Early View

Original research article

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An international survey on nasal nitric oxide measurement practices for the diagnosis of primary ciliary dyskinesia

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Take home message There is considerable variability in routine practices for measuring nasal nitric oxide (nNO) in patients suspected of primary ciliary dyskinesia. Guidance is needed for professionals using different techniques, equipment and methodology in children.

Running title: Survey describing nasal nitric oxide measurement

Abstract

Nasal nitric oxide (nNO) measurements are routinely used in the assessment of patients suspected to have primary ciliary dyskinesia (PCD), but recommendations for performing such measurements have not focused on children and do not include all current practices. To guide the development of a European Respiratory Society-supported technical standard for nNO measurements in children, an international online survey was conducted to better understand current practices for measuring nNO among providers involved in PCD diagnostics.

Seventy-eight professionals responded, representing 65 centres across 18 countries, mainly located in Europe and North America. Nearly all centres measured nNO in children and more than half of them performed measurements before 5-years of age. The test was often postponed in children with signs of acute airway infection. In Europe, the electrochemical technique was more frequently used than chemiluminescence. A similar proportion of centres performed measurements during exhalation against a resistance (49/65) or during tidal breathing (50/65) with 15 centres using only exhalation against a resistance and 15 centres using only tidal breathing. The cut-off values used to discriminate PCD was consistent across centres using chemiluminescence analyzers and these centres reported results as an output ($\text{nL}\cdot\text{min}^{-1}$). However, cut-off values were highly variable across centres using electrochemical devices, and nNO concentrations were typically reported as ppb.

This survey represents the first to determine real-world use of nNO measurements worldwide and revealed remarkable variability in methodology, equipment, and interpretation. These findings will be useful to standardize methods and training.

Key words: bronchiectasis, diagnostic tool, children, adult, chronic bronchitis, mucus clearance

Introduction

Primary ciliary dyskinesia (PCD) is a rare inherited disorder affecting motile cilia. It is characterised by impaired mucociliary clearance leading to chronic upper and lower respiratory tract infections from infancy; laterality defects occur in 50% of individuals and subfertility is common. The diagnosis is often delayed or missed because the presentation can be variable and individual symptoms non-specific [1]. The diagnosis currently relies on the identification of typical ultrastructural cilia defects by transmission electron microscopy (TEM) or biallelic pathogenic variants in a known PCD-associated gene. Ciliary beat analysis using high speed video microscopy and immunofluorescent labelling of ciliary proteins are also used as diagnostic tools. However, these methods require highly specialised resources that are not widely available [2, 3].

Nitric oxide (NO) production is reduced in the nasopharynx and paranasal sinuses of most people with PCD [4]. Thus, measurement of nasal nitric oxide (nNO) production has become a useful adjuvant test in those presenting with compatible symptoms, once cystic fibrosis or immune defects have been excluded [5, 6]. There are established, validated methods using chemiluminescence analysers, in which nNO transnasal flow is measured in series during a respiratory manoeuvre with velum closure [7]. The North American PCD Foundation Clinical Center Network and the North American Research Network (Genetic Disorders of Mucociliary Clearance Consortium) has recently updated these recommendations. Their technical standard focuses on chemiluminescence devices for nNO measurements while exhaling against a resistor in children 5-years of age or older, and tidal breathing methods in pre-school children or others who are unable to exhale into a resistor [8]. Alternative approaches, such as breath-holding, pursed-lip breathing through the mouth, voluntary elevation of the soft palate by a

trained subject, or nasal exhalation during humming, have also been suggested but are used less often [7]. In addition, these standards highlighted other important factors, such as the flow at which nasal air was sampled or the impact of ambient NO levels [7].

Recently, more affordable portable devices have emerged that measure NO using electrochemical technology. For these procedures, the test is predefined by industrial specifications without the benefit of a graphical (online) display of the NO level, hence hindering the investigator's ability to select the optimal NO sample [9–11]. While such devices are CE marked and approved for use in Europe, they have not been fully validated against established PCD diagnostic tests in large multicentre trials. As a consequence, there is still a need to standardise and validate nNO measurements using these analysers [8, 10].

Perhaps more important, infants and younger children cannot correctly perform respiratory manoeuvres to close the velum as recommended in existing guidelines, leaving no choice but to sample the nNO during tidal breathing [12]. Similarly, there is no standardized approach to measure or interpret nNO measured during tidal breathing.

