Atopy seems to be a significant factor associated with a raised exhaled NO, with or without asthma (31, 32). In patients with chronic obstructive pulmonary disease, F_{ENO} has been reported to be high in exacerbations compared with stable patients (33), and one report suggested that F_{ENO} falls after inhaled steroids in stable chronic obstructive pulmonary disease (34). Other diseases associated with high F_{ENO} include the following: bronchiectasis in one study (35), but not in other reports (36, 37); viral respiratory tract infections (38, 39); systemic lupus erythematosus (40); liver cirrhosis (41–44); acute lung allograft rejection (45); and post-transplant bronchiolitis obliterans (46). Low levels of F_{ENO} have been described in cystic fibrosis (27, 47–50), HIV infection (51), and pulmonary hypertension (52–54). Exhaled NO has been shown to correlate with other outcomes in mild asthma (e.g., induced sputum eosinophilia [55] and bronchial reactivity [56]) in non-steroid-treated subjects.

In general, exhaled NO has not correlated with lung function parameters in asthma.

It is increasingly recognized that the measurement of exhaled mediators in general and NO in particular constitutes a novel way to monitor separate aspects of diseases, such as asthma, chronic obstructive pulmonary disease, and interstitial lung diseases, that are not assessed by other means, such as lung function. The additional information about the pathogenesis of these diseases and their modification by therapy justifies further research, development of new technologies, and broadening of the scope of mediators that are measured in different diseases.

In asthma, where exhaled NO promises to be very useful, it has been proposed to use this marker to diagnose asthma (57) to monitor the response to antiinflammatory medications (25), to verify adherence to therapy (58), and to predict upcoming asthma exacerbations (59–61). It is also proposed that adjusting antiinflammatory medications guided by the monitoring of non-invasive markers, such as sputum eosinophils and exhaled NO, could improve overall asthma control.

Nasal NO

Nasal NO concentrations are very high relative to the lower respiratory tract in humans (23, 62), with the highest levels reported in the paranasal sinuses (63, 64). Nasal NO may have physiologic roles, such as preserving sinus sterility (65) and modulating ciliary motility (66). Nasal NO concentration has been proposed as a surrogate marker of nasal inflammation in allergic rhinitis (67–71), but results are inconsistent (72). In contrast, subjects with primary ciliary dyskinesia and cystic fibrosis have extremely low nasal NO (73, 74), and in the former, nasal NO may become a useful clinical test (75).

SECTION 2: RECOMMENDATIONS FOR A STANDARDIZED PROCEDURE FOR THE ONLINE MEASUREMENT OF EXHALED NO IN ADULTS

Requirements for the Clinical Use of Exhaled NO Measurements

Exhaled NO measurements have primarily been performed in the research setting. In Europe, clinical testing was commenced in the late 1990s, and the U.S. Food and Drug Administration approved the first NO analyzer (Aerocrine AB, Stockholm, Sweden) for clinical monitoring of antiinflammatory therapy in asthma in 2003. Online measurement refers to F_{ENO} testing with a real-time display of NO breath profiles, whereas offline testing refers to collection of exhalate into suitable receptacles for delayed analysis (76). The use of F_{ENO} measurement as a clinical tool requires the adoption of a standardized measurement technique followed by collection of reference data in all age groups. To date, several authors have published exhaled NO values in healthy subjects, but variable measurement techniques and methods reduce the utility of the data (77–87). The achievement of a consensus as detailed in this document should enable multicenter collaborative studies using standardized techniques to obtain normal ranges for exhaled NO. Ideally, there should be interinstitutional agreement of mean F_{ENO} within 10% for each age group. To establish exhaled NO as a clinical tool, guidelines should be developed. The evidence that supports moving F_{ENO} from “bench to bedside” was recently reviewed (88).

Standardization of Exhaled NO Terminology and Units

In general, the term “exhaled” is preferred to “expired,” as this facilitates literature searches. Nomenclature and symbols used in exhaled NO literature have been variable. The following guide-

lines have been formulated to bring this field in line with standard physiologic nomenclature.

Online measurement. The $F_{E_{NO}}$ is expressed in parts per billion, which is equivalent to nanoliters per liter. The exhalation flow rate used for a particular test can be expressed as a subscript of the flow rate in liters/second: for example, $F_{E_{NO,0.05}}$. Exhaled is denoted by E, and inspired by I, in qualification of the test results: for example, $F_{E_{NO}}$ and $F_{I_{NO}}$.

NO output represents the rate of NO exhaled, is denoted by \dot{V}_{NO} , and calculated from the product of NO concentration in nanoliters per liter and expiratory flow rate in liters per minute corrected to BTPS, as follows: \dot{V}_{NO} (nl/minute) = NO (nl/L) \times airflow rate (L/minute).

Terms such as NO release, NO excretion, NO secretion, and NO production are to be discouraged when referring to \dot{V}_{NO} . \dot{V}_{NO} may be particularly useful in situations where NO excretion is measured over longer periods of time and during varying flow situations (e.g., tidal breathing).

Offline NO collection. $F_{E_{NO}}$ refers to the fractional NO concentration in exhalate from a vital capacity collection. If the exhalation is at a constant flow rate, this should be added as a subscript (e.g., $F_{E_{NO,0.35}}$) with the flow rate in liters/second.

General Principles Regarding Exhaled NO Measurement

Source of exhaled NO. Current thinking is that NO is formed in both the upper and lower respiratory tract (73, 89–95) and diffuses into the lumen by gaseous diffusion down a concentration gradient, thus conditioning exhaled gas with NO (96–98). There may be significant contribution from the oropharynx (91, 99). Alveolar NO is probably very low because of avid uptake by hemoglobin in pulmonary capillary blood (92, 100). Although gastric NO levels are very high (101), this does not appear to contaminate exhaled NO, probably because of closed upper and lower esophageal sphincters. Recently, several investigators have described models of NO exchange. Their work is summarized in SECTION 6 (NEW DEVELOPMENTS IN EXHALED NO).

Nasal NO contamination. Nasal NO can accumulate to high concentrations relative to the lower respiratory tract (63, 64, 73, 102–105). The issue of the relative contribution of nasal NO to exhaled NO has been addressed in many publications (23, 62, 63, 102, 104, 106–109). Accordingly, techniques that aim to sample lower respiratory NO should prevent contamination of the sample with nasal NO (106, 107).

Ambient NO. Because environmental NO can reach high levels relative to those in exhaled breath, standardized techniques must prevent the contamination of biological samples with ambient NO. The ways of achieving this are method-specific and are discussed in each section. Notwithstanding which technique is used, ambient NO at the time of each test should be recorded.

Expiratory flow rate dependence. Exhaled NO concentrations from the lower respiratory tract exhibit significant expiratory flow rate dependence (107, 110), and the same is true for the nasal cavity (111, 112). This flow dependence is characteristic of a diffusion-based process for NO transfer from airway wall to lumen (see model description in SECTION 6) and can be simply understood by faster flows minimizing the transit time of alveolar gas in the airway, and thereby reducing the amount of NO transferred. The rate of NO output, however, is greater at higher flow rates analogous to respiratory heat loss (107). In view of this flow dependency, the use of constant expiratory flow rates is emphasized in standardized techniques.

Breath hold. Breath-hold results in NO accumulation in the nasal cavity, lower airway, and probably in the oropharynx, which causes NO peaks in the exhalation profiles of NO versus time (63, 73, 90, 113–115). For this reason, the use of breath hold is discouraged in the standardized techniques described in

this document, although it has been used in more experimental methods for exhaled NO measurement (115).

Non-Disease-related Patient Factors Influencing Exhaled NO Values

The following sections are pertinent to online and offline exhaled NO measurement in both adults and children. Some of the factors mentioned later may affect nasal NO levels and will be discussed separately in SECTION 5.

Age/sex. In adults, there is no consistent relationship between exhaled NO level and age, but it has been reported that, in children, $F_{E_{NO}}$ increases with age (82, 85, 86, 116). In adults, there are conflicting reports regarding the effects of sex (87, 117–119), menstrual cycle (117, 120–122), and pregnancy (120, 123), so these patient characteristics should be recorded at the time of measurement.

Respiratory maneuvers. Because spirometric maneuvers have been shown to transiently reduce exhaled NO levels (124–127), it is recommended that NO analysis be performed before spirometry. The same stipulation applies to other taxing respiratory maneuvers, unless these can be shown not to influence exhaled NO. The $F_{E_{NO}}$ maneuver itself and body plethysmography do not appear to affect plateau exhaled NO levels (125, 127).

