

## Early View

Research letter

# Nasal nitric oxide measurement variability to establish a standard for reliable results

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Please cite this article as: Beydon N. Nasal nitric oxide measurement variability to establish a standard for reliable results. *ERJ Open Res* 2022; in press (<https://doi.org/10.1183/23120541.00028-2022>).

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## **Nasal nitric oxide measurement variability to establish a standard for reliable results**

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**"take home" message:** A repeatability of 10% for NO measurements obtained with the velum closed in the same or both nostrils is relevant, while measurements taken during tidal breathing should aim for a repeatability of 20% and 30%, respectively.

## **To the editor**

Nasal nitric oxide (nNO) measurement is a first line test used to increase the post-measurement probability of primary ciliary dyskinesia in subjects with symptoms consistent with this diagnosis [1]. The accuracy of nNO measurement is essential since it will orientate the work-up towards tests that are usually highly specialised and sometimes invasive tests. Accuracy of biological measures relies on the technical and on the biological variability. While the accuracy of NO analysers is known better for chemiluminescence devices (eg.  $< 1$  ppb with 1% linearity from 0.1 to 5000 ppb, CLD 88<sup>®</sup> Eco Medics<sup>LTD</sup>) than for widely used electrochemical devices [2] (eg.  $\pm 5$  ppb for values  $< 50$  ppb and 10% for values  $> 50$  ppb, Niox Vero<sup>®</sup>, Circassia<sup>LTD</sup>), little is known on the biological variability of nNO measurements, except for increased nNO output variability in adult with rhinitis compared to healthy subjects and the positive effect of training on the level of nNO taken during expiration against a resistance (nNO-ER) in children [3, 4]. The PCD Foundation Clinical Center Network and Genetic Disorders of Mucociliary Clearance Consortium recommends for nNO-ER to sample both nostrils and to perform at least two measurements per nostril with the aim of obtaining a 10% repeatability [4]. For measurements performed during tidal breathing (nNO-TB) it is recommended to record peak values within 10% and aim for inter-nostril repeatability of 10% [4].

Using, in most cases, the coefficient of variation (CV) of three measurements to assess the variability of nNO in children and adults, small studies ( $n \leq 50$ ) reported intra-nostril CV during breath hold (BH) (CV 3.8%, 9% and 12.5%) [5-7], ER (CV 5%) [7] or TB (CV 9.9% and 11%) [7, 8], and inter-nostril CV of nNO-BH (CV 10%) [9, 10]. Two large studies conducted in children and adults established nNO-ER CV at 6.9% [IQR 4.1–14.5%] for six nNO-ER measurements performed three in each nostril ( $n=226$ ) [11], and 10.4% (SD 14.2%) for three measurements performed in same nostril ( $n=282$ ) [12]. The latter also established the CV (SD) of

nNO-BH at 6.7 (8.8)% and of nNO-TB at 12.3 (15)%. In line with these results, we previously found an inter-nostril repeatability up to 10% more frequently for nNO-ER (93%) and nNO-BH (78%) than for nNO-TB (57% mean of 5 peaks –TB5p, and 68% mean of 10s period –TB10s) [13], possibly because of the constantly variable nasal flow associated with the permanent open velum during TB. To date, the frequency of peaks within 10% on nNO-TB traces has never been evaluated.

Since many factors can influence the value of nNO such as the size and the local airflow aerodynamic of the nostrils, or the degree of velum closure, and because, in large studies, the CV or the 10% repeatability of nNO measurements depended of the methods of measurement, we sought to evaluate in a large paediatric population tested in routine practice the relevance of the 10% repeatability criterion. We studied values obtained using different methods of measure (two methods with velum close (VC), ie. ER and BH, and two TB methods, ie. TB5p and TB10s, the latter to mimic electrochemical sampling). We also evaluated relationships between nNO repeatability and age (different size of nostrils and ability to close the velum) or level of nNO (more difficulty in reaching % repeatability in low nNO values).

We retrieved retrospectively from our database of children referred for nNO measurement between 2009 and 2022, all nNO results checked for trace quality by the author (NB). Each child could contribute for one or more visits, and for nNO-ER and/or nNO-TB and/or nNO-TB measurements on one (if repeated) or both nostrils.

Nasal NO was measured using a chemiluminescence NO analyser (NIOX Flex<sup>®</sup>, Aerocrine<sup>LTD</sup> Solna, Sweden, until 2014, then CLD 88<sup>®</sup>, Eco Medics AG<sup>LTD</sup>, Duernten, Switzerland, with a sampling flow of 0.3 L.min<sup>-1</sup> and 0.33 L.min<sup>-1</sup>, respectively) in subjects without obstructed nose (inspection of the ventilation one nostril obstructed alternatively).