A European Respiratory Society (ERS)-supported International task force (TF) was recently created and charged to produce a technical standard for nNO measurements in children suspected to have PCD, reviewing various equipment and methodologies. Little is known regarding current practices for measuring nNO worldwide, so to better understand current practice and needs, we surveyed professionals who routinely use nNO as an adjuvant tool for PCD work-up. We assessed equipment and routine procedures used to measure and interpret nNO in various settings, focusing on children, and assessing how clinicians resolve technical and environmental factors which might influence the result in younger populations. False

positive tests mainly due to technical issues would likely expose children to unnecessary and costly invasive tests. Alternatively, false negative tests would delay the diagnosis of PCD.

Material and Methods

Questionnaire

In December 2020, three members of the TF (NB, AH, JSL) constructed a brief survey which was circulated between TF members for comments, revisions, and amendments. The final version was created as an electronic survey using Microsoft Forms software in January 2021.

After questions concerning the respondents and their service, the survey included 37 stem questions and seven sub-questions, each depending on the answer to a stem. The last item of the questionnaire was a free text box for any relevant comments (survey is in Supplementary File – Annex 1).

Briefly, the questionnaire asked about the number of clinical tests undertaken annually at the centre, the designation and the training of the professionals performing the measurements, age categories of the population routinely tested, equipment available for measuring nNO at the centre, and response to environmental considerations (respiratory infections, ambient NO). Additional information included the testing protocol and number of measurements performed on each patient, the respiratory manoeuvres performed to measure nNO and repeatability criteria. Lastly, the survey queried standardisation of the nNO measurement interpretation and reporting.

Participants

We sent an explanatory email with a link to Chairs to circulate their international clinical members and research collaboratives; these included the ERS-funded BEAT-PCD Clinical

Research Collaboration (CRC), ERS group 7.1 (Paediatric respiratory physiology & sleep) and group 9.1 (Respiratory function technologists/Scientists), the National Institutes of Health-funded Genetic Disorders of Mucociliary Clearance Consortium, and the PCD Foundation's Clinical Center Network. In addition, paediatric pulmonologists from countries represented in the American Thoracic Society (ATS) Virtual International Pediatric Pulmonology Network (VIPPN) roster contacted their national respiratory societies and other national working groups to distribute the invitation to participate in the survey.

The email asked individuals to complete the survey if they were responsible for measuring nNO at their centre; if measured by somebody else, they were asked to forward the link to that colleague.

Analyses

Responses collected between January and March 2021 were extracted into an Excel file. The number and proportion of centres are reported in percentage with the 95% confidence interval (CI). Comparisons between groups were performed using Chi-Square test or Fisher exact test, as appropriate, using GraphPad Prism (version 6.01). The *P*-value confirmed a statistical relationship when ≤ 0.05 (two-sided).

Results

Questionnaire respondents

There were 147 individuals who followed the link to the survey. Seventy-eight (53%) were eligible and completed the survey. These respondents were from 65 different centres and 18 countries; we used only one respondent per centre for analysis when there was duplication to

avoid bias. Respondents were mostly from centres in Europe and North America. All participating centres and the main results are available in Tables 1 and 2.

Sixty-seven (46%) individuals did not perform nNO measurements, representing 56 different centres located in 11 different countries (Algeria, Australia, Bolivia, Bulgaria, France, Ireland, Morocco, the Netherlands, UK, Spain, Thailand, and Turkey), and they were therefore not eligible to complete the survey. Two individuals started the survey, but did not complete the questionnaire; their surveys were not included in the final analysis.

Centre activity and personnel training

More than 25 annual tests were regularly performed in most of the responding centres, some exceeding 100 tests per year (main results per centre are shown in the supplementary Table E1). As many as four physicians or staff members performed nNO measurements in two-thirds of the centres, but a larger number of professionals could be routinely involved (between 12 to 25 providers) without relationship to the number of tests performed annually. Notably, 11/27 centres performing fewer than 25 tests per year had between 6 and 15 professionals/staff obtaining the nNO measurements.

The professional backgrounds of staff who perform nNO measurements are listed in Table 1. Physiologists and respiratory scientists most often obtained the measurements, and, along with paediatric pulmonologists, accounted for the main professionals answering the survey. A training program to teach personnel how to perform nNO measurements existed in more than half of the centres. It was usually delivered in a standardised format by a specific member of the team or consortium. However, at 7 sites, all using electrochemical devices, the only source of training was provided by an industry representative. Comparisons between centres using chemiluminescence or electrochemical devices are included in the Supplementary File.