Airway caliber. It has been demonstrated that $F_{E_{NO}}$ levels may vary with the degree of airway obstruction or after bronchodilation (26, 125, 126, 128–134), 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.

Food and beverages. Patients should refrain from eating and drinking before NO analysis. An increase in $F_{E_{NO}}$ has been found after the ingestion of nitrate or nitrate-containing foods, such as lettuce (with a maximum effect 2 hours after ingestion) (99, 135), and drinking of water and ingestion of caffeine may lead to transiently altered $F_{E_{NO}}$ levels (136, 137). It is possible that a mouthwash may reduce the effect of nitrate-containing foods (99). 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 $F_{E_{NO}}$ in patients with asthma and healthy subjects (138, 139).

Circadian rhythm. Although $F_{E_{NO}}$ levels are higher in nocturnal asthma, there was no circadian rhythm in two studies (140, 141), but another study did report a circadian pattern (142), 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.

Smoking. Chronically reduced levels of $F_{E_{NO}}$ have been demonstrated in cigarette smokers in addition to acute effects immediately after cigarette smoking (22, 143–145). Despite the depressant effect of smoking, smokers with asthma still have a raised $F_{E_{NO}}$ (146). Subjects should not smoke in the hour before measurements, and short- and long-term active and passive smoking history should be recorded.

Infection. Upper and lower respiratory tract viral infections may lead to increased levels of exhaled NO in asthma (38, 39). Therefore $F_{E_{NO}}$ measurements should be deferred until recovery if possible or the infection should be recorded in the chart. HIV infection is associated with reduction in exhaled NO (51).

Other factors. The manipulation of physiologic parameters has been shown to affect $F_{E_{NO}}$. Changing pulmonary blood flow has no effect in humans (147), but hypoxia decreases exhaled NO (92, 148), and this may occur in subjects at high altitude, particularly those prone to high-altitude pulmonary edema (149–151). The application of positive end-expiratory pressure has

been shown to increase $F_{E_{NO}}$ in animals (152–154), but airway pressure in humans does not affect exhaled NO plateau levels (107, 110, 136) according to most reports, although one study suggests the opposite (155). Many studies have examined the effect of exercise on $F_{E_{NO}}$ and nasal NO (24, 52, 109, 114, 156–162). During exercise, according to one report, $F_{E_{NO}}$ and nasal $F_{E_{NO}}$ fall, whereas NO output increases, and this effect may last up to 1 hour (163). Others have reported that $F_{E_{NO}}$ remains stable after exercise (164). It would seem prudent to avoid strenuous exercise for 1 hour before the measurement.

Medications and exhaled NO. The potential effect of drugs on NO cannot be excluded, and so all current medication and time administered should be recorded. Exhaled NO falls after treatment with inhaled or oral corticosteroids in subjects with asthma (20, 21, 25, 29, 165–167) and after inhaled NO synthase inhibitors (168). Leukotriene-axis modifiers also reduce $F_{E_{NO}}$ (169, 170). NO donor drugs (171) and oral, inhaled, and intravenous L-arginine (172, 173) increase $F_{E_{NO}}$ and nasal $F_{E_{NO}}$ (174). Even if a certain medication does not affect NO production, it might affect the apparent level of NO through other mechanisms, such as changes in airway caliber (125, 128–130, 133).

Recommended Technique for Online Adult Exhaled NO Measurement

Online methods refer to exhalations where the expirate is continuously sampled by the NO analyzer, and the resultant NO profile versus time or exhaled volume, together with other exhalation variables (e.g., airway flow rate and/or pressure), is captured and displayed in real time. This enables the test administrator to monitor the exhalation to ensure conformation to the required flow rate and pressure parameters and the achievement of an adequate NO plateau. Suboptimal exhalations can be immediately identified and discarded. The online method requires more stringent analyzer specifications (see SECTION 7).

Inspired gas source. Although there is evidence that ambient NO levels do not affect the single-breath plateau levels of exhaled NO (107), the use of NO free air (containing < 5 ppb) for inhalation is preferable. This is because when inhaling gas containing high levels of NO, an early NO peak is observed in the exhaled NO profile versus time, probably because of the ambient NO present in the instrument and patient dead space (107). This peak takes time to wash out, which increases the time elapsed until a plateau is reached, with resulting need for prolongation of the exhalation. In all studies, it is advisable to record ambient levels of NO.

Inhalation phase. The patient should be seated comfortably, with the mouthpiece at the proper height and position. A nose clip should not be used, because this may allow nasal NO to accumulate and promote leakage of this NO via the posterior nasopharynx. However, if a subject cannot avoid nasal inspiration (seen as an early expiratory peak) or nasal exhalation, a nose clip may be used. The patient inserts a mouthpiece and inhales over 2 to 3 seconds through the mouth to total lung capacity (TLC), or near TLC if TLC is difficult, and then exhales immediately, because breath holding may affect $F_{E_{NO}}$. TLC is recommended because this is the most constant point in the respiratory cycle and patients accustomed to spirometry are familiar with inhaling to this volume.

Exhalation phase. Two factors are critical in ensuring reproducible and standardized measurements of lower respiratory tract exhaled NO: (1) exclusion of nasal NO and (2) standardization of exhalation flow rate.

The exclusion of nasal NO is important in view of the high nasal NO levels relative to the lower respiratory tract (23, 63, 73, 102–104, 111). This nasal NO can enter the oral expiratory air via the posterior nasopharynx. Closure of the velopharyngeal

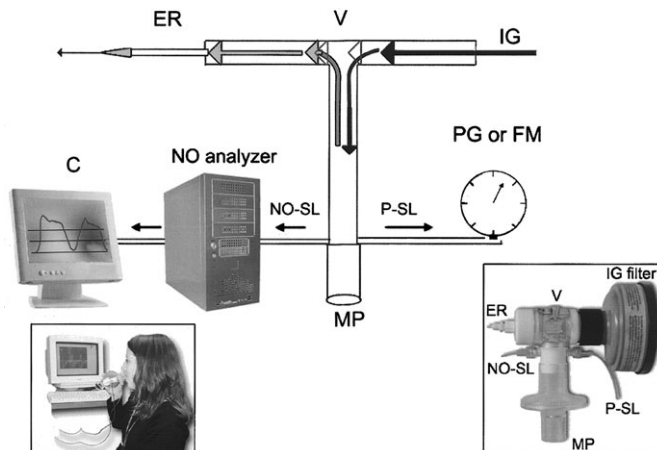


Figure 1. Diagram of a configuration for the breathing circuit used in the restricted-breath technique (see text for explanation of technique). *Right bottom insert* showing a restricted-breath configuration courtesy of Ionics Instruments (Boulder, CO). C = computer; ER = expiratory resistance; FM = flow meter; IG = inspired gas; MP = mouthpiece; NO-SL = nitric oxide sampling line; PG = pressure gauge; P-SL = pressure sampling line; V = three-way valve.

aperture during exhalation is one way to minimize the nasal NO leakage. This can be achieved by exhaling against an expiratory resistance with subjects asked to maintain a positive mouthpiece pressure (106, 107). It is common practice to display pressure or expiratory flow rate to the subject, who is requested to maintain these within a certain range. The procedure causes velum closure as validated by nasal CO_2 measurement (107) and nasal argon insufflation studies (106). The resultant mouthpiece pressure should be at least 5 cm H_2O to ensure velum closure and exclude contamination of the expirate with nasal NO. However, a pressure above 20 cm H_2O should be avoided because this may be uncomfortable for patients to maintain. One apparatus for the restricted breath apparatus technique is shown in Figure 1. In addition to the restricted exhalation method, another acceptable technique is continuous nasal aspiration (175, 176), which reduces nasal NO leakage either by removing NO and thus preventing the accumulation of nasal NO or by itself causing velopharyngeal closure. A third published method used the inflation of a balloon in the posterior nasopharynx (63, 102). However, the latter two methods are less practical for routine clinical use.

Exhaled NO plateau values vary considerably with exhalation flow rate because of variation of airway NO diffusion with transit time in the airway (107, 110, 177). Therefore, standardization of exhalation flow rate is critical for obtaining reproducible measurements. Low flow rates (< 0.1 L/second) amplify the measured NO concentrations and are believed to aid in discriminating among subjects (98, 178). In addition, the resulting higher $F_{E_{NO}}$ values avoid measurement close to the detection limits of current NO analyzers. On the negative side, lower flow rates result in longer exhalation times to reach an NO plateau (107), and the prolongation of the exhalation may be uncomfortable for some patients with severe disease. Low flow rates are also associated with a decreased NO output (107, 110).

