Airway inflammation is a central process in asthma and other lung diseases (1). 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 (2), CO (3–5), ethane, and pentane (6–8), but also non-volatile substances in the liquid phase of exhalate, termed breath condensate, for example, hydrogen peroxide (9–12). The noninvasive nature of the measurement of exhaled mediators makes them ideally suited for the serial monitoring of patients.

The presence of endogenous nitric oxide (NO) in exhaled breath of animals and humans was first described in 1991 (2). Soon after, several publications reported high levels of orally exhaled NO in subjects with asthma as compared with unaffected subjects (13–18) and a fall in these high levels after treatment with corticosteroids (15, 19, 20). Similar findings have been described in the pediatric age group (21–24). In patients with chronic obstructive pulmonary disease (COPD), exhaled NO has been reported to be high in exacerbations compared with stable patients (25). Other diseases associated with high exhaled NO include bronchiectasis in one study (26) but not in another (27), viral respiratory tract infections (28, 29), systemic lupus erythematosus (30), liver cirrhosis (31–34), and acute lung allograft rejection (35). Low levels of exhaled NO have been described in cystic fibrosis (21, 36–39), human immunodeficiency virus (HIV) infection (40), and pulmonary hypertension (41, 42). Exhaled NO has been shown to correlate with other parameters in mild asthma, for example, induced sputum eosinophilia (43) and bronchial reactivity (44) in non-steroid-treated subjects.

Nasal nitric oxide concentrations are high relative to the lower respiratory tract in humans (18, 45), with the highest levels reported in the paranasal sinuses (46, 47). Nasal NO may have physiologic roles, for example, preserving sinus sterility (48) and modulating ciliary motility (49). Nasal NO concentration has been proposed as a surrogate marker of nasal inflammation such as occurs in allergic rhinitis (50–54). In contrast, subjects with immotile cilia syndromes and cystic fibrosis have low nasal NO (55, 56).

Although there are numerous publications on exhaled NO, the study of exhaled and nasal NO measurement has been characterized by a variation in published exhaled NO levels in health and disease, much of which is attributable to the lack of a standardized technique of measurement. Many investigators have employed or recommended different methodologies for exhaled NO measurement (57–64); there is little published about nasal NO measurement techniques.

In order for the field of NO measurement to advance, it was felt that an international consensus on the appropriate measurement techniques was required as a basis for the collection of normative data and the application of standardized techniques to measurements made in various disease states. A task force of the European Respiratory Society published European recommendations in 1997 (58).

The recommendations in this document were formulated by international investigators in the field of exhaled and nasal nitric oxide, at a workshop sponsored by the American Thoracic Society (ATS)/American Lung Association (Toronto, Canada, May 1998). Also attending as committee members to assist in the technical recommendations were scientists from nitric oxide analyzer manufacturers: Aerocrine, Eco Physics, Sensor Medics, and Sievers Instruments. The workshop consisted of five committees: adult online exhaled nitric oxide measurement, offline exhaled nitric oxide measurement, pediatric online exhaled nitric oxide measurement, nasal nitric oxide measurement, and technical recommendations. The initial draft of the document was prepared during the workshop by the moderators of each of the committees and presented to the entire forum at the last session of the workshop. Modifications were made subsequent to frequent communication between the members of the breakout sessions, and in light of comments from external reviewers. The revised document was circulated to all workshop members for review and presented to the ATS board of directors in July 1999.

The standardization of techniques opens the way for the collection by numerous centers of comparable data from normal subjects and those with disease states. The workshop participants felt that adequate knowledge and experience were available to warrant presentation of the guidelines that follow. The document is divided into a general section that deals with aspects common to all sections, followed by those dealing with adult online and offline measurement, pediatric measurement, nasal NO measurement, and technical aspects of NO analysis. Wherever possible, the small number of recommendations are based on published material including abstracts as referenced; in the absence of clear data, we have relied on the experience of participants in the workshop. Where aspects concerning exhaled NO measurement are undetermined, this has been clearly stated in text.

While these recommendations will allow uniformity of measurement techniques in future studies, this document does not
intend to invalidate previous or ongoing studies that have employed 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 of these recommendations to be made on scientific grounds.