Population tested and equipment used

Both children and adults were tested in two-thirds of the responding centres, and roughly a quarter of centres tested only children. Children younger than 5-years of age could be tested in approximately half of the centres (34/65). The majority of centres would postpone measurements if concerned about confounders, especially recent respiratory tract infections, which typically delayed testing for 2 to 4 weeks.

More responding centres used only an electrochemical device compared to sites that used only chemiluminescence devices. The NiOX VERO (Circassia[®]) was used at most centres (35/41) as an electrochemical device, while CLD 88 (Ecomedics[®]) was the predominant chemiluminescence analyser (30/33). Higher annual number of tests performed and younger age of children tested were more likely in centres using a chemiluminescence device (Supplementary File).

The sampling flow of the device was usually between 0.25 and 0.40 L.min⁻¹ among the two-thirds of centres that disclosed this information (Table 1). The survey did not determine whether the sampling flow was regularly checked before tests, but most North American sites measured this as part of their standard operating procedure. Ambient NO was measured daily or before each nNO measurement in nearly two-third of the centres, particularly in centres having a chemiluminescence device (Table 2 and Supplementary File).

Nasal nitric oxide measurements

The sampling of both nares with at least two measurements per naris was performed in most centres (Table 2), but sites that had only an electrochemical device on site performed fewer repeated measurements (Supplementary File). Only four centres performed a single measurement in one nostril (Table E1). All centres reported using either exhaling against a

resistance or tidal breathing methods to measure nNO (Figure 1). Tidal breathing with an open mouth was used in 44% of centres that performed this method. The maximum acceptable intra-measurement repeatability of nNO was set at 10% by most sites (32/45).

Nasal nitric oxide results and interpretation

A standardised report for nNO results was used by approximately two-thirds of the centres. Nasal NO was more frequently reported as a concentration (ppb) than as an output ($\text{nL}\cdot\text{min}^{-1}$) (Table 2), particularly by those sites that used electrochemical devices (Supplementary File).

The cut-off value for nNO output were set at $77 \text{ nL}\cdot\text{min}^{-1}$ for exhalation against a resistance or for tidal breathing methods at most centres. Nevertheless, cut-offs for “normal” nNO values varied among sites, especially among centres using electrochemical analysers, which more often expressed the nNO cut-off as a concentration. The cut-off values, respiratory manoeuvres, and units used to report nNO results are shown in Figure 2.

Discussion

Repeatedly low nNO values in people with key clinical features are highly suggestive of PCD. nNO measurements have become a useful first-line diagnostic tool, provided the measurement is performed accurately. Accurate measurements are imperative to avoid false positive and false negative results.

Results of an international survey, mostly answered by European and North American centres, were collected, and analysed to determine the variability in nNO measurement practices (Table 1 and E1). In North America, standard operating procedures and technical standards manuscript that focuses on use of the chemiluminescence technique is likely to account for the

more uniform responses across these centres compared to the rest of the world. [7, 8] Indeed, the individual practices highlighted, such as age of children tested, recording of ambient NO, number of measurements performed in a subject, and reporting standards, may be due to the use of specific devices or techniques (chemiluminescence or electrochemical). More worrisome was that a large proportion of centres did not have standardized protocols or provide formal training for staff performing nNO measurements. These findings confirm the need for standardized and well validated methods for nNO measurements, and these are particularly lacking for the electrochemical technique.

Most responding centres routinely tested children. Interestingly, more than half of the centres measure nNO in children under 5 years of age despite the absence of guidelines or established cut-off values for infants and pre-school children [2, 3].

At nearly two-thirds of the centres, nNO measurements were postponed if the child had a recent or intercurrent respiratory infection. Recent respiratory infections have been shown to reduce nNO values in infants, but the time required for the injured sinonasal epithelium to recover and return to the usual NO production level is unknown [13].

Ambient NO level was assessed in roughly two-thirds of the centres. Ambient NO can increase measured nNO, but there is no consensual cut-off for high ambient NO or an effective way to “correct” the measurement [14–17]. Nevertheless, the majority of centres measuring ambient NO levels (34 out of 47 centres) took into account its’ value when interpreting nNO results [7, 18]. Not all sites reported ambient NO levels (Table 2).

Repeatability was assessed in the vast majority of centres by sampling both nares and performing more than one measurement per naris. All published guidelines recommend repeating nNO measurements to confirm reliability and accuracy of the measured value.