Recommended Expiratory Flow Rate

A flow rate of 0.05 L/second (BTPS) was chosen for the 1999 ATS statement, on the basis of knowledge at that time, to be a reasonable compromise between measurement sensitivity and patient comfort. There are now reports that this flow rate is

acceptable to children and adults, and reproducible (76, 85, 86, 107, 126, 179–185). However, FE_{NO} measurement can be performed at higher or lower flow rates if this is desirable in certain situations, and the use of different flow rates allows the derivation of flow-independent parameters (see SECTION 6). Exhaled NO measurements at both low- and high-exhalation flow rates can distinguish subjects with asthma from normal subjects with a high sensitivity and specificity (76). In all cases, however, the expiration flow should be clearly recorded and reported in any publications.

A constant expiratory flow rate can be achieved in different ways. One commonly used method to achieve a constant expiratory flow rate is by displaying a target mouthpiece pressure or flow rate to the subject (e.g., using a gauge or computer display), while the subject exhales via a fixed expiratory resistance (107, 110). The constant pressure and therefore flow rate is achieved by biofeedback of pressure or flow rate parameters to the subject who maintains these parameters within specified limits. It may be preferable to target flow rate rather than pressure because this is the main determinant of FE_{NO} levels. Other ways of controlling exhalation flow rate, which may be useful in young children and subjects who cannot easily control flow rate, include the use of dynamic resistors (186), operator-controlled flow rate (183), mass flow controllers (187), Starling resistors (95), and servo-controlled devices (188).

Exhalation pressure does not affect NO plateau measurements according to some (107, 110, 136), although one report suggests the opposite (155). Until this matter is clarified, individual investigators may select pressures between 5 and 20 cm of H_2O , with the appropriate expiratory resistance to achieve the desired flow rate.

With biofeedback of expiratory pressure or flow rate, most subjects are able to maintain low flow rates that vary little from the desired target. In general, an exhalation is deemed adequate if the mean exhalation flow rate is 0.05 L/second ($\pm 10\%$) during the time of the NO plateau generation, and instantaneous flow rate is not less than 0.045 L/second or greater than 0.055 L/second at any time during the exhalation. If it is not possible to keep within these values, the results should still be recorded and the failure to achieve this flow rate criterion noted in the record.

Interpretation of NO Single-Breath Profiles

Constant flow rate exhalations, however achieved, result in a single-breath NO profile (exhaled NO vs. time plot) that consists of a washout phase followed by an NO plateau, which is usually reproducible and flat (Figure 2A) but may slope up or down (Figure 2B). The washout phase is sometimes followed by an early NO peak before the plateau (Figure 2C). This peak may be derived from the nasal cavity if the subject inhales through the nose or if the velum is open initially as the exhalation starts. In addition, NO in the inhaled air source and NO accumulating in the oral cavity and lower airway, if the subject pauses at TLC, may also generate an early peak. Early peaks are ignored, and only NO plateaus are interpreted.

Plateau Definition

The duration of exhalation must be sufficient (at least 4 seconds for children < 12 years and > 6 seconds for children > 12 years and adults). This corresponds to an exhaled volume of at least 0.3 L in adults at an exhalation flow rate of 0.05 L/second to allow the airway compartment to be washed out and a reasonable plateau achieved. In general, patients can exhale comfortably up to 10 seconds, and this may be necessary for the achievement of a stable NO plateau. The plateau concentration in NO should be evaluated over a 3-second (0.15 L) window of the exhalation profile according to the following guidelines: The plateau can

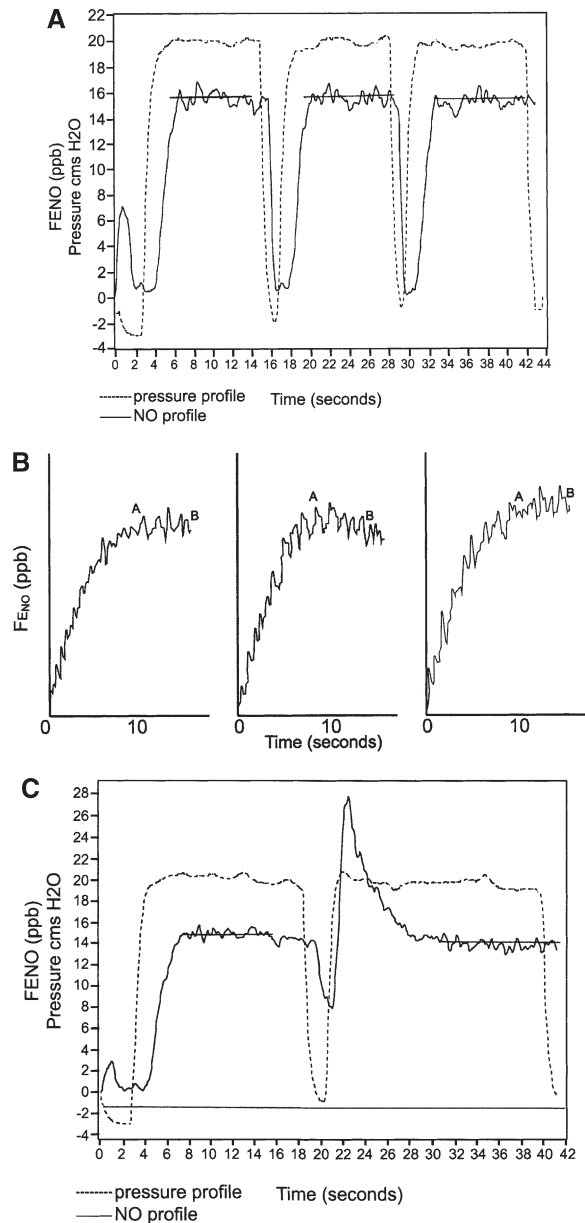


Figure 2. (A) NO concentration (ppb) and airway opening pressure versus time for three separate exhalations in the same subject, showing reproducible profiles and plateaus. (B) Schematic diagram of exhaled NO and pressure profiles showing horizontal, up- and down-sloping NO plateaus with the start (A) and the end (B) of an NO plateau as defined in the text. (C) NO concentration and airway opening pressure versus time. The left-hand trace was performed with an oral inspiration of gas containing < 5 ppb NO. The right-hand trace shows an early peak, which is generated by asking the subject to inhale nasally. This fills the conducting airways with nasal NO, which is then exhaled. Inhaling ambient NO can produce a similar peak. The NO plateau is essentially unaltered once the early peak has washed out.

be considered to begin at Point A and end at Point B (see Figure 2B). The plateau can be flat, positive sloping, or negative sloping. However, the magnitude of the slope should be minimized using the following criteria: Points A and B should be chosen to define the first 3-second window in the exhaled concentration profile such that the absolute magnitude of A–B is less than 10%. In addition, no point within the 3-second window

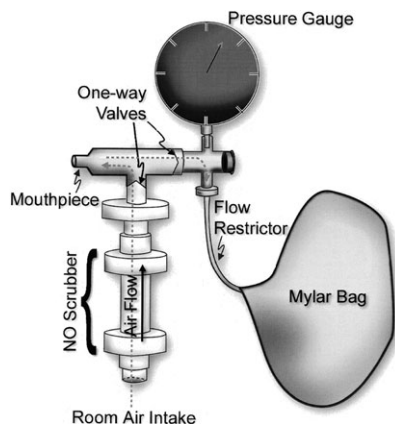


Figure 3. A diagram of a simple apparatus for the collection of exhaled gas for offline NO measurements. The inspired gas is scrubbed of NO and expiratory flow rate and pressure are controlled by means of an in-line pressure gauge and flow resistor. See text for details.

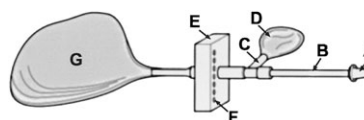


Figure 4. An example of an offline collection device with a side reservoir for the separation of dead-space gas. Exhaled gas flows via a mouth-

piece (A) through a ridged tube (B) fitted with a low-resistance side port. A small balloon attached to this side port (C) fills with gas derived from the anatomic dead space. As this balloon reaches capacity, subsequent gas is diverted through a flow transducer (E) with display (F) and in-line flow resistor into the main collection balloon (G). Reproduced by permission from Reference 181.

should deviate from either the value at Point A or B by more than 10%. The plateau concentration, $F_{E_{NO}}$, is then defined as the mean concentration over this 3-second window. Once a 3-second plateau is achieved, there is no reason to continue the exhalation.