1. GENERAL ASPECTS OF EXHALED AND NASAL NITRIC OXIDE MEASUREMENT

Requirements for the Clinical Use of Exhaled NO Measurements

At present, exhaled and nasal NO measurements have been performed in the research setting. Online measurement refers to exhaled NO testing with a real-time display of exhaled NO breath profiles, whereas offline testing refers to collection of exhalate into suitable receptacles for delayed analysis. The use of exhaled NO measurement as a clinical tool requires the adoption of a standardized measurement technique followed by collection of normative data in all age groups. The achievement of a consensus as detailed in this document will enable an international multicenter collaborative study using standardized techniques. Ideally, there should be interinstitutional agreement of mean exhaled NO within 5% for each age group. Also, clinical indications for the measurement of exhaled NO should be validated.

Standardization of Exhaled NO Terminology and Units

Nomenclature and symbols used in articles reporting exhaled NO have been variable. The following guidelines have been formulated to bring this field of study in line with standard physiological nomenclature.

Online measurement. The fractional exhaled NO concentration (\(F_{\text{ENO}}\)) is expressed in parts per billion (ppb), which is equivalent to nanoliters per liter (nl/L). The exhalation flow rate employed for a particular test can be expressed as a subscript of the flow in liter/sec, for example, \(F_{\text{ENO},0.05}\). Expired and exhaled are both denoted by \(\varepsilon\), and inspired by \(I\), in qualification of the test results, for example, \(F_{\text{ENO}}\) and \(F_{\text{NO}}\).

NO output represents the rate, that is, the amount of NO exhaled per unit time, and is denoted by \(V_{\text{NO}}\). It is calculated from the product of NO concentration in nanoliters per liter and expiratory flow rate in liters per minute, corrected to BTPS.

\[
V_{\text{NO}} = [\text{NO}] \times \text{airflow rate} \times \frac{\text{nl/min}}{\text{nl/L} \times \text{L/min}}
\]

Terms such as “NO release,” “NO excretion,” “NO secretion,” and “NO production” are to be discouraged when referring to \(V_{\text{NO}}\).

Offline NO collection. \(F_{\text{ENO}}\) refers to the fractional NO concentration in exhalate from a vital capacity collection. If the exhalation is at a constant flow, this should be added as a subscript, for example, \(F_{\text{ENO},0.35}\) with the flow rate in liters per second.

Nasal NO. The fractional concentration of nasal NO is termed nasal \(F_{\text{ENO}}\). Nasal NO output is the rate of nasal NO exhaled and should be represented as nasal \(V_{\text{NO}}\).

General Principles Regarding Exhaled NO Measurement

Source of exhaled nitric oxide. Current thinking is that NO is formed in both the upper and lower respiratory tract (55, 65–71) and diffuses into the lumen by gaseous diffusion down a concentration gradient, thus conditioning exhaled gas with NO (60, 72, 73). A lveolar NO is very low owing to avid uptake by hemoglobin in pulmonary capillary blood (68, 74). Although gastric NO levels are very high (75), this does not appear to contaminate exhaled NO (75), probably owing to closed upper and lower esophageal sphincters.

Nasal NO contamination. Nasal NO can accumulate to high concentrations relative to the lower respiratory tract (46, 47, 55, 76–79). The distribution of the relative contribution of nasal NO to exhaled NO has been addressed in many publications (18, 45, 46, 57, 59, 62, 76, 78, 80). Accordingly, techniques that aim to sample lower respiratory NO should prevent contamination of the sample with nasal NO (57, 59).

Ambient nitric oxide. As 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 employed, 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 dependence (59, 81) and the same holds for the nasal cavity (82, 83). This variation in exhaled NO has been attributed to faster flows minimizing the transit time of alveolar gas in the airway. The rate of NO output, however, is greater at higher flow rates, but not in direct proportion; this is analogous to respiratory heat loss (59). In view of this flow dependency, the use of constant expiratory flow rates is emphasized in standardized techniques.

Breathhold. Breathhold results in NO accumulation in the nasal cavity, lower airway, and probably in the oropharynx and this results in NO peaks in the exhalation profiles of NO versus time (46, 55, 61, 66, 84). For this reason, the use of breathhold is discouraged in the standardized techniques described in this document.

Patient Factors Influencing Exhaled NO Values

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

Age/sex. In adults, there is no consistent relationship between exhaled NO level and age, sex (85), menstrual cycle, or pregnancy (63, 86) but these patient characteristics should be recorded at the time of measurement. One study reported that, in children 7–13 yr of age, \(F_{\text{ENO}}\) increased with age (87).