Better between-naris repeatability has been reported in methods involving velum closure compared to the tidal breathing method [14], which may reflect the variable airflow in nares during tidal breathing. Regardless of the method used, nNO can significantly vary between nares due to mechanical or anatomical factors, demonstrating the necessity to sample both nares, and report the highest value.

Because the recommended method to measure nNO when exhaling against a resistance requires the subject to be cooperative, it was expected that centres testing young children would use alternative approaches, such as tidal breathing. This was reflected in survey results which showed that these two methods (tidal breathing and exhaling against a resistance) were equally used across the responding centres (Figure 1). The tidal breathing method has been extensively used and results have been shown to correlate with measurements obtained using the exhalation against a resistance method, though the tidal breathing method may be less accurate in distinguishing PCD children from unaffected children [12, 14]. Fifteen centres exclusively used the tidal breathing method, even though there are no published guidelines describing its accuracy or interpretation of results in children of all ages [9, 12–14, 19–24].

Finally, cut-off values varied between centres, depending on the technique and equipment used, and the method of measurement (Figure 2). The cut-off measures were much more uniform across centres that expressed nNO levels as an output ($\text{nL}\cdot\text{min}^{-1}$) when compared to those reporting NO concentration (ppb), which reflect the differences between chemiluminescence versus electrochemical techniques, respectively (see Supplementary File). The unique cut-off in $\text{nL}\cdot\text{min}^{-1}$ was determined in a large multicentre study that included results from different chemiluminescence analysers sampling gas at 0.50, 0.33 or 0.30 $\text{L}\cdot\text{min}^{-1}$ [25]. The sampling flow of the device has an effect on NO output and measured concentration,

but it has been demonstrated that in this narrow range of sampling flows, typical for measurements in children (Table 1), nNO output can confidently be compared [25]. However, significantly larger sampling flows would hamper the comparison between cut-off values [14, 26]. It is to be noted that in the survey, one-third of the responding centres did not know about the sampling flow of their device (Table 1), and others did not record it (Table 2), thus hampering inter-centre comparisons of results. Discrepant cut-off values for PCD have been published depending on the study population (number of subjects, genetic background, diagnostic criteria and health status) included in the ROC analyses and the sensitivity and specificity chosen by the investigators [9, 10, 14, 20, 21, 27, 28].

The cut-off values used for nNO output were similar, regardless of the method used, exhalation against a resistance or tidal breathing, even though cut-off values measured during tidal breathing are often lower than cut-off values measured during exhalation against a resistance in which the velum is closed [12, 14, 19–21]. However, accurately measuring nasal output flow during tidal breathing is problematic, because the tidal breath superimposes local flow to the device's sampling flow, resulting in variability that can increase when subjects close or open their mouths [19]. We do not know whether these limitations would strongly impact the difference between nNO cut-off values using tidal breathing or exhalation against a resistance method, but similarity between the two methods when values are expressed using $\text{nL}\cdot\text{min}^{-1}$ is doubtful.

The strength of this study is the large number of international professionals who responded. There were few missing responses which ensures the reliability of the data presented. However, the questionnaire was not distributed to paediatric otolaryngologists or out-of-hospital practices; therefore, our results may not fully reflect the true variability in. It is likely

that these settings would more frequently use electrochemical devices and different protocols, possibly with fewer measurements per subject, and variable cut-off values, thus adding to the practice variability that was noted.

In conclusion, this survey clearly shows the need to standardise and validate nNO testing in children worldwide for both chemiluminescence and electrochemical analysers. Standardization will improve test consistency, reliability and accuracy. Education and training are also needed. The current international efforts to produce technical standards for nNO testing in children for PCD will be critical toward achieving this goal.

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Tables

Table 1 – Main results for routine performance of nasal nitric oxide measurements in 65 centres responding completely in the survey

	Number of centres	Percentages of centres	95% CI of percentages
Countries			
United Kingdom	14	22	[12; 33]
United State of America	13	20	[11; 32]
France	9	14	[7; 25]
The Netherlands	7	11	[4; 21]
Belgium	4	6	[2; 15]
Australia	3	5	[1; 13]
Others with 1 or 2 respondents*	14	22	[12; 33]
Number of tests performed per year			
<25 tests	27	42	[29; 54]
>25 tests	38	58	[46; 70]
Of which >100 tests	15	23	[14; 35]
Qualification of professionals performing nasal NO measurement			
Physiologists/Respiratory therapists	21	32	[21; 45]
Pulmonary function technicians	9	13	[6; 24]
Paediatric Pulmonologists	5	8	[3; 17]
Nurses	4	6	[2; 15]
Others or association of professionals	26	40	[30; 53]
Training programme for measuring nasal NO			
Yes, standardised	26	40	[30; 53]
Yes, informal	9	13	[6; 24]
No	30	46	[34; 59]
Population tested			
Children and adults	44	68	[55; 79]
Only children	17	26	[16; 39]
Only adults	4	6	[2; 15]
Younger age of children tested in 61 centres			
≤ 2 years	25	41	[29; 54]
2-5 years	9	15	[7; 26]
≥ 5 years	27	44	[31; 58]
Test postponed if recent respiratory infection			
Total	64		
No	20	31	[20; 44]
Yes	44	69	[56; 80]
<i>Delay for 2 weeks</i>	<i>22/44</i>	<i>50</i>	<i>[35; 65]</i>
<i>Delay for 4 weeks</i>	<i>15/44</i>	<i>34</i>	<i>[20; 50]</i>

Technique used to measure NO	Total	64		
	Electrochemical	31	48	[36; 61]
	Chemiluminescence	23	36	[24; 49]
	Both	10	15	[8; 27]
Sampling flow of the device				
	Unknown	22	34	[23; 47]
	0.25 to 0.40 L.min ⁻¹	30	46	[34; 59]
	> 0.40 L.min ⁻¹	13	20	[11; 32]

Abbreviations: CI: Confidence Interval; NO: nitric oxide

* Canada, Chile, Czech Republic, Switzerland, Brazil, Denmark, Germany, Ireland, Mexico, Palestine, Portugal, and Tunisia

Table 2 – Methods used to measure and report nitric oxide results in 65 study centres

	Number of centres	Percentages of centres	95% CI of percentages
Measurement of ambient NO			
Daily or before each test	41	63	[50; 75]
Not systematically	6	9	[3; 19]
Never	18	28	[17; 40]
Number of nostril sampled during one test			
Always in both nostrils	41	63	[50; 75]
Not systematically in both nostrils	15	23	[13; 35]
Never in both nostrils	9	14	[6; 25]
Number of measurements per nostril			
One	14	22	[12; 33]
Two	32	49	[37; 62]
More than two	19	29	[19; 42]
Methods used in centres to sample nasal NO (more than one method per centre)			
Exhalation against a resistor	49	75	[63; 85]
Tidal breathing	50	77	[65; 86]
Breath hold	10	15	[8; 26]
Humming	2	3	[0; 11]
Between-measurement repeatability within individuals			
Not reported	17	26	[16; 39]
Reported, expressed as ppb	3	5	[10; 13]
Reported, expressed as %	35	54	[41; 66]
Reported, expressed as ppb and %	10	15	[8; 26]
Report of nasal NO results includes			
Practical issues during measurements*	55	85	[74; 92]
Sampling flow of the device	31	48	[35; 60]
Ambient NO	27	42	[29; 54]
Nasal NO results in nL.min ⁻¹	16	25	[15; 37]
Nasal NO results in ppb	33	50	[38; 63]
Nasal NO results in nL.min ⁻¹ and ppb	16	25	[15; 37]

Abbreviations: CI: Confidence Interval; NO: nitric oxide

* Practical issues such as poor cooperation, difficulty with the respiratory manoeuvre, obstructed nostrils, crying, etc.

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Figures

Figures 1. Methods used to measure nasal nitric oxide across 65 centres

ER: Exhalation against a resistance; BH: Breath Hold; TB: Tidal Breath; HUM: Humming

Figure 2. Cut-offs of nasal nitric oxide values used by PCD centres to screen according to the respiratory manoeuvre and expression of the result

ER: exhalation against a resistance; BH: breath hold; TB: tidal breathing

Dark columns are number of centres using cut-offs expressed as ppb during an exhalation against a resistance (A, n = 20), breath hold (C, n = 10) or tidal breathing (E, n = 22). Grey columns are number of centres using cut-offs expressed as nL.min^{-1} during an exhalation against a resistance (B, n = 22), breath hold (D, n = 3) or tidal breathing (F, n = 10).

Figure 1

Figure 2

**An international survey on nasal nitric oxide measurement practices for the diagnosis of
primary ciliary dyskinesia**

Supplementary File

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Results

Comparisons between centres using chemiluminescence or electrochemical devices

In 7 sites, the only source of training was an industry representative, most often in centres using an electrochemical device (5/23 versus 1/31 centre using a chemiluminescence device and one centre unable to state which technology was used).

The chemiluminescence technique was more frequently used in centres performing more than 25 tests per year compared to centres performing less than 25 annual tests (25/38 versus 8/27; $P = 0.004$), and in centres measuring nNO in infants under 2 years of age when compared to centres testing children 2 years or older or 5 years or older (13/16 versus 8/18 and 12/27, respectively; $P = 0.040$).

The sampling flow was known by the respondents in 42 centres, more frequently in centres using only chemiluminescence (21/23) as compared to half of the sites using the electrochemical technique (21/41; $P = 0.002$).

When comparing centres using only chemiluminescence devices ($n = 23$) to those using only electrochemical devices ($n = 31$), the former more frequently sampled always or often both nostrils (22 versus 15; $P < 0.001$) and never sampled only once a single nostril (0 versus 13; $P < 0.001$).

At almost every site using a chemiluminescence device, ambient NO was measured daily or before each measurement (33/34) while it was only done in 17/41 sites using an electrochemical device ($P < 0.0001$).

Centres reporting ambient NO levels were more likely to have a chemiluminescence device (22 centres with chemiluminescence device among the 27 centres who reported ambient NO) compared to centres not reporting ambient NO levels (11 centres with chemiluminescence device among the 38 centres who did not reported ambient NO) ($P < 0.0001$).

Centres using only a chemiluminescence device more frequently reported results only in $\text{nL}\cdot\text{min}^{-1}$ (14 vs 7) when compared to those using only an electrochemical device (1 vs 28) ($P < 0.0001$). In contrast, centres using an electrochemical device more frequently reported results only in ppb (25 vs 6) compared to centres using only chemiluminescence (5 vs 19) ($P < 0.0001$).

ANNEX 1

	QUESTIONS	ANSWERS
	Basic Information about you	
	Name, surname (optional)	
	Centre/ hospital (Name, Town, Country)	
	Do you perform nasal NO (nNO) measurement in patients with suspicion of Primary Ciliary Dyskinesia?	YES / NO
	If NO, please do not answer the survey but kindly forward it to people performing nasal NO measurement at your centre	
	Staff	
1	You are?	Drop down or button: 1 answer
		Technician
		Physiologist/Respiratory therapist
		Pulmonologist
		Paediatric pulmonologist
		Paediatrician
		ENT
		Nurse
		Physiotherapist
		Post-doctoral trainee
		Other (detail):
2	At your centre who is performing nasal NO?	(Drop down or buttons: ≥1 answers)
		Technician
		Physiologist/Respiratory therapist
		Pulmonologist
		Paediatric pulmonologist
		Paediatrician
		ENT
		Nurse
		Physiotherapist
		Post-doctoral trainee
		Other (detail):
3	All staff considered; how many people are measuring nNO at your centre?	number
	Generalities on Tests	
4	How many tests are performed in your centre per year?	<10 / 10 - <25 / 25-100 / 100-200 / > 200

5	Do you sometimes postpone nNO testing for particular circumstances such as: recent respiratory infection, block nose, high ambient NO etc...?	YES / NO
6	How long do you want patients to be infection free before undertaking nNO measurement? (weeks)	Drop down 1 answer: I don't delay for infections, 1, 2, 3, 4, 5, 6, >6 weeks
	<i>Age of patients tested</i>	
7	Do you test children?	YES / NO
	if YES starting age for testing	Drop down 1 answer: 0-6 months 6-12 months, 1year, 2y, 3y, 4y, 5y, 6, >6y
8	Do you test adults?	YES / NO
	<i>Device (technique and brand) used</i>	
9	Do you use chemiluminescence device?	YES / NO
	If YES specify (e.g. Ecomedics, Sievers)	text
10	Do you use electrochemical device?	YES / NO
	If YES specify (e.g. NIOX Vero)	text
	If you are unsure whether your device is chemiluminescent / eletrochemical, please specify brand	text
	<i>Methods to perform nNO measurements</i>	
11	Which methods do you regularly use to measure nNO?	
	blow against resistor or party-favour	YES / NO
	breath hold	YES / NO
	hum	YES / NO
	tidal breathing	YES / NO
	If YES, your preferred method is?	1 answer drop down
		mouth open
		closed
		indifferent
		pursed lips
12	