For $F_{E_{NO}}$ values of less than 10 ppb, the 10% plateau criterion may be difficult to fulfill because of instrument variability and patient flow rate control variability; in such cases, a change of 1 ppb or less between Points A and B is an acceptable plateau. Online electronic analysis of NO profiles allows automatic identification of a valid NO plateau according to these criteria. At the recommended flow rate of 0.05 L/second, plateaus are generally flat and clearly discernible (Figure 2A).

Repeated, reproducible exhalations should be performed to obtain at least two NO plateau values that agree within 10% of each other. Exhaled NO is then calculated as the mean of two values (Figure 2A). For certain purposes, it may be better to achieve three reproducible $F_{E_{NO}}$ values (e.g., measurement of flow-independent NO exchange parameters at multiple flow rates). At least 30 seconds of relaxed tidal breathing off the NO measurement circuit should elapse between exhalations to allow subjects to rest. Care must be taken not to exhaust the patient when repeated exhalations are unsatisfactory.

SECTION 3: RECOMMENDATIONS FOR THE OFFLINE MEASUREMENT OF $F_{E_{NO}}$

Background to Offline Exhaled NO Collection

NO measurements can be made from exhaled gas collected in a reservoir and subsequently analyzed for NO concentrations. Since the publication of the previous ATS statement, several groups have confirmed that the measurements obtained from offline determinations closely parallel those made from online measurements when obtained with an identical flow rate (76, 85, 86, 185, 189, 190). In patients with asthma, changes in $F_{E_{NO}}$ after withdrawal of antiinflammatory inhaled corticosteroids, measured with the technique previously stipulated, parallel those changes observed in other markers of airway inflammation, including sputum eosinophils and the concentration of methacholine required to cause a 20% fall in the forced expired volume in 1 second (PC_{20}). It is noted that, in the hands of some investigators, offline and online measurements are not identical even when matched for flow rate, and thus cannot be considered interchangeable (76, 189). Other investigators, however, have reported good agreement between online and offline techniques (191).

Advantages and Disadvantages of Offline Collection

In contrast to online techniques, offline collection offers the following: (1) the potential for expirate collection at sites remote

from the analyzer (which may include the hospital ward, clinic, workplace, school, and remote laboratory); (2) independence from analyzer response times; and (3) more efficient use of the analyzer because gas may be collected from several patients simultaneously and less analyzer time per patient is required.

Potential problems with offline methods include the following: (1) contamination from gas not derived from the lower airway; (2) error introduced by the sample storage; and (3) reduced capacity to allow for instantaneous feedback and assessment of technique. Current recommendations regarding the standardized expirate collection and storage for the offline measurement of $F_{E_{NO}}$ are presented in the following section.

Procedures for Collection of an Offline Exhaled NO Sample

Expiratory maneuver. For ambulatory patients, it is recommended that gas for $F_{E_{NO}}$ be collected by asking the subject to inhale orally to TLC and then immediately perform a slow vital capacity maneuver against an expiratory resistance into an appropriate reservoir without a breath hold. The reservoir is sealed and subsequently analyzed for $F_{E_{NO}}$. Details pertaining to this maneuver and to the equipment needed for this measurement are presented later in this section, and one simple apparatus for the offline collection is shown in Figure 3.

Inspired gas. Because high concentrations of NO in the inspired gas (> 20 ppb) (6–8) significantly increase offline exhaled NO measurements, it is recommended that the inspired gas NO concentration be controlled below this level. This can be accomplished by placing an NO-scrubbing filter in the inspiratory limb of the collection apparatus (Figure 3) or by allowing the subject to inhale from a reservoir of NO-free gas. The exhaled gas sample should be collected immediately after the subject has inhaled at least two tidal breaths of NO-free air.

Partitioning the expiratory sample. Some investigators have partitioned the expirate, discarding the initial 150 to 200 ml with spring-loaded or manually activated valves or low-compliance reservoirs placed in series with the main collection vessel (Figure 4); the remainder of the exhaled gas is reserved for NO analysis. By physically removing dead-space gas, this technique may reduce contamination of the lower airway sample by NO derived from ambient or nasal sources. Using partitioned offline techniques, some investigators have reported $F_{E_{NO}}$ values closer to flow-matched online values as compared with those obtained with nonpartitioned offline techniques (181, 190, 192). However, the appropriate volume of gas to be discarded is not known and likely varies from subject to subject. Furthermore, nonpartitioned techniques have been demonstrated to correlate well with online values and provide identical sensitivity and specificity regarding detection of disease (76, 181, 189, 190). Thus, partitioned samples, which are more complex to perform and do not offer any diagnostic advantage, can, however, be considered a valid alternative to the standard nonpartitioned collection.

The Recommended Offline Exhaled NO Expiration Flow Rate

It is now well established that the concentration of NO recovered in the expirate decreases with collections at higher flow rates (107, 110). The recovered $F_{E_{NO}}$ likely represents the dynamic equilibrium between alveolar NO, the production of NO (or its release) in the airway, and the diffusion of NO into the gas flowing through the airway (93–95, 193). Thus, it is critical that, during offline collections, the exhalation flow rate is known, held constant during the exhalation, and standardized between serial measurements. Although lower flow rates produce larger numeric differences between individuals with asthma and healthy individuals as compared with measurements made at higher flow rates, the intersubject variability also increases at lower flow rates and thus the ability to discriminate health from disease appears to be robust at all flow rates between 50 and 500 ml/second (76, 181). Because $F_{E_{NO}}$ is less sensitive to flow rate variation above 250 ml/second and faster flow rates allow most subjects to expire their vital capacity over less than 20 seconds, an expiratory flow rate of 0.350 L/second is suggested with flow rate not falling below 0.315 L/second and not exceeding 0.385 L/second at all times during the exhalation for collection of the entire vital capacity. However, it is recognized that alternate flow rates may produce values that appropriately vary with disease states (182, 189, 192). Thus, as long as flow rate is controlled, measured, and standardized across measurements, other flow rates may be considered valid for offline NO collection in adults.

Expiratory Pressure and Nasal NO Contamination

Because concentrations of NO in the nasopharynx may be high relative to those recovered in the lower airway, the nasopharyngeal gas must be excluded from the expirate collected using an offline technique. The maintenance of an oropharyngeal pressure of at least 5 cm H_2O during the exhalation minimizes nasal contamination of the sample by ensuring closure of the soft palate (106, 107). This oropharyngeal pressure elevation can be achieved by placing a flow resistance in the neck of the reservoir bag (20, 124). When such a resistance is used, airway opening pressure should be monitored during the exhalation (i.e., as shown in Figures 3 and 4). By ensuring a constant pressure during the exhalation, a constant flow rate will also be achieved. Nose clips are not required as long as measures are taken to ensure that (1) the soft palate is closed and gas from the nasopharynx is isolated from the collected sample and (2) the inspiration and expiration occurs through the mouth.

Storage Vessel

The reservoir used to collect the exhaled gas sample must be nonreactive and relatively impermeable to NO. Suitable materials include Tedlar and Mylar (20, 108, 124). It has been demonstrated that although new Mylar balloons allow for sample stability for at least 48 hours, such reservoirs that have been repeatedly used produce stable values over 8 to 12 hours (20, 194, 195). In this regard, it is possible that vessel integrity may further deteriorate over time. Unless the investigator can demonstrate that the specific, individual vessels used have the capacity to allow for stable NO values (both regarding loss of sample to the atmosphere and to contamination by ambient NO) over a greater time period, it is recommended that offline samples be assayed within 12 hours from the time of collection.

Practical Guide for Offline NO Collection Apparatus

Two “recipes” to help investigators construct their own offline apparatus are provided in the online supplement to this document entitled “A Practical Guide for Building an Offline Collection Device for Nitric Oxide Measurement.”

SECTION 4: RECOMMENDATIONS FOR ONLINE AND OFFLINE EXHALED NO IN CHILDREN

In 2002, a joint ERS/ATS taskforce on exhaled and nasal NO measurement in children published a statement providing practical recommendations and suggestions for measurement of $F_{E_{NO}}$ concentration in pediatric patients, particularly in young children who cannot actively cooperate. The need for developing practical, noninvasive markers to reflect asthmatic airway inflammation is universally recognized, and this is especially important in young children who wheeze, in whom other objective diagnostic tools, such as spirometry or induced sputum, cannot be easily applied in clinical practice. Several methods can be used to measure $F_{E_{NO}}$, and the choice depends on the age and cooperation of the child. For children who cannot perform the standardized single-breath on- or offline exhalations, alternative methods are now available. The reader may refer to the original report for more extensive details on NO measurements in children (3), and this statement is available on the ATS Web site (<http://www.thoracic.org/statements>).