Most of the data showed a non-Gaussian distribution. Results are medians [25<sup>th</sup>; 75<sup>th</sup>] percentiles and numbers (percentages). nNO results are displayed in concentration (ppb). The inter- or intra-nostril repeatability is the difference between two measurements expressed as percentage of the maximal inter- or intra-nostril value, respectively. The nNO-TB between-peak variability is the CV of five peaks and the minimum peak as a percentage of the maximum peak (Min%Max). We used non-parametric tests to study correlations (Spearman correlation) and to compare values (Mann Whitney, Kruskal-Wallis, Friedman and Wilcoxon matched-pairs sign rank tests), and the Chi<sup>2</sup> or Fisher's exact test to compare proportions of children (GraphPad Prism<sup>®</sup> version 6.01, San Diego, CA, USA). Families were informed of the possible retrospective use of their children's nNO results and gave consent electronically.

We included 409 children, 238 (58.2%) males, who had repeated nNO measurements in both nostrils and/or in the same nostril using the same method (ER, BH or TB) and who contributed for 550 visits (186 children had 1 visit, 148 had 2 visits, 216 had 3 visits or more) at a median age of 7.9 [5.7; 11.5] years. We assessed the inter- and intra-nostril repeatability using respectively, 271 and 237 nNO-ER measurements, 227 and 141 nNO-BH measurements, 395 and 140 nNO-TB5p measurements, 337 and 96 nNO-TB10s measurements. Children tested with TB method were significantly younger than children tested with ER or BH methods (6.3 [4.2; 9.6], 8.6 [6.4; 12.3], and 9.9 [7.4; 13.2] years, respectively;  $P < 0.0001$ ). Median nNO-ER, nNO-BH, nNO-TB5p and nNO-TB10s were 325.0 [76.2; 652.6], 280.0 [87.4; 596.0], 162.4 [44.0; 354.2] and 120.4 [32.6; 287.3] ppb, respectively.

Inter-nostril repeatability was significantly different among the four methods of measurements (nNO-ER 5.1 [2.0; 10.0]%, nNO-BH 5.6 [2.3; 13.4]%, nNO-TB5p 17.1 [7.2; 37.4]%, and nNO-TB10s 19.4 [7.8; 36.4]%,  $P < 0.0001$ ) with no difference within the two VC methods ( $P = 0.21$ ) or

the two TB methods ( $P = 0.55$ ). There was no significant correlation between the inter-nostril repeatability and age or maximal nNO value for all methods (all  $r$  between -0.12 and 0.08, all  $P > 0.08$ ), except for a poor but significant correlation between inter-nostril nNO-TB variability and age (nNO-TB5s,  $r = 0.11$ ,  $P = 0.025$  and nNO-TB10s,  $r = 0.15$ ,  $P = 0.005$ ).

Intra-nostril repeatability was different according to the method of measurement (nNO-ER: 5.7 [2.6; 11.8]%, nNO-BH: 5.6 [1.8; 11.0]%, nNO-TB5p 9.6 [3.4; 19.2]%, nNO-TB10s 10.0 [4.0; 22.6]%,  $P < 0.0001$ ), without any difference within the two VC methods or the two TB methods ( $P = 0.33$  and  $P = 0.47$ , respectively). There was no significant correlation between the intra-nostril repeatability and age or maximal nNO value for all methods (all  $r$  between -0.13 and 0.05, all  $P > 0.13$ ) except for poor but significant correlations between intra-nostril nNO-TB5s repeatability and age ( $r = 0.23$ ,  $P = 0.007$ ) or maximal value ( $r = 0.17$ ,  $P = 0.042$ ).

The distributions of nNO-ER, nNO-BH and nNO-TB repeatability, and the between-peak variability of nNO-TB according to different criteria are shown in Table 1.

This study showed that VC methods had better within-occasion repeatability than TB methods, while both VC methods and both TB methods had similar repeatability. The proportion of children with nNO inter-nostril repeatability lower than 10% was slightly lower to that previously published [12], but acceptable for VC methods. Conversely, 30% inter-nostril repeatability for nNO-TB measurements would be necessary to achieve proportions of successful measurements similar to those of nNO-VC. Results were similar for intra-nostril repeatability, with 20% repeatability of nNO-TB needed to achieve similar repeatable measurement frequency as VC methods (Table 1). However, between a third and a quarter of repeated measurements failed the repeatability criterion, in favour of testing both nostrils and twice each.