Respiratory maneuvers. Because spirometric maneuvers have been shown to transiently reduce exhaled NO levels (88, 89), 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 exhaled NO maneuver itself does not appear to affect plateau exhaled NO levels (89).

Airway caliber. It has been demonstrated that exhaled NO levels may vary with the degree of airway obstruction (20, 90), or after bronchodilatation (89, 91, 92), perhaps owing to a mechanical effect on NO output. Accordingly, the time of last bronchodilator administration should be recorded.

Food and beverages. There are insufficient data in the literature to make a firm recommendation concerning whether or for how long patients should refrain from eating and drinking before NO analysis. A n increase in exhaled NO has been found after the ingestion of nitrate- or nitrate-containing foods, such as lettuce (with a maximum effect 2 h after ingestion) (93), and drinking of water may lead to transiently altered NO levels (94). It is possible that a mouthwash may reduce the effect of nitrate-containing foods (93). Until more is known, it is pru-
dent when possible to refrain from eating and drinking for 1 h before to exhaled NO measurement, and to question patients about recent food intake. A alcohol ingestion reduces exhaled NO in patients with asthma and in unaffected subjects (95, 96).

Circadian rhythm. Studies are in progress to examine the effect of circadian rhythm on exhaled NO, so it is uncertain whether measurements need to be standardized for time of day (97, 98). It is therefore prudent to perform serial NO measurements at the same time of the day when possible and to always record the time.

Smoking. Chronically reduced levels of exhaled NO have been demonstrated in cigarette smokers in addition to acute effects immediately after cigarette smoking (17, 99–101). Subjects should not smoke in the hour before the study and short- and long-term active and passive smoking history should be recorded.

Infection. U pper and lower respiratory tract infections may lead to increased levels of exhaled NO (28, 29). Therefore exhaled NO measurements should be deferred until recovery if possible or the infection should be remarked on in the record.

Other factors. Manipulation of physiological parameters has been shown to affect exhaled NO. Changing pulmonary blood flow has no effect in humans (102), but hypoxia decreases exhaled NO (68, 103). The application of positive end-expiratory pressure (PEEP) has been shown to increase exhaled NO in animals (104–106), but airway pressure in humans does not affect exhaled NO plateau levels (59, 81, 94). Many studies have examined the effect of exercise on exhaled NO and nasal NO (41, 80, 84, 107–114). During exercise, exhaled NO and nasal NO fall while NO output increases. The duration of this effect after exercise is unknown. It is therefore prudent to avoid strenuous exercise for 1 h 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 nitric oxide falls after treatment with inhaled or oral corticosteroids in asthmatic subjects (15, 16, 19, 23, 115–117) and after inhalation NO synthase inhibitors (118). The effect of other anti-inflammatory agents is not yet published. NO donor drugs (119) and oral, inhaled, and intravenous L-arginine (64, 120) increase exhaled NO and nasal NO (121). 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 (89–92, 122).

2. RECOMMENDATIONS FOR A STANDARDIZED PROCEDURE FOR THE ONLINE MEASUREMENT OF EXHALED NITRIC OXIDE IN ADULTS

Moderator: P. E. Silkoff, M.D.

Online methods refer to exhalations where the expiration 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 and/or pressure) are captured and displayed in real time. This allows the test administrator to monitor the exhalation to ensure conformation to the required flow 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 6).
tients with severe disease. Low flow rates are also associated with a decreased NO output (59, 81).

**Recommended Expiratory Flow Rate**

A flow rate of 0.05 L/s (BTPS) was thought, on the basis of current knowledge, to be a reasonable compromise between measurement sensitivity and patient comfort. However, exhaled NO measurement can be performed at higher or lower flow rates if this is desirable in certain situations. In all cases, however, the expiration flow should be clearly recorded and reported in any publications.

A constant expiratory flow can be achieved in different ways. One commonly used method to achieve a constant expiratory flow is to display a target mouthpiece pressure or flow to the subject (e.g., using a gauge or computer display) while the subject exhales via a fixed expiratory resistance (59, 81). The constant pressure and therefore flow is achieved by biofeedback of pressure or flow parameters to the subject, who maintains these parameters within specified limits.

Exhalation pressure does not affect NO plateau measurements (59, 81, 94), and so individual investigators may select pressures between 5 and 20 cm H\(_2\)O, with the appropriate expiratory resistance to achieve the desired flow.

With biofeedback of expiratory pressure or flow, 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/s (± 10%) during the time of the NO plateau generation and the instantaneous flow is not less than 0.045 L/s or greater than 0.055 L/s 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 criterion noted in the record.

Recent theoretical considerations suggest that it is possible to derive other parameters such as an airway diffusion rate, effective mucosal surface concentration, and alveolar NO levels by the measurement of exhaled NO at multiple flow rates (69–71, 73). However, the additional contribution of exhaled NO measurement at multiple flows relative to measurement at one flow alone is of unproven value at this stage to justify recommendation for general application, and it should therefore remain a research tool at present.

**The Interpretation of NO Single-Breath Profiles**

Constant flow exhalations, however achieved, result in a single-breath NO profile (exhaled NO versus time plot) that consists of a washout phase followed by an NO plateau, which is usually reproducible and flat (Figure 2) but may slope up or down (Figure 3). The washout phase is sometimes followed by an early NO peak before the plateau (Figure 4). This peak may be derived from the nasal cavity if the subject inhales through the nose or the velum is open initially as the exhalation starts. In addition, NO in the inhaled air source (see INSPIRED GAS SOURCE, above), 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.

The duration of exhalation must be sufficient (at least 6 s) to obtain a plateau in the NO versus time profile of at least 3 s; the plateau starts at point A and ends at point B and may be flat, up sloping, or down sloping (see Figure 3). Once a 3-s plateau is achieved, there is no reason to continue the exhalation.
The plateau is defined as the first portion of the NO versus time profile where \( A - B \) or \( B - A \), related to the smaller of \( A \) and \( B \), is \(< 10\%\). Also, at no time between points \( A \) and \( B \) should the NO values be greater than the NO value at \( A \) or \( B \) (Figure 3). For exhaled NO value \(< 5 \) ppb, the 10% plateau criterion may be difficult to fulfill; 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 valid NO plateaus according to these criteria. At the recommended flow of 0.05 L/s, plateaus are usually flat and clearly discernible (Figure 2).

Repeated reproducible exhalations should be performed, resulting in three NO plateau values that agree within 10% of the mean value. Exhaled NO is then calculated as the mean of these three values (Figure 2). At least 30 s 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.

3. RECOMMENDATIONS FOR OFFLINE MEASUREMENT OF EXHALED NITRIC OXIDE IN ADULTS

Moderator: J. M. Drazen, M.D.

Figure 3. Schematic diagram of exhaled NO profiles, showing (from left to right) horizontal, downsloping, and upsloping NO plateaus with the start (A) and the end (B) of an NO plateau as defined in text.

Figure 4. 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 that is generated by asking the subject to inhale nasally. This fills the conducting airways with nasal NO, which is then exhaled. A similar peak can be produced by inhaling ambient NO. The NO plateau is essentially unaltered once the early peak has washed out.

Nitric oxide determinations can be made from exhaled gas collected in a reservoir and subsequently analyzed for NO concentrations. Several groups have used reservoir collection techniques to study nitric oxide in humans and, while the absolute \( F_{ENO} \) values vary in different reports, the relative changes in \( F_{ENO} \) and clearly discernible (Figure 2).

Advantages and Disadvantages of Offline Collection

As compared with online techniques, offline collection offers (1) the potential for expire collection at sites remote from the analyzer, (2) independence from analyzer response times, and (3) more efficient use of the analyzer, as gas may be collected from several patients simultaneously and less analyzer time per patient is required.

Potential problems with offline methods include (1) contamination with gas not derived from the lower airway, (2) error introduced by sample storage, and (3) an inability to allow for instantaneous feedback and assessment of technique. Recommendations regarding the standardization of exhalation collection and storage for the offline measurement of \( F_{ENO} \) are presented in the following sections.

Procedures for Collection of the Sample

For ambulatory patients, it is recommended that gas for \( F_{ENO} \) be collected by asking the patient to inhale orally to TLC and then immediately perform a slow vital capacity maneuver against an expiratory resistance into a reservoir bag without a breathhold. The reservoir is sealed and subsequently analyzed for \( F_{ENO} \). Details pertaining to this maneuver and to the equipment needed for this measurement are presented in the following sections, and one simple apparatus for the offline collection is shown in Figure 5.

Inspired Air

When measuring exhaled NO offline, evidence provided by several groups demonstrates that high inhaled concentrations of NO affect \( F_{ENO} \) measurements; determinations made with air containing more than 20–40 ppb NO are significantly higher than values obtained when subjects are inspire air containing negligible amounts of NO (59, 88, 127). It is likely that this effect results from contamination of dead space gas in the collection device with high-NO ambient gas. A spontaneouly occurring indoor ambient NO concentrations in urban centers may reach several hundred parts per billion, it is critical to actively control the NO concentration of the inspired gas when the collected sample contains dead space gas. This can be accomplished by asking the subjects to breathe from a source of low-NO air or through an NO scrubbing filter for 15 s (or a minimum of two tidal breaths) before the collection of the expire (88).

Expiratory Flow and Oropharyngeal Pressure

It is known that the concentration of NO recovered in the expire decreases with collection at higher flow rates (59, 81). This change in the recovered \( F_{ENO} \) likely represents the dynamic equilibrium between the production of NO (or its release) in the airway and the diffusion of NO into the gas flowing through the airway (128).

The Recommended Flow Rate

During offline collections, the flow rate of the exhalate must be known. For collection of the entire vital capacity, an expi-
It has been demonstrated that new Mylar balloons allow for sample stability for at least 48 h (15). Because no standardized vessels are available, the investigator must ensure that the reservoirs used are leak-free (both with regard to loss of sample to the atmosphere and to contamination by ambient NO), stable, and nonreactive. This can be accomplished by assaying several samples of varying NO concentration serially in the period of time and under ambient NO conditions appropriate for the experimental protocol employed. It should be noted that a given vessel may deteriorate over time; individual vessel integrity needs to be established at the time of its experimental use.

4. RECOMMENDATIONS FOR ONLINE AND OFFLINE MEASUREMENT OF EXHALED NITRIC OXIDE AND NASAL NITRIC OXIDE IN CHILDREN

A s with adults, there are several pediatric studies that show increases in exhaled NO in children with asthma compared with unaffected children, and a decrease in exhaled NO in children receiving inhaled corticosteroids (21–24, 38, 129–131). In addition, exhaled NO is generally lower than normal in children with cystic fibrosis (21, 38). The techniques for measurement used in these studies include (1) online measurement, which has proven difficult for some preadolescent subjects (87, 132); (2) nasal NO contamination, which compares favorably with online single-breath exhalation (15, 22, 132, 133); and (3) analysis of online tidal breathing NO profiles against expiratory pressure (23, 134, 135).

Recommended Online Exhaled NO Measurement in Children

General aspects. The patient should remain comfortably seated, breathing room temperature air for 5 min before the test to acclimatize to laboratory conditions. The inspired gas should contain < 5 ppb NO (59).

Patients 12 yr old or older. N O exhaled by children ≥ 12 yr old should be measured by the same technique recommended for adults (see Section 2). If the patient is unable to perform the adult test, then testing should be performed as for children less than 12 yr old.

Patients less than 12 yr old. A n expiratory flow rate of 50 ml/s, a dead space of 10 ml, and a 2-s plateau duration should be used for children less than 12 yr old (87, 136). Note that this differs from the adult protocol only in that the dead space and plateau duration are lower. The flow rate of 50 ml/s ensures an acceptable time to plateau and an acceptable rate of decline in lung volumes (87, 132), particularly for children with significant pathology who have vital capacities of less than 1 L (137). The expiratory pressure is maintained between 5 and 20 cm H₂O to ensure velum closure (59). Subjects inhale to TLC, and then exhale at a constant rate of 50 ml/s until at least a 2-s NO plateau has been achieved and exhalation has lasted for at least 4 s. Repeated exhalations are performed until three NO plateau values agree at the 10% level or two agree at the 5% level. There should be at least a 30-s interval between tests, to allow patients to rest. The mean NO value is then recorded. For children unable to sustain a steady expiratory flow, forced vital capacity offline collection may be ideal (see below).

Recommended Offline Collection of Exhaled NO in Children

Offline NO collection has advantages in certain situations (see Section 3). Many studies of NO exhalation by children have
been performed by offline methods (22, 138). The adult standard for offline NO collection (see Section 3), which has features that ensure nasal NO exclusion and constant flow exhaustion, is recommended for children.

**Children Unable to Cooperate**

Children of any age may be unable to cooperate with the online and offline techniques, which require expiratory flow control. For these children the following two techniques may be employed.

**Tidal breathing offline collection.** The subject performs relaxed tidal breathing of NO-free air during nasal occlusion. The subject inspires orally via a one-way valve and expires against a resistance of at least 2 cm H\(_2\)O. Tidal expire is collected in a light-impermeable Mylar bag or other suitable collection vessel. The mixed expired NO concentration is measured from the collection vessel after 2 min of tidal breathing.

**Online tidal breathing method.** Studies of online tidal breathing measurement against an expiratory pressure are highly repeatable, successfully exclude nasal air, and have shown robust differences between exhaled NO in asthmatic and normal subjects (23, 134, 135). The subject wears a nose clip to prevent nasal inhalation and breathes air passed through an NO scrubber and segregated from exhaled air by one-way valves. Nitric oxide levels are analyzed continuously during mouth breathing by a chemiluminescence analyzer, sampling at a constant flow. The exhaled air that is not withdrawn by sampling is discarded through a one-way valve to prevent contamination with ambient air. To keep the soft palate closed, an inspiratory line is discarded through a one-way valve to prevent contamination with ambient air.

**Exhaled Nitric Oxide Measurements in Ventilated Patients and Canopy Measurements**

The state of the art in the areas of exhaled NO measurements in ventilated patients and canopy measurements is rapidly advancing but is not considered sufficient to provide detailed recommendations. However, it is agreed that (1) studies of \(V_{\text{NO}}\) in children should include information about the intake flow rate of the NO analyzer and the site of the sampling port; (2) studies in ventilated patients must be done without bias flow in the circuit; and (3) intrinsic losses of NO in the circuit between the patient and the sampling site must be measured and reported.

**Nasal NO Measurement in Children**

Nasal NO has been measured in children (21, 36, 37, 51, 55, 130, 138, 139) in the context of acute sinusitis, cystic fibrosis, and Kartagener syndrome. The use of audiovisual aids designed for children should facilitate the measurements.

**Recommended method.** The adult method is recommended for children who are able to cooperate (see Section 5), with a transnasal flow of 3 L/min and exclusion of lower respiratory tract air from the nasal cavity by closure of the velum (see Section 5) by any method shown to be reliable. Thus, Baraldi and coworkers have successfully used the nasal aspiration method during breathhold with closed glottis in children 5 yr of age and older (138, 139).

**5. RECOMMENDATIONS FOR STANDARDIZED MEASUREMENT OF NASAL NITRIC OXIDE**

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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. The supralveolar airway can generate NO concentrations severalfold greater than in the lower respiratory tract, in the parts per million range (18, 45, 47), and the NO concentration is particularly high in the para-nasal sinuses (47, 77). The nasal airway is a complex system of communicating cavities, that is 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 (140). The nasal cavity has a unique vasculature that results in variation in nasal cavity volume, and alteration in nasal blood flow and/or volume could theoretically affect nasal NO production and absorption.

**General Considerations**

Measurement of nasal NO output requires generation of air flow through the nasal cavity (transnasal air flow). Flow through the nasal cavities in series can be achieved by aspirating or insufflating air via one nares 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 can be achieved by exhaling via one or both nasal cavities, by aspirating via the mouth with air entrained into both nares during breathholding, or by aspirating from one or both nares with the mouth open during breathholding. A present, little is known about effects of transnasal airflow direction on NO output. The transnasal flow in parallel mimicks natural nasal breathing. In subjects with unilateral or bilateral nasal obstruction, transnasal airflow may be decreased or absent, making measurement of nasal NO output more difficult or impossible.

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 versus time, analogous to that seen in the lower respiratory tract (Figure 6). The nasal NO concentration is inversely related to the transnasal airflow rate (82, 83, 141) (Figure 7). However, different flow rates may have different aerodynamic profiles, resulting in changes in the physics of air flow (e.g., laminar versus turbulent flow) and different pathways of flow through the nasal passages (142).

**Figure 6.** 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 (200 ml/min). Amb. NO = ambient NO.
American Thoracic Society

The transnasal flow dependence of nasal NO (83).

Figure 7. The transnasal flow dependence of nasal NO (83).

The dynamics of this flow may affect nasal NO output (143, 144). For all of the preceding reasons, any standardized method should include rigorous control of the transnasal airflow rate.

Nasal NO Output

The product of transnasal flow rate ($V$) and measured NO concentration allows calculation of NO output ($\dot{V}_{NO}$). Present evidence suggests that NO is relatively constant over a range of transnasal flow rates between 1 and 5 L/min (82, 143–145). There is reasonable agreement, using different measurement techniques, that nasal NO output is in the range of 205–455 nl/min in healthy primates (45, 55, 141, 146). At transnasal flow rates ≤ 0.3 L/min, NO may be taken up by nasal tissues, reducing the calculated $\dot{V}_{NO}$ (141). At higher flow rates, NO may increase progressively (145, 147).

The Importance of Velum Closure in Nasal NO Measurement

With transnasal airflow in series, 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 several ways:

1. Slowly exhaling orally against a resistance (59)
2. Pursed lips breathing via the mouth (148)
3. Breathholding with velum closed (46)
4. Voluntary elevation of the soft palate by a trained subject (145)

During the nasal NO test, measurement of nasal CO$_2$, which should remain low, can be used to verify velum closure.

Recommended Method for Measurement of Nasal NO

While several methods have been described for nasal NO measurement, the recommended method involves aspiration at constant flow from one naris with gas entrained via the other naris. This is currently the most prevalently used and best validated method (50, 53, 58, 83, 138, 139, 143, 147–150), 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$_2$O has been chosen as the preferred method (141), as this has been shown to close the velum reliably (59). A biofeedback display of airway pressure to the patient 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. The apparatus required for the recommended nasal measurement technique is shown in Figure 8.

Description of Method

Two nasal olives with a central lumen are securely placed in the nares, and used to aspirate air via one naris and entrain air via the other. These olives should be composed of a soft, non-traumatizing material, and of sufficient diameter and shape to occlude the naris. The seated subject inserts a mouthpiece, inhales to TLC, and exhales against expiratory resistance while targeting a mouth pressure of 10 cm H$_2$O to close the velum. While this exhalation is proceeding, air is aspirated at constant flow 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 (83). This insufflation method may be desirable when nasal cavity obstruction leads to dynamic alar collapse during the aspiration technique.

Figure 8. The transnasal airflow pattern employed in the recommended method for nasal NO measurement (see text for explanation). NOA = nitric oxide analyzer.
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 (145).

**Transnasal Airflow Rate**

A target airflow rate of 3 L/min (50 ml/s) should be used in the measurement of nasal NO output, as this flow provides a steady plateau level of NO concentration in most patients within 20 s. This flow rate is also close to the physiologic range of ventilation flow through one side of the nasal cavity in a resting adult human and provides a turbulent flow pattern that facilitates ventilation of the nasal cavity (143). If a steady plateau of NO concentration is not achieved at this flow rate, other flow rates (in the range of 3–6 L/min) may be used to obtain a steady plateau NO concentration, as NO output is relatively stable in individual subjects over this flow range (82, 143–145). The precise flow used should be recorded with the NO measurement for each subject.

**Factors Influencing Nasal NO Values**

A small decrease in nasal NO has been observed in smokers (e.g., papaverine) increased nasal NO output in one report (154) but nasal NO was unchanged when the supine posture was assumed (47, 77, 153), but also to decrease NO output (155, 156).

Saline does not appear to affect nasal NO output (151) but nasal NO concentration falls during intense physical exercise (80, 82, 114). It is therefore prudent to refrain from exercise for 1 h before measurements are made.

**Local Nasal Factors Affecting Nasal NO**

A small decrease in nasal NO has been observed in smokers (145).

6. **EQUIPMENT RECOMMENDATIONS FOR MEASUREMENT OF EXHALED NO**

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Current NO analyzers employ the principle of chemiluminescence to measure NO. However, NO measurement based on alternative technologies may be developed in future. Equipment needs will vary according to the applications and test procedures. The following recommendations therefore refer to the proposed application.

**Online Analysis of Exhaled and Nasal NO in Adults and Children**

Table 1 displays the current minimum specifications required for accurate online measurement of exhaled NO and nasal NO. Exhalation flow rates for adults and children (0.05 L/s) are measured online at 37°C, 760 mm Hg saturated (BTPS), in keeping with other pulmonary function measurements.

<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Specification</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exhalation Flow Rate</strong></td>
<td>0.05 L/s</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>37°C</td>
</tr>
<tr>
<td><strong>Humidity</strong></td>
<td>760 mm Hg saturated (BTPS)</td>
</tr>
</tbody>
</table>
Breath-by-Breath Analysis

Breath-by-breath analysis may be necessary for young children and in ventilated subjects although the latter are not covered in this document. Each investigator will need to select an analyzer that is adequate in terms of response time and sampling rate for the particular frequency of the breaths or mechanical ventilation.

Offline Analysis

For offline analysis, the preceding specifications for online analysis apply with regard to sensitivity, accuracy, and range. The analyzer response time and tubing/setup lag time are not relevant. The specific requirements include the ability to display a steady NO plateau of at least 3 s by adjustment of the sample inlet flow, thus permitting a reliable signal. This is especially important with small volume samples, for example, from young children.

Material Requirements

Acceptable materials for tubing, connectors, and so on, include Teflon, stainless steel, siliconized materials, and Teflon-coated materials (for greater flexibility). Latex-related materials are not acceptable, owing to interference, including reaction with NO.

Calibration Requirements and Procedures

Zero NO gas. A reliable zero NO gas is essential for NO measurements. It is recommended that, rather than relying on medical-grade air, NO scrubbers (KMnO4 and/or charcoal) be used to generate zero NO gas. A good zero NO gas can be prepared by passing ambient air through an ozone generator, which converts any NO to NO2 before entry into the analyzer (NO knockout method).

Upper point calibration. Upper point calibration requires specially prepared NO calibration gases, most commonly in nitrogen. Commonly available concentrations range from 2 to 100 ppm, although levels as low as 100 ppb are available. Although standard gases may be supplied at the 100 ppm, although levels as low as 100 ppb are available. Al

Nitrogen. Commonly available concentrations range from 2 to

Sensitivity 1 ppb (noise, <0.5 ppb)

Signal/noise ratio > 2

Accuracy Exhaled NO: Better than 1 ppb Nasal NO: Better than 0.1 ppm

Range Exhaled NO: 1-500 ppb Nasal NO: 0.1-50 ppm

Response time* < 500 ms

Lag time* To be measured and reported by the investigator

Drift Less than 1% of full scale per 24 h

Reproducibility Exhaled NO: Better than 1 ppb Nasal NO: Better than 0.1 ppm

Flowthrough sensor To be measured by manufacturer and reported in publications

* Response time is defined as the delay from introduction of a square-wave signal and achievement of 90% of the maximum signal, inclusive of electronic delays and physical delays because of sample introduction, but not including tubing length. Lag time includes delays due to transit time through sample tubing in a particular application.

Influence of Extraneous Factors on NO Analysis

Ambient conditions. The instruments are fairly sensitive to ambient conditions, including exposure to sunlight, temperature, humidity, and so on. Due diligence should be taken to confirm stable ambient conditions, failing which the zero point should be rechecked before each sample is taken. For example, ambient temperature should not vary by more than 1°C from the time of calibration.

Humidity. With regard to sample humidity, drying the sample by passing it through a filter containing crystals (e.g., Drierite) may absorb NO and is therefore not recommended. The possible error in NO measurement due to humidity should be addressed by each manufacturer. One approach is to use a Nafion tube in the sample line, which equilibrates the sample with ambient humidity. In any case, steps should be taken to ensure that calibration gases (dry, ambient temperature) and samples (saturated, 37°C) are at the same humidity and temperature.

Interfering substances. Interfering substances include volatile anesthetic gases, which may be hazardous to the measurement system with regard to chemical reactions, oxidation of analyzer and tubing materials, and so on. Tolerances to quenching by CO2 and water vapor, which affect NO analysis (157), should be <1% NO per 1% level of interfering substance. Alcohol-containing disinfectants interfere with NO analysis (158).

Ancillary Features and Equipment

NO analysis specifications as detailed here are essential to the reliable measurement and reporting of exhaled NO data. However, some additional features will facilitate NO measurements according to the recommendations for a standardized technique in this statement. The following list of features would be part of an integrated NO measurement, analysis, and data-handling system:

Output: Provision of both analog and digital output, RAM storage card

Data collection capability: The following features may be helpful:

- Transmission of collected NO output data (NO, pressure, flow) to computer or monitor for real-time display
- Automatic sensing and indication of quality of exhalation, achievement of valid NO plateau according to that
defined in this statement (see Section 2), allowing termination of exhalation.
- Data storage
- Data analysis software allowing manipulation and display of results, etc.

**Biofeedback of exhalation parameters:** For adult and pediatric measurement of exhaled NO and nasal NO, biofeedback of exhalation parameters may be essential for those systems that generate constant flow in this manner.

**Sample flow rate:** A utomatic monitoring and display of NO analyzer sample flow rate, e.g., by including a rotameter.

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