Exhaled NO Measurement in Children Older than 4 to 5 Years

Single-breath online measurement. The single-breath online measurement method is considered the preferred method in all children who can cooperate. The child should be comfortably seated and breathe quietly for approximately 5 minutes to acclimatize. The child inhales to near TLC, and immediately exhales at a constant flow rate of 50 ml/second, until an NO plateau of at least 2 seconds can be identified during an exhalation of at least 4 seconds. Inspired gas should contain low NO concentration (< 5 ppb). The expiratory pressure should be maintained between 5 and 20 cm H_2O to close the velum. Repeated exhalations (2–3 that agree within 10%, or 2 within 5%) are performed with at least 30-second intervals, and mean $F_{E_{NO}}$ is recorded (2). A target expiratory flow rate of 50 ml/second⁻¹ has a good reproducibility and discriminatory power in children (179, 186). However, single-breath online measurement may be difficult in preschool children who often have difficulty in maintaining flow rate or pressure within the required limits (183, 196, 197). Audiovisual aids to facilitate inhalation to TLC and expiratory flow control, and the use of dynamic flow restrictors that allow the child to exhale with a variable mouth pressure while maintaining a constant expiratory flow rate, may overcome these problems (183). Dynamic flow restrictors are simple manual or mechanical devices that vary their resistance depending on the blowing pressure, and their use is recommended in children.

Offline method with constant flow rate. The offline method with constant flow rate is the offline method of choice. The child blows air through a mouthpiece into a receptacle made of material that does not react with NO, while nasal contamination is prevented by closing the velum by exhaling against at least 5 cm H_2O oral pressure (196, 197). The gas collection receptacle may be a Mylar or Tedlar balloon. The size of the balloon is not critical, but should preferably be similar or larger than the subject's vital capacity. Wearing a nose clip and breath holding are not recommended, because they potentially affect nasal contamination (194). NO concentrations in balloons can be stable for several hours (194), and the measurements can take place at a distant site (e.g., school or home). Flow rate standardization improves the reproducibility of offline methodology, with results similar to those of online constant flow rate methods in schoolchildren and adolescents (85). A major improvement in offline collection can be expected from the incorporation of a dynamic flow restrictor in the collection system (185), which has proved highly feasible in children as young as 4 years. When using dynamic flow restrictors, there seems no justification to use

higher flow rates with offline collection than with online collection. Therefore, the pediatric measurement taskforce reached a working consensus and proposed flow rates of 50 ml/second⁻¹ for both off- and online collection (3).

Alternative Methods for Preschool Children and Infants

Online measurement of FE_{NO} during spontaneous breathing. This method has been applied in children aged 2 to 5 years (79). FE_{NO} may be measured online during spontaneous breathing while the exhalation flow rate is adjusted by changing the exhalation resistance (79). This allows scrutiny of the breath-to-breath profiles to ensure a stable and reproducible breathing pattern. The child breathes slowly and regularly through a mouthpiece connected to a two-way valve. NO-free air is continuously flushed through the inlet of the valve. Quiet breathing at a normal frequency is attempted. The exhalation flow rate is targeted at 50 ml/second⁻¹ (range, 40–60) by continuously adapting the exhalation resistance manually or by automatic flow rate controllers. The method still requires passive cooperation inasmuch as the child needs to breathe slowly and regularly through a mouthpiece, which is a limiting factor. The use of biofeedback by allowing the child to visualize the tidal breathing pattern may facilitate a slow and regular respiration. Measurements during spontaneous breathing may introduce variability, because there is no control over the lung volume at which the flow rate is measured. NO levels measured during spontaneous breathing may not equate to single-breath online measurements, and separate characterization of this method is required, including description of normal values in healthy children.

Tidal breathing techniques with uncontrolled flow rate. Currently, there is no standardized tidal breathing method to recommend for the clinical setting in infants and young children, and further research is needed to solve some methodologic issues (198). The tidal breathing method in infants is potentially simple and noninvasive, and both on- and offline methods have been applied without the use of sedatives (199–201).

OFFLINE. Exhaled air can be collected via a mouthpiece or a facemask connected to a non-re-breathing valve that allows inspiration of NO-free air from an NO-inert reservoir to avoid contamination by ambient NO. Exhaled breath samples are collected into an NO-inert bag fitted with the expiratory port once a stable breathing pattern is present. The expiratory port of the valve provides an expiratory resistance (201, 202). This resistance will only help to avoid nasal contamination if the mask does not cover the nose. With a fast-response NO analyzer, small samples of exhaled air (e.g., 5 breaths) are sufficient for analysis.

ONLINE. Recently, online tidal breathing techniques for measuring NO and the breathing pattern in unsedated newborns and infants have been described with good reproducibility (200, 203). Infants breathe into a facemask that covers the nose and mouth. NO concentrations should be recorded during phases of quiet tidal breathing only.

Reproducibility is a significant problem with tidal breathing methods as reported by some investigators (198, 204). Because FE_{NO} is flow-dependent, with tidal breathing there will be scatter of data from variation in flow rates. The disadvantage of mixed expiratory air is that it may be contaminated by ambient NO and NO from the upper airway. Although inspiratory NO contamination can be limited by inhalation of NO-free air, there are currently no data on the relative contribution of upper airway NO to the mixed expiratory NO concentration in infants. Until more is known about the contribution of upper airway NO to FE_{NO} levels, it seems sensible to exclude nasal NO by collecting only orally exhaled air. This can be accomplished by using a facemask that covers the mouth alone, with nostrils occluded, or by using a two-compartment facemask (201).

Single-breath technique during forced exhalation. There is limited experience with single-breath methods in infants. A modification of the raised-volume, rapid, thoracoabdominal compression technique, as used for lung function measurement (184), has been used to measure FE_{NO} during a single forced exhalation (187). With the described method, FE_{NO} levels were measured online, and NO plateaus achieved during constant expiratory flow rate were determined for flow rates that varied between 10 and 50 ml/second (187). In addition, a two-compartment facemask was used separating nasal and oral compartments, and flow-independent parameters of FE_{NO} were calculated. This technique can be incorporated into standard protocols that use the raised-volume, rapid, thoracoabdominal compression methodology. On the negative side, sedation is needed, and it is unclear at the present time how this technique compares with the single-breath technique used in older children and adults, or with tidal breathing techniques in the same age group.

SECTION 5: RECOMMENDATIONS FOR THE STANDARDIZED MEASUREMENT OF NASAL NO

Background to Nasal NO Measurement

The supralaryngeal airway can generate NO concentrations several-fold greater than the lower respiratory tract, in the parts-per-million range (23, 62, 64), and the NO concentration is particularly high in the paranasal sinuses (64, 103). The nasal airway is a complex system of communicating cavities (i.e., the nasal cavities, paranasal sinuses, middle ear, and nasopharynx). Each of these areas may contribute to nasal NO output. Measurements of nasal NO output or concentration cannot provide evidence as to the source of the gas (e.g., nasal cavity and/or paranasal sinuses) or the biochemical processes that generate the NO output (205). The nasal cavity has a unique vasculature, which results in variation in nasal cavity volume, and alteration in nasal blood flow and/or volume could theoretically affect nasal NO production and absorption.

The evaluation and comparison of standardized methods for measurement of upper airway NO output are less developed compared with measurements of lower airway NO output, but interest in this area is increasing. For most indications (e.g., allergic rhinitis, cystic fibrosis, sinusitis), nasal NO is still to be considered an interesting research tool, but there is one exception. In patients with primary ciliary dyskinesia, nasal NO is consistently lower by 90 to 98% compared with control subjects (73, 75, 206–211). Furthermore, the use of a nasal NO test in primary ciliary dyskinesia is so promising that it should be recommended as a screening tool for this disorder.