In conclusion, in a large cohort of patients with a wide range of nNO values, we confirmed that an inter-measurement repeatability of 10% is acceptable for inter- or intra-nostril nNO-ER or nNO-BH measurements. These results cannot be extended to nNO-TB (except for between peaks variability), which would require 30% inter-nostril repeatability or 20% intra-nostril repeatability to achieve similar frequencies of repeatable measurements. However, since these repeatability criteria are not met in about a quarter of cases, a second measurement on the same side or on the other side can deviate significantly from the first, in favour of sampling both nostrils and at least twice each, in order to record the best value. This standard could change the interpretation of nNO in children with low/borderline results on a single measure.

**Acknowledgments:** We are grateful to Jessica Assouline, Michèle Boulé, Houda Guillo, Marie-Claude La Rocca, Marc Koskas, Fatma Lacin, Lucia Maingot, and Noria Medjahdi for their help in supervising the children's nasal nitric oxide measurements, and to Claire Goaguen, Pascale Jacquemart, Fanny Koëth, Valérie Le Bail, Isabelle Schmitt, and Françoise Vallée for their technical assistance, all working in Unité d'Exploration Fonctionnelle Respiratoire, Hôpital Armand Trousseau, Paris, France

## References

1. Lucas JS, Barbato A, Collins SA, *et al.* European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. *Eur Respir J* 2017; 49: 1601090.
2. Beydon N, Ferkol T, Harris AL, *et al.* An international survey on nasal nitric oxide measurement practices for the diagnosis of primary ciliary dyskinesia. *ERJ Open Res* 2022; 8: 00708–02021.
3. Djupesland PG, Chatkin JM, Qian W, *et al.* Aerodynamic influences on nasal nitric oxide output measurements. *Acta Otolaryngol (Stockh)* 1999; 119: 479–485.
4. Shapiro AJ, Dell SD, Gaston B, *et al.* Nasal Nitric Oxide Measurement in Primary Ciliary Dyskinesia. A Technical Paper on Standardized Testing Protocols. *Ann Am Thorac Soc* 2020; 17: e1–e12.
5. Kharitonov SA, Walker L, Barnes PJ. Repeatability of standardised nasal nitric oxide measurements in healthy and asthmatic adults and children. *Resp Med* 2005; 99: 1105–1114.
6. Harris A, Bhullar E, Gove K, *et al.* Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC Pulm Med* 2014; 14:18.
7. de Winter-de Groot KM, van der Ent CK. Measurement of nasal nitric oxide: evaluation of six different sampling methods. *Eur J Clin Invest* 2009; 39: 72–77.
9. Karadag B, James AJ, Gültekin E, *et al.* Nasal and lower airway level of nitric oxide in children with primary ciliary dyskinesia. *Eur Respir J* 1999; 13: 1402–1405.
10. Struben VMD, Wieringa MH, Mantingh CJ, *et al.* Nasal NO measurement by direct sampling from the nose during breathhold: Aspiration flow, nasal resistance and reproducibility. *Eur Arch Oto-Rhino-Laryngol* 2006; 263: 723–728.
11. Boon M, Meyts I, Proesmans M, *et al.* Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest* 2014;44:477–485.
12. Marthin JK, Nielsen KG. Choice of nasal nitric oxide technique as first-line test for primary ciliary dyskinesia. *Eur Respir J* 2011; 37: 559–565.
13. Beydon N, Chambellan A, Alberti C, *et al.* Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. *Pediatr Pulmonol* 2015; 50: 1374–1382.



**Table 1 – Proportions of repeatable nasal nitric oxide measurements according to different criteria of repeatability and between-peak variability in the 409 study children**

	Repeatability of nNO measurements		
	$\leq 10\%$	$\leq 20\%$	$\leq 30\%$
INTER-NOSTRIL			
nNO-ER, n = 271	208 (76.8%)		
nNO-BH, n = 227	157 (69.2%)		
nNO-TB5p, n = 395	138 (34.9%)		267 (67.6%)
nNO-TB10s, n = 337	107 (31.8%)		226 (67.1%)
INTRA-NOSTRIL			
nNO-ER, n = 237	171 (72.2%)		
nNO-BH, n = 141	105 (74.5%)		
nNO-TB5p, n = 140	78 (55.7%)	137 (78.6%)	
nNO-TB10s, n = 96	51 (53.5%)	69 (71.9%)	
	Intra-measurement variability of five peaks during TB		
nNO-TB5p CV, n =157	135 (86%)	148 (94.3%)	
nNO-TB5p Min%Max, n = 157	105 (66.9%)	137 (87.3%)	

nNO: nasal nitric oxide; ER: expiration against a resistance; BH: breath hold; TB: tidal breathing; CV: coefficient of variation; Min%Max: minimum peak as a percentage of the maximum peak