

# Measurement of exhaled nitric oxide in young children during tidal breathing through a facemask

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Measurement of exhaled nitric oxide (eNO) offers a non-invasive means for assessment of airway inflammation. The currently available methods are difficult to apply in preschool children. We evaluated four methods potentially applicable for eNO measurement during tidal breathing in young children. eNO was assessed during tidal breathing in 24 children, 2–7 yr old, using a facemask which separated nasal and oral airflow. Facemasks with and without a one-way valve allowing exhalation through the nose were used. Expiratory flow control was not attempted. Measurements of eNO were performed both on-line and off-line. In 11 children, 8–12 yr old, measurements were compared with the standard single breath on-line method. eNO was significantly lower applying the one-way valve in on-line and off-line measurements in comparison with measurements without the valve [4.6 and 3.9 parts per billion (ppb) vs. 6.9 ppb and 6.5 ppb]. The mean within subject coefficient of variation (CV) was significantly lower in on-line measurements with the one-way valve (9.6%) compared with the other three methods (18.8, 27.7 and 29.3% respectively). Measurements with a facemask fitted with a one-way valve yielded similar eNO levels as the standard single breath method (7.0 ppb vs. 6.9 ppb) and reproducibility (9.8% vs. 7.1%). In conclusion, reproducible measurements of eNO can be obtained without control of expiration flow using a facemask fitted with a one-way valve on the nasal compartment. The likely explanation to this is that the one-way valve reduces the admixture of nasal NO, thereby improving the reliability of eNO measurements.

**Peter Fleng Daniel, Bent Klug and  
Niels Henrik Valerius**

Department of Pediatrics, Copenhagen University  
Hospital, Hvidovre, Denmark

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Peter Fleng Daniel, Department of Pediatrics,  
Copenhagen University Hospital, Hvidovre, Denmark  
Tel.: +45 3070 6679  
Fax: +45 5671 1479  
E-mail: fleng@dadlnet.dk

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Exhaled nitric oxide (eNO) could become a useful tool in diagnosing and monitoring airway inflammation in asthma (1–8). The methods employed for measurement of eNO can be categorized as either on-line or off-line methods. In on-line methods measurement of eNO takes place simultaneously with the breathing manoeuvre, whereas in the off-line methods eNO is collected in reservoirs for subsequent analysis.

Recommendations for standardized procedures for measurement of eNO (9, 10) address the dependency of eNO on exhalation flow and the possibility of mixture of oral and nasal NO and therefore outline recommendations on flow rate, flow duration and exhalation pressure.

However, methods that comply with these recommendations require considerable collaboration of the subject and are therefore only applicable to adults (11) and older children (12). Children younger than 5 yr of age can rarely meet the demands set by the recommendations and there have been few reports on methods applicable in young children (13–15).

Measurements of lung function can be performed in young children during tidal breathing using a facemask (16). This approach might be applicable for measurements of eNO. However, this method is hampered by the admixture of air from the nasal cavity which contains concentrations of NO 10–100 times higher (17, 18) than NO in air from the lower airways.

The purpose of this study was to evaluate four methods for measurement of eNO during tidal breathing in children 2–7 yr of age. To avoid admixture of nasally derived NO with NO from the lower airways, we used a parted facemask that separated nasal and oral airflow.

## Material and methods

### Subjects

Twenty-four children (11 girls and 13 boys), 2–7 yr of age [mean (s.d.): 4.7 (1.6) yr], completed all methods.

Comparison of the single breath on-line and the facemask with the one-way valve on-line methods was carried out in 11 children (seven girls and four boys) 8–12 yr of age [mean (s.d.): 10.9 (1.2) yr].

The parents completed a questionnaire on former or current airway disease and allergy. Written consent was obtained from the parents of the children.

The study was approved by The Scientific Ethical Committee for the Municipalities of Copenhagen and Frederiksberg (KF 01-325/98).

### Study design

*Collection and measurement of eNO.* The children were seated and asked to perform normal tidal breathing through the facemask. At least 1 min was allowed to pass to obtain normal breathing and clearing of ambient air. With both facemasks, collection and measurement of eNO were performed as (i) on-line measurement of eNO and (ii) concomitantly with the on-line measurement the exhaled air was collected in NO impermeable bags for subsequent off-line analysis.

Three satisfactory measurements, as described below, were required for each method. Each on-line measurement had a duration of 60 s and was executed as consecutive measurements without removing the mask. A fixed flow rate was not applied.

Off-line measurement was made by directly connecting the gas sampling tube to the gas sampling bag and calculating the mean value of eNO over 15 s.

*Measurement of eNO using facemask with one-way valve and using single breath on-line method.* On-line measurements using the facemask with the one-way valve were carried out as described above.

For single breath on-line measurements the children were seated and were asked to breathe

normally through a mouthpiece for at least 3 min to get accustomed with the setup. They were then asked to inspire to total lung capacity and immediately exhale at a constant flow of 100 ml/s.

### Methods

*Equipment.* Nitric oxide was measured on the Exhalyzer system (ECO Physics, Dürnten, Switzerland). In this setup the eNO is measured with a CLD AM 77 chemiluminescence analyser (ECO Physics), flow and pressure with a Spiroson SCIENTIFIC ultrasonic flowhead (nnd Medizintechnik AG, Zürich, Switzerland) and NO-free air is supplied as scrubbed ambient air contained in a 30 l collapsible bag (Hans Rudolph Inc., Kansas City, MO, USA). During inhalation, the NO-free air was conducted through a flexible tube to a one-way valve in the ingoing limb of a T-piece (Fig. 1), through the ultrasonic flowhead and on to a facemask or mouthpiece. Upon exhalation the exhalant was led through the other one-way valve in the outgoing limb of the T-piece and into collapsible 5 l NO impermeable PVF gas sampling bags (ADTECH, Stroud, Gloucestershire, UK). These bags were flushed with NO-free air before use. A fraction of the breathed air was removed from the airflow at a constant rate of 330 ml/min through a tube connected to the flowhead close to the mouth, and led to the NO analyser. The off-line collection period was 60 s or until the sampling bag was filled. At the same point mouth pressure was measured. A facemask (Hans Rudolph Inc.) covering both nose and mouth was applied. The mask was parted by a diaphragm into two separate compartments to prevent mixing of oral and nasal airflow (Fig. 2). Two different facemasks were used: one modified with a valve, allowing a part of the exhaled air to pass through the nose, and the other without. Total dead space was 58 ml.

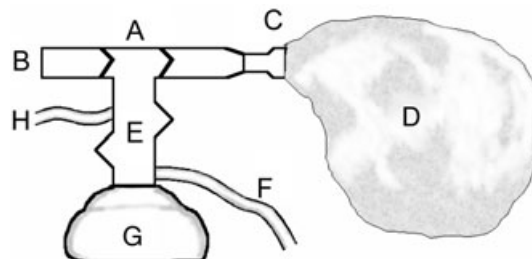


Fig. 1. Experimental setup: A, T-piece with one-way valves in the ingoing and outgoing limbs, respectively; B, inlet for NO-free air; C, restrictor; D, gas sampling bag; E, ultrasonic flowhead; F, NO sampling tube; G, facemask; H, pressure measurement tube.

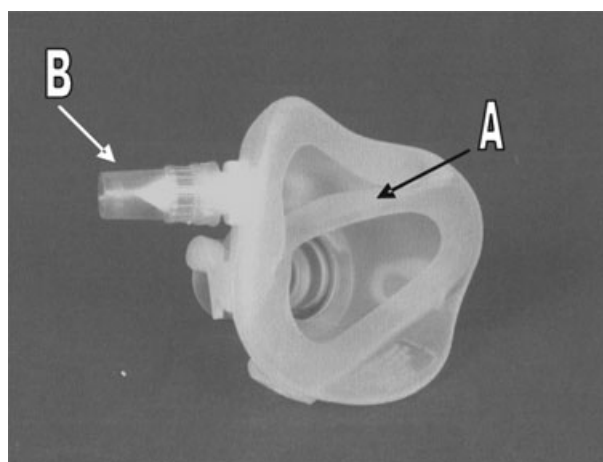


Fig. 2. Mask: Type with valve. A, diaphragm separating nasal and oral airflow; B, one-way valve mounted on the nasal compartment.

For single breath on-line measurements, a 9-cm long flexible silicone tube was used. The tube at one end was fitted with a stainless steel cuff to prevent occlusion. Total dead space was 29 ml.

Exhalation was performed through a resistance placed in the outgoing limb of the T-piece to obtain a mouth pressure of 5 cmH<sub>2</sub>O or more. As described in the *Software* section below, each eNO on-line measurement was calculated from five eNO plateaus according to the custom-made software. The data of the corresponding five pressure curves were scrutinized to confirm a pressure of 5 cm H<sub>2</sub>O or more before further data processing.

The NO analyser was calibrated daily according to the manufacturer's guidelines with a 10 ppm NO gas and calibration was verified with a 10 ppb NO gas (AGA, Lidingö, Sweden). NO free air supplied by the Exhalyzer-system was used throughout. The collapsible bag with NO-free air was kept only 75% filled to prevent overflow into the gas sampling bags.

**Software.** Measurements of NO, pressure and flow were registered with the Spiroware computer program (nidd Medizintechnik AG) on a computer connected to the Exhalyzer system. A custom-made software program was used for calculating the on-line eNO measurements (riis-edb, Nærum, Denmark) on a Mathcad 8 Professional platform (MathSoft Inc., Cambridge, MA, USA). Each on-line measurement had a duration of 60 s. The program was designed to detect the first five plateaus of eNO with a duration of 900 ms, in which the slope and the variation of each plateau should be no more than 15%

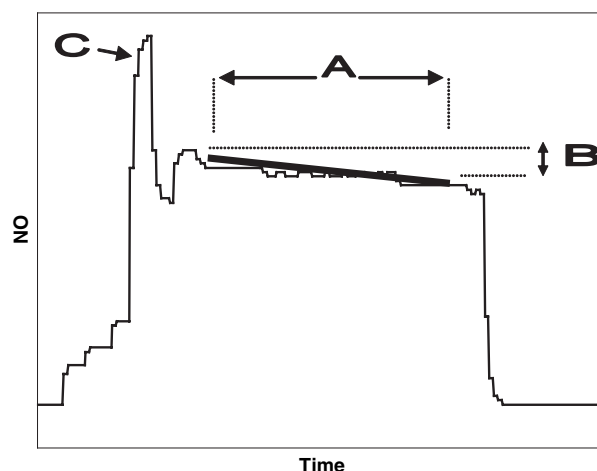


Fig. 3. Registration of NO during 1 breath cycle. A, duration of plateau; B, slope of plateau; C, peak representing initial admixture of nasal NO.

(Fig. 3); finally a maximum variation of 20% between the five plateaus was accepted.

For single breath on-line measurements an exhalation of 5 s was required. A plateau of 2 s at the end of the exhalation was identified and the mean of this plateau was calculated.

**Statistical analysis.** Coefficient of variation (CV) for each method is the mean of the compiled CV of every subject's three measurements with each method. The methods were compared by two-sample paired *t*-tests and are presented as the differences, SD of the differences and *p*-values. Age dependency was analysed by linear regression. Statistical analysis is performed on a computer package (SAS Institute Inc., Cary, NC, USA). *P* < 0.05 was considered significant.

## Results

Twenty-four of 28 children completed measurements with all four methods (two aged 2 yr; nine aged 3 yr; three aged 4 yr; four aged 5 yr; four aged 6 yr; and two aged 7 yr). Four children aged 2, 3, 4 and 5 yr were not able to complete all four methods. Twenty-one of the children were healthy; three had a history of asthma but no current symptoms.

The median values of eNO varied between 3.9 ppb in the off-line method using the mask with a valve and 6.9 ppb in the on-line method without the valve (Table 1 and Fig. 4). Irrespective of the type of measurement (on-line or off-line) eNO was significantly lower when using the facemask equipped with a valve. The differences between methods were statistical significant except from the difference between the on-line

Table 1. Median eNO values and CV of each method (n = 24)

|                             | Facemask with one-way valve |               | Facemask without one-way valve |               |
|-----------------------------|-----------------------------|---------------|--------------------------------|---------------|
|                             | On-line                     | Off-line      | On-line                        | Off-line      |
| Median (1–3 quartile) (ppb) | 4.6 (3.4–5.5)               | 3.9 (3.1–4.4) | 6.9 (4.9–8.6)                  | 6.5 (5.5–9.4) |
| CV (s.d.) (%)               | 9.6 (5.5)                   | 18.8 (12.4)   | 23.7 (17.4)                    | 29.3 (14.1)   |

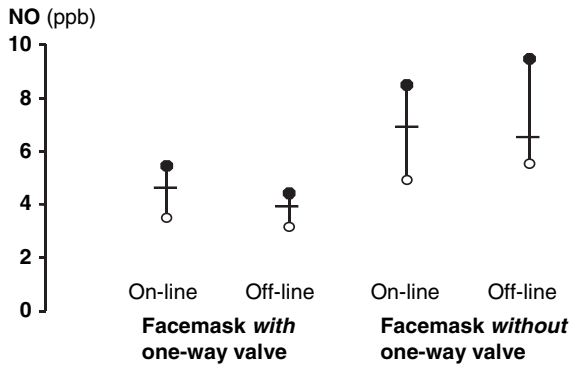


Fig. 4. Median eNO-values, 1. and 3. quartiles.

and the off-line methods using a facemask without a valve (Table 2).

The mean within subject CV was lowest in the on-line method with the valve (9.6%) and highest in the off-line method without valve (29.3%) (Table 1). The on-line method with the valve had a significantly lower CV than the other methods, and the CV of the off-line method with the valve was significantly lower than the CV of the off-line method without the valve (Table 2).

Eleven children completed measurements with both the single breath on-line and the facemask with the one-way valve methods. One of these children suffered from grass-induced rhinitis one

Table 2. Two sample paired t-tests comparing eNO and CV between methods. Mean differences between methods and SD of the differences. p: p-values of the differences. \*: denotes mean differences, SD of differences and p-values on comparing CV between methods. In method 1 and 2 a facemask fitted with a valve was applied for measurement of eNO on-line (1) and for concomitant collection of NO in impermeable bags subsequently analysed off-line (2). In method 3 and 4 a facemask without a valve was applied for measurement of eNO on-line (3) and for concomitant collection of NO in impermeable bags subsequently analysed off-line (4)

|             | Difference (SD) (ppb) | p       | Difference (SD)* % | p*      |
|-------------|-----------------------|---------|--------------------|---------|
| Methods 1–2 | 0.7 (2.6)             | 0.012   | 9.3 (13.9)         | 0.0034  |
| Methods 1–3 | –2.4 (3.6)            | 0.0048  | 14.1 (18.6)        | 0.0012  |
| Methods 1–4 | –2.0 (3.7)            | 0.0079  | 19.7 (17.8)        | <0.0001 |
| Methods 2–3 | –3.1 (3.8)            | <0.0001 | 4.8 (23.7)         | 0.34    |
| Methods 2–4 | –2.7 (3.1)            | <0.0001 | 10.4 (19.7)        | 0.023   |
| Methods 3–4 | 0.4 (3.1)             | 0.88    | 5.6 (19.5)         | 0.20    |

Table 3. Median eNO values and CV of each method. p-values of the two sample paired t-tests on the differences between methods (n = 11)

| Method                      | Standard single breath on-line | Facemask on-line with valve | p-value |
|-----------------------------|--------------------------------|-----------------------------|---------|
| Median (1–3 quartile) (ppb) | 6.9 (4.7–10.4)                 | 7.0 (4.6–8.1)               | 0.36    |
| CV (s.d.) (%)               | 7.1 (3.1)                      | 9.8 (4.1)                   | 0.10    |

had formerly suffered from wheezing. None of the children had airway symptoms at the time of measurement. Neither eNO nor CV showed significant differences between methods (Table 3). No age dependency was found in either group of children.

## Discussion

In the present study we evaluated four methods for measurement of eNO during tidal breathing in young children. The results show that under these conditions with no active cooperation of the children it is possible to obtain reproducible eNO measurements.

Previous studies have shown that an increase in expiratory flow rate results in a fall in eNO plateau values (19–23). Recommendations on eNO measurement have prescribed exhalation at a fixed flow rate (9, 10). However, young children are not capable of sustaining a fixed flow rate and this recommendation is therefore not applicable to young children, unless some technique for controlling expiratory flow is applied (14, 24). Only the single breath on-line measurements were performed at a fixed flow in the present study and only in older children.

We designed a software program for measuring eNO plateaus. The requirements to the software were elaborated to secure (i) a plateau of a certain length, (ii) a defined slope and variation of the plateau and (iii) that the five plateaus were not random plateaus, thus assuring that measurements represented eNO from the lower airways. The lack of controlled flow during measurements with the facemasks may, however, have increased the variability of measurements.

Presently no standard method is available to measure eNO levels during tidal breathing. Baraldi et al. (25) measured eNO on single plateaus between the third and the sixth minute of tidal breathing, and recently Hall et al. (26) reported eNO values based on mathematical calculations of single plateaus.

Nasal NO concentrations can be 100 times higher than NO concentrations in the lower airways (17, 18). Therefore, admixture of nasally derived NO can result in falsely high levels of

eNO. To overcome this problem other groups have performed measurements during tidal breathing in children wearing a nose-clip (27, 28). However, the use of a nose-clip is poorly accepted by young children and does not eliminate exchange of air through the open passage between the nasopharynx and the oropharynx. Moreover, some groups report that an expiratory resistance which secures a mouth pressure of 4–5 cmH<sub>2</sub>O or more results in closure of the soft palate and a reduction of the admixture of nasally derived NO (19, 29).

To separate nasal and oral airflow we used a parted facemask. In methods without a valve fitted on the nasal compartment eNO was higher compared with those methods where the mask was fitted with a valve on the nasal compartment (Table 1). During exhalation it is likely that a sustained high pressure in the nasal compartment occurs in the facemask without a valve, thus preventing closure of the soft palate. This enables admixture of nasal NO to the oral exhalant. Furthermore, the facemask is made of a soft material. The pressure with which it is held against the face may vary during measurements, and thus contribute to fluctuations in the pressure in the nasopharynx. We believe that the valve allows the pressure of the nasopharynx to equilibrate with the ambient pressure. Combined with an expiratory resistance which secures a mouth pressure of 4–5 cmH<sub>2</sub>O or more, this results in closure of the soft palate and a reduction of the admixture of nasally derived NO.

On-line methods showed higher eNO values than off-line methods (Table 1 and Fig. 4). One might have expected that eNO values were higher in the off-line methods compared with the on-line methods because of the peak of NO occurring at the start of an exhalation (Fig. 3). This peak increases the NO concentration in the bag sample but does not affect the on-line eNO plateau measurement. However, the peak of NO is short-lived compared with the total duration of the exhalation and thus only contributes with a small fraction to the NO in the gas sampling bag. Furthermore, the dead space between the mask and the two one-way valves in the T-piece (Fig. 1) contains NO-free air at the end of inspiration. This NO-free air will be conducted into the sampling bag upon exhalation and lower the concentration of NO.

Analysis on the differences between methods on levels of eNO showed significant differences except between the on-line and off-line methods using the facemask without a valve.

The CV was lower (Table 1) when using the facemask with the valve in comparison with the facemask without the valve, which is most likely the result of the reduced admixture of nasal NO. The lower CV in on-line compared with off-line methods can be attributed to the fact that variations in the breathing pattern may influence the off-line measurements whereas the data processing software automatically excludes extreme NO curves in on-line measurements.

Some authors (12, 20, 30) have found age dependency on eNO in children; others have not (25, 31, 32). In the present material, no age dependency could be found. This could, however, be explained by i) the number of subjects in each group and ii) each group's narrow age range.

In a group of 121 healthy 3–6-year-old children (including a few 2- and 7-year-old children), we used the method with the facemask fitted with the one-way valve. In this group age dependency on eNO was not found ( $p = 0.58$ ) (data on file).

Until now there have been few reports on eNO measurements in young children. One study has recently described a flow controlled technique for on-line measurement during tidal breathing (14). However, some cooperation from the child seemed to be necessary and also a skilled investigator should handle the flow control unit if not automated. Another study used off-line measurements during tidal breathing through a facemask covering the mouth (15). In accordance with our results they reported a high CV in a similar group of children. The CV of the on-line method with the facemask fitted with a valve was comparable with that reported by others (20, 33).

Finally the method with the facemask fitted with the one-way valve showed no significant difference in eNO values or CV when compared with the single breath on-line method.

In conclusion, on-line measurement while using a facemask fitted with a valve compared favourably with the other three methods by low CV. Furthermore, eNO measured by this method yielded lower values of eNO by reducing the influence of nasal NO. Comparing the on-line method with the facemask fitted with a valve with the recommended constant flow, single breath on-line method showed accordance as regards eNO values and variability.

Thus, the on-line method with the facemask fitted with a valve is applicable in young children and can improve the reliability of eNO measurements during tidal breathing.

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