General Considerations of Nasal NO Measurement

The measurement of nasal NO output requires generation of airflow through the nasal cavity (transnasal airflow rate). Flow through the nasal cavities in series is achieved by aspirating or insufflating air via one naris while the velum is closed, so that air circulates from one naris to the other around the posterior nasal septum. Transnasal flow with the nasal cavities in parallel, which mimics natural breathing, can be achieved by exhaling via one or both nasal cavities (nasal exhalation) by aspirating via the mouth with air entrained into both nares during breath holding, or by aspirating from one or both nares with the mouth open during breath holding. Nasal exhalation is the best validated of the methods where a transnasal flow is used (212–214). Typically, the subject first exhales nasally through a tight facemask covering the nose, and nasal FE_{NO} is measured. After this, an oral exhalation is performed, and exhaled FE_{NO} is measured. Nasal NO output is calculated by subtracting oral levels from nasal levels. Caution should be taken to avoid leakage of air

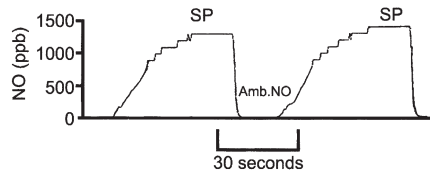


Figure 5. Two reproducible NO profiles versus time from a nasal NO measurement using Method 1 showing a washout phase and a steady NO plateau (SP). In this case, the sampling line of the NO analyzer was used to generate the flow rate (200 ml/minute). Amb = ambient.

around the mask during exhalation. An advantage with this method is that exhalation can be performed at the flow recommended for online orally exhaled NO, which facilitates comparisons between upper and lower airway NO output.

With all methods, a constant transnasal flow rate produces a washout phase followed by the establishment of a steady NO plateau seen in the profile of NO concentration versus time, analogous to that seen in the lower respiratory tract (Figure 5). Nasal NO concentration is inversely related to the transnasal airflow rate (111, 112, 215), as shown in Figure 6, and similar to that seen with exhaled NO (107). However, different flows may have different aerodynamic profiles, resulting in changes in the physics of airflow rate (e.g., laminar vs. turbulent flow) and different pathways of flow through the nasal cavity (216). The aerodynamics of this flow may affect the nasal NO output (217, 218). For all the previously described reasons, any standardized method used should rigorously control transnasal airflow rate.

Nasal NO Output

The product of transnasal flow rate (\dot{V}) and measured NO concentration allows calculation of nasal NO output (nasal \dot{V}_{NO}). Present evidence suggests that nasal \dot{V}_{NO} is relatively constant over a range of transnasal flow rates between 0.25 and 3 L/minute (111, 217–219). There is reasonable agreement, using different measurement techniques, that nasal \dot{V}_{NO} is in the range of 200 to 450 nl/minute in healthy primates (62, 73, 215, 220). At higher flow rates, nasal \dot{V}_{NO} may increase progressively (219, 221).

Terminology

The fractional concentration of nasal NO can be expressed as nasal FE_{NO} if the measurement is obtained by nasal exhalation. If the measurement is obtained by transnasal flow in series, the term *nasal NO* is preferable. Nasal NO output, however, obtained is termed nasal \dot{V}_{NO} . The flow rate can be included with the measurement symbols as a subscript (e.g., nasal $NO_{0.25L}$ or nasal $\dot{V}_{NO_{(0.25L)}}$).

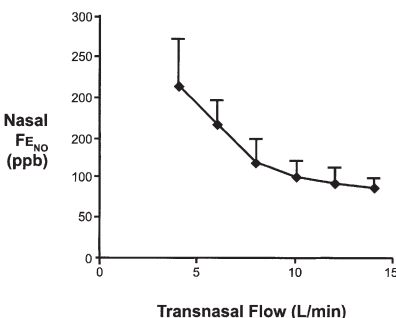


Figure 6. The transnasal flow dependence of nasal NO (112).

The Importance of Velum Closure in Nasal NO Measurement

With transnasal airflow in series (where air enters one naris and exits the other as described previously), velum closure is required to prevent loss of nasal NO via the posterior velopharyngeal aperture or entry of lower respiratory air into the nasal cavity. Velum closure can be achieved in the following ways:

1. Slowly exhaling orally against a resistance (107)
2. Pursed-lip breathing via the mouth (222)
3. Breath holding with velum closed (63)
4. Voluntary elevation of the soft palate by a trained subject (219)

During the nasal NO test, measurement of nasal CO_2 , which should remain low, can verify velum closure.

Recommended Method for Measurement of Nasal NO

Although several methods have been described for nasal NO measurement, the current recommended method is aspiration at constant flow rate from one naris with gas entrained via the other naris (transnasal flow in series). This method is currently the most prevalent and best-validated method (1, 67, 70, 112, 217, 219, 222–226) and samples nasal NO in isolation from the lower respiratory tract. Velum closure is required to prevent leak of nasal NO via the posterior velopharyngeal aperture. Although several methods can be used to close the velum, slow oral exhalation against a resistance of at least 10 cm H_2O has been chosen as the preferred method (215) because this has been shown to reliably close the velum (107). A biofeedback display of airway pressure to the subject facilitates maintenance of a steady exhalation pressure within the desired range. Notwithstanding, any method that has been reliably demonstrated to close the velum is acceptable.

Description of method. Two nasal olives with a central lumen are securely placed in the nares and used to aspirate via one naris and entrain air via the other. These olives should be composed of a nontraumatizing material and be of sufficient diameter and shape to occlude the naris in most subjects. The seated subject inserts a mouthpiece, inhales to TLC, and exhales through an expiratory resistance while targeting a mouth pressure of 10 cm H_2O to close the velum. While this exhalation is proceeding, air is aspirated at constant flow rate via one olive by a suction pump. A side port just distal to the aspirating olive samples gas for the NO analysis. An acceptable alternative to aspiration of air via a suction pump is insufflation of air from a constant flow, positive-pressure source (e.g., medical-grade compressed air) into one nostril and sampling of nasal NO as air exits the other nostril (112). This insufflation method may be desirable when nasal cavity obstruction leads to dynamic alar collapse during the aspiration technique. However, insufflation of air under positive pressure may increase the likelihood of leakage of nasal air across the velum, and thus requires confirmation of velum closure (219).

Recommended Transnasal Airflow

A target airflow rate of 0.25 to 3 L/minute is recommended in the measurement of nasal NO output, because this flow rate provides a steady plateau level of NO concentration in most subjects within 20 to 30 seconds. Lower airflow rates will result in higher absolute NO values, which may be an advantage when identifying decreased nasal NO (e.g., in patients with primary ciliary dyskinesia). In addition, the relative influence of ambient NO is less when using lower airflow rates. On the other hand, when using very low airflow rates, a steady plateau can be difficult to establish within a reasonable time. The precise flow rate used

should be recorded with the NO measurement for each subject. In all cases, the transnasal flow rate used should be recorded.

Factors Influencing Nasal NO Values

As with lower respiratory tract NO, the factors which specifically affect nasal NO are not well defined, and the following discussion should serve to alert investigators to their possible influence on results.

Ambient air. Methods that use ambient air as the gas source for the transnasal flow may introduce considerable NO concentrations (up to several hundred ppb) into the nasal cavity. It is conceivable that this extraneous NO may influence nasal physiology, but more important, reduce the gradient for NO diffusion from nasal epithelium to lumen. In an extreme situation, if ambient NO concentrations were greater than nasal mucosal wall concentrations, no net excretion of nasal NO would occur. For these reasons, it is preferable to use a clean air source. In any case, ambient NO should always be recorded at the time of each test and must be taken into account when assessing results.

Circadian change. A circadian effect on nasal NO was found by one study (214) but not another (78). It is reasonable to record the time and to attempt to measure nasal NO at the same time each day when performing serial measurements.

Posture. It would seem advisable to study patients in the seated position, which is the most convenient. In one study, nasal NO was unchanged when assuming the supine posture (227), although this position increases nasal volume (228).

Age. In children, nasal NO does not appear to be age-dependent after the age of 11 years, but for those children younger than 11 years, it may affect NO output (47). In adults, there have been reports that nasal NO is not age-dependent (78, 229).

Sex. There is no effect of sex on nasal NO (78, 229). The effects of menstrual cycle or pregnancy on nasal NO output are unknown, so these characteristics should be noted in the record.

Body size/surface area. NO output, corrected for body surface area, is higher in children younger than 11 years (144), but further studies are required in adults and children. In any case, height and weight should always be reported to allow calculations of NO output/body surface area (\dot{V}_{NO}/m^2).

Exercise. Nasal NO concentration falls during heavy physical exercise (109, 111, 162). It is therefore prudent to refrain from exercise for 1 hour before the measurements.

Local nasal factors affecting nasal NO.

Alterations in local nasal physiology could affect nasal NO, or may be mediated by nasal NO.

NASAL VOLUME. Changes in nasal cavity volume could affect nasal NO by altering NO uptake into nasal blood and modulating the nasal epithelial surface area. Also, the communication of the nasal cavity with the communicating sinuses, which produce NO, could be altered. Evidence concerning the influence of nasal volume on nasal NO is contradictory at present. Nasal NO output was not volume-dependent, provided a true steady-state plateau was achieved, in one study (227), but has been reported to be volume-dependent at low transnasal flows in another (230), possibly because of changes in nasal aerodynamics (217).

NASAL AERODYNAMICS. The physics of airflow through the nasal cavity could alter the sampling of nasal NO. At low flow rates, laminar flow rate may predominate, and certain areas of the cavity may contribute less NO to the sample. Also at low flow rates, the pressure fluxes in the nasal cavity will be less than at high flow rates, possibly reducing the efflux of gas from the paranasal sinuses. Variations in nasal aerodynamics may explain some of the flow dependency of nasal NO output (217). Humming appears to increase nasal NO, most likely by increasing NO influx to the nasal cavity from the paranasal sinuses

(231–234). When measuring nasal NO during humming, the nasal exhalation method is recommended.

Medications and Nasal NO

Medications have been shown to affect NO and should be recorded. Those reported to have an effect on nasal NO include nasal decongestants (56, 216), which decrease nasal NO output by approximately 15% (227, 230). The routine use of decongestants to facilitate nasal NO measurement itself requires further study. Nasal steroids have been reported to have no effect in normal subjects in one study (73), but to reduce nasal NO output after 2 weeks' therapy in normal subjects (104) and subjects with asthma (29) in other reports. Antibiotic therapy had no effect on nasal NO in one study in normal subjects (104), but nasal NO rose after treatment of sinusitis in another (223). Vasodilators (e.g., papaverine) increased nasal NO output (235), whereas histamine had no effect in another study (230). Saline does not appear to affect nasal NO output (227), but lidocaine may have a differential effect on nasal and sinus NO output (205).

NO synthase inhibitors. N(G)-nitro-L-arginine methyl ester (L-NAME) by nasal spray has been reported to have no effect in some studies (64, 103, 230), but to decrease NO output in others (235, 236).

L-arginine. L-arginine is the substrate for NO synthesis. Systemic administration increased nasal NO output by 35% in one study (174), but had no effect when applied by nasal spray in normal subjects (230).

Smoking

A small decrease in nasal NO has been observed in smokers (219).

Nasal NO Perturbation in Disease States

There is a profound decrease in nasal NO in the ciliary dyskinesia syndromes, and nasal NO may become a useful screening test for this disorder as stated previously (74, 75, 207, 237, 238). The findings in allergic rhinitis, however, have been inconsistent, and it is uncertain what role nasal NO will play in the management of this condition (67, 70, 72, 213, 224, 239–244). Nasal NO has been reported to be low in nasal polyposis (245), HIV infection (246), panbronchiolitis (247), and cystic fibrosis (27, 47, 48, 206, 248–251).

SECTION 6: NEW DEVELOPMENTS IN EXHALED NO

Modeling NO Excretion

In an effort to understand the strong dependence of F_{ENO} concentration on exhalation flow rate (107), several recent reports have described NO exchange dynamics using a two-compartment model of the lungs (an airway and an alveolar compartment) (93, 95, 193, 252). The alveolar compartment is described by a single parameter, the steady-state alveolar concentration: $Calv,ss$ or $Falv,ss$ (ppb or mm Hg). The airway compartment is described with two parameters: the airway diffusing capacity (or transfer factor), $DawNO$ (ml NO/s/mm Hg or ml NO/s/ppb), and either the airway wall concentration, $CawNO$ or $FawNO$, or the maximum airway wall flux, $J'awNO$ (product of $DawNO \cdot CawNO$ expressed in ml NO/s). The potential advantage of this approach is twofold. First, a greater level of specificity can be achieved for inflammatory diseases that affect primarily the airways or the alveolar regions of the lungs. Second, the parameters are independent of exhalation flow rate. The flow-independent parameters can be derived by measuring exhaled NO concentration at multiple exhalation flow rates using several different techniques. These approaches have been used to describe NO exchange dy-

namics in healthy subjects as well as in several diseases, including asthma (93, 253–259), allergic alveolitis (254, 260), cystic fibrosis (180), scleroderma (260), allergic rhinitis (255), and chronic obstructive pulmonary disease (255, 261). The flow-independent parameters have recently been measured in infants (187) and in ventilated patients (262). Although promising in principle, this approach remains at an early stage of development, and should therefore be considered a research tool at present.

Measurement of Exhaled NO in Mechanically Ventilated Patients

Exhaled NO measurement in patients who are critically ill and mechanically ventilated provides exciting opportunities and unique challenges. A diagnostic role for $F_{E_{NO}}$ is proposed in the areas of infection, sepsis, acute lung injury, and ischemia reperfusion injury (263, 264). Online (262), offline, and “hybrid” methods have been used in ventilated patients.

Online tidal $F_{E_{NO}}$ in ventilated patients. Online measurements are performed with fast-response chemiluminescent analyzers able to resolve the NO concentration profile within one respiratory cycle by sampling continuously at a constant flow rate while ventilation is standardized over a measurement epoch. Simultaneous recording of CO_2 concentration and flow profile may facilitate data analysis and interpretation. However, it should be recognized that sample-tubing characteristics, distinct lag phase of NO, CO_2 and flow measurements, and variable response times of analyzers create different curve characteristics and time delay of the respective traces. A rapid rise in the inspiratory flow rate and a sharp decrease in end-tidal CO_2 concentrations can be used to adjust and synchronize the different traces both during recording or data processing after the measurements (171, 203, 265–272).

Online measurement of NO with breath holding in ventilated patients. This simple maneuver might be quite useful in some ventilated patients, especially when there are limitations of the tidal breath measurements (273). Because the plateau phase can be explained by a steady state between NO production/release to the gas phase, and consumption/removal from this phase presumably by pulmonary blood flow rate, it provides useful information regarding NO without the confounding effect of different ventilation parameters and modes.

Real-time analysis of mixed exhaled gas for NO in ventilated patients. In this method, a mixing chamber is connected to the exhaust port of a ventilator and a portable real-time NO analyzer is used to sample NO concentration in the gas exiting the mixing chamber after steady-state concentrations have been reached within the chamber. This method allows for measurement over several breaths, reflecting a true average. The system is microbe-resistant with addition of a filter and frequent change of tubing to prevent bacterial overgrowth in the chamber. Problems of lag times between measurement of CO_2 and NO are avoided.

Offline methods for NO measurement in ventilated patients. Offline measurements offer several added advantages compared with online measurements. The main advantage is that the samples can be collected away from the analyzer and then taken to the analyzer for measurement at a different time or location. Offline methods are usually less dependent on analyzer response times, and also allow more efficient use of analyzers (less analyzer time per measurement) and for measurement of other gases (274, 275). In spontaneously breathing individuals, there is a good correlation between online and bronchoscopic NO levels and those obtained by offline methods (92, 276, 277), but this finding has not been evaluated in ventilated individuals. Exhaled gas from ventilated individuals for offline analysis of NO can be collected by different methods and the port of sampling also varies (278–282). Furthermore, single or multiple

breaths can be collected for analysis. There is no standard method used by the different investigators to collect exhaled gases, and the variability of NO levels obtained by these methods is very high. These observations underscore the need for standardization of the collection methods for measuring exhaled NO in ventilated patients. For this field to advance further, it is believed that international efforts toward a consensus on the following factors is needed: (1) characteristics of proposed exhaled breath markers in mechanically ventilated patients; (2) guidelines on standardization of breath analysis measurement techniques; and (3) the potential role of exhaled breath markers in assessing and monitoring disease progression and response to therapy in critically ill patients.

SECTION 7: EQUIPMENT RECOMMENDATIONS FOR THE MEASUREMENT OF $F_{E_{NO}}$

Background

Most NO analyzers in research and clinical use employ the principle of ozone-/NO₂-based chemiluminescence to measure NO. However, NO measurement based on alternative technologies, including luminol-/H₂O₂-based chemiluminescence, tunable diode laser absorption spectrometry, and laser magnetic resonance spectroscopy, is currently available or in development. New technologies offer potential advantages regarding increased portability, reduced cost, and autocalibration. Equipment needs will vary according to the applications and desired test procedures.

As newer NO analyzers based on different measurement technologies become available, different specifications and specific guidelines may be required. Moreover, future equipment specifications will depend critically on clinical requirements. For example, analysis of $F_{E_{NO}}$ during tidal breathing and in mechanically ventilated patients will require equipment with faster response times.

Thus, the following recommendations are limited to the proposed application of measurement of $F_{E_{NO}}$ and nasal NO in spontaneously breathing subjects using ozone-/NO₂-chemiluminescence-based analyzers.

Current Equipment Specifications for Online and Offline Analysis of Exhaled and Nasal NO in Adults and Children

Table 1 displays the current minimum specifications required for accurate measurement of $F_{E_{NO}}$ and nasal NO under various clinical scenarios. During online measurement of exhaled NO, recommended exhalation flow rates for adults and children older than 12 years (0.05 L/second) and children younger than 12 years (0.05 L/second) are measured at 37°C, 760 mm Hg, saturated (BTSP) in keeping with other measurements of lung function.

Equipment Considerations for Breath-by-Breath NO Analysis

Breath-by-breath analysis may be useful for research purposes in young children and in mechanically ventilated subjects. It is suggested that reliable analysis of $F_{E_{NO}}$ during tidal breathing and in mechanically ventilated patients requires equipment with faster response times and less noise to enhance signal/noise ratio. Investigators are cautioned to select an analyzer that provides the necessary response times and sampling rates for the specific respiratory frequency or mechanical ventilation conditions.

Equipment Considerations for Offline NO Analysis

For delayed, offline analysis of $F_{E_{NO}}$ in previously collected exhaled gas or nasal gas samples, similar specifications are required concerning sensitivity, accuracy, and range as for the respective online analysis. In contrast, instrument response time and system lag time (e.g., tubing, physical setup) are less exacting. An impor-

TABLE 1. REQUIRED SPECIFICATIONS FOR NO ANALYZERS

Parameter	F _{ENO}	Nasal NO
Sensitivity	1 ppb (noise < 0.5 ppb)	10 ppb
Signal/noise ratio	≥ 3:1	Same as exhaled NO
Accuracy	Better than 1 ppb	Better than 10 ppb
Range	1–500 ppb	10 ppb–50 ppm
Instrument response time*	< 500 ms	< 500 ms
System lag time†	To be measured and reported by the investigator	Same as F _{ENO}
Drift	< 1% of full scale/24 h	Same as F _{ENO}
Reproducibility	Better than 1 ppb	Better than 10 ppb
Flow-through sensor	To be measured by manufacturer and reported in publications	Same as exhaled NO

Definition of abbreviation: F_{ENO} = fractional exhaled nitric oxide.

* Defined as the delay from introduction of a square-wave signal until achievement of 90% of the maximum signal, inclusive of electronic delays, and inherent instrument physical delays because of sample introduction, but not including tubing length.

† Defined as the delay due to sample transit time through tubing and connections in a particular application system or setup.

tant specific requirement of offline analysis is the confirmation of a plateau in NO concentration of at least 3 seconds duration, thus providing a reliable, reproducible signal. This can be accomplished by adequate sample mixing as well as adjustment of the analyzer inlet sample flow. This is especially important with small-volume samples, such as from young children.

Material Requirements for NO Analysis

NO is a highly reactive molecule. Recommended materials for analyzer and system components in contact with sample gas (e.g., reaction cells, tubing, sample containers) include stainless steel, siliconized materials, glass, Teflon, and Teflon-coated materials. Plastics (e.g., polyvinyl chloride, polyacrylamide) may also be acceptable, but investigators should rigorously confirm that individual components made of such materials do not liberate NO nor react with NO in gas samples. Rubber and latex-related materials are not considered acceptable, because of a significant risk of interference, including reaction with NO in gas samples.

Calibration Requirements and Procedures

Zero gas. A reliable NO-free calibration gas is essential for NO measurements. It is recommended that zero gas be generated by exposing ambient air to NO scavengers (e.g., KMnO₄ and/or charcoal) or to an ozone generator, which converts NO to NO₂ (NO knockout method). The use of medical-grade air as a zero gas is not recommended because of the reported presence of highly variable NO concentrations, up to many parts per billion, depending on the source and purification system. Ideally, the instrument should control baseline drift.

Calibration range. For each analyzer, it is recommended that an initial linear validation be performed using at least a three-point calibration (zero and two higher NO concentrations). This requires specially prepared standard NO calibration gases, most commonly diluted in nitrogen. Commonly available concentrations currently range from 200 ppb to 500 ppm. Standard gases are supplied at various guaranteed accuracy levels (e.g., ± 2, ± 5%). An accuracy of ± 2% or better is recommended to optimize accuracy and reproducibility of exhaled and nasal gas sample NO analysis. Calibration gases should also be guaranteed stable for a defined period of time (e.g., > 6 months). It is highly desirable that NO analyzers be calibrated in the expected range of sample values: 10 to 100 ppb for exhaled samples and 0.4 to 50 ppm for nasal samples. We recognize that stable, standard NO concentration gases in the range of clinical exhaled NO samples (< 100 ppb) are not easily or widely available. High-accuracy gas dilution systems that permit generation of parts-per-billion gases from parts-per-million standards are commercially available. We also recognize the extremely high linearity

of commercially available NO analyzers, often over a range of several log concentrations, such that the use of parts-per-million range NO standard calibration gases for analysis of parts-per-billion range samples is considered acceptable, albeit suboptimal.

Frequency of calibration. A daily calibration using the zero gas and one other standard NO concentration is recommended, but not absolutely essential; each analyzer manufacturer should recommend a desirable frequency. However, regular confirmation of stable ambient conditions (e.g., temperature, barometric pressure, humidity) is highly recommended. In the presence of unstable ambient conditions, frequent recalibration should be considered, and at the very least, the zero point should be rechecked before each sample.

Factors affecting calibration. The O₃/NO₂ chemiluminescence signal is very sensitive to changes in reaction chamber pressure, of which the major determinant is analyzer inlet gas sample flow. Thus, it is recommended that NO analyzer calibration and sample analysis always be performed at a constant inlet sample flow. Moreover, this inlet sample flow rate should be checked at regular intervals, depending on stability of ambient laboratory conditions.

Influence of Extraneous Factors on NO Analysis

Ambient conditions. Ozone chemiluminescence analyzers are sensitive to ambient conditions, including temperature, humidity, exposure to sunlight, and so forth (283).

Exhaled and nasal gas samples are presumed to be fully saturated with water vapor. However, the drying of samples by passing through various drying agents (e.g., Drierite) is not recommended because of possible absorption of NO. An acceptable approach is the equilibration of sample humidity with ambient humidity (e.g., Nafion tubing in the sample line). In all measurement systems, calibration gases (ambient temperature and pressure, dry [ATPD]) and clinical gas samples (body temperature [37°C] and pressure, saturated [BTPS]) should be measured under uniform humidity and temperature conditions.

It is recommended that the quantitative effect of humidity on NO measurement should be measured and reported by the manufacturer of each NO analyzer. Quenching by water vapor should be less than 1% of measured NO signal per 1% volume H₂O (283).

Interfering substances. These substances include the following: volatile anesthetic gases, which may be hazardous to the measurement system regarding chemical reactions; oxidation of analyzer and tubing materials; risk of spontaneous combustion (e.g., O₃, electrical sparks in NO analyzers); and so forth. Quenching by CO₂ should be less than 1% NO per 1% CO₂

(283). Alcohol-containing disinfectants can interfere with NO analysis (284).

Recommended Ancillary Features and Equipment

The NO analysis specifications detailed previously are essential to the reliable measurement and reporting of $F_{E,NO}$ data. However, some additional features will facilitate NO measurements according to the recommendations for a standardized technique in this document. The following list of features would be part of an integrated $F_{E,NO}$ measurement, analysis, and data-handling system.

Output. Provide both analog and digital output.

Data collection capability. The following features may be helpful:

The transmission of collected NO output data (NO, pressure, flow rate) to computer or monitor for real-time display

Automatic sensing and indication of quality of exhalation and achievement of valid NO plateau according to that defined in this document (see SECTION 2), allowing termination of exhalation

Data storage. Data analysis software allowing manipulation and display of results and so forth.

Biofeedback of exhalation parameters. For adult and pediatric measurement of $F_{E,NO}$ and nasal NO, biofeedback of exhalation parameters may be essential for those systems that generate constant flow in this manner.

Sample flow rate. As mentioned previously, O_3 -/ NO_2 -based chemiluminescence NO analyzers are very sensitive to sample flow rate. Several acceptable flow-control systems exist, including dynamic (Starling-type) flow resistors and mass flow controllers. In either system, automatic monitoring and display of NO analyzer sample flow (e.g., by including a rotameter) would be essential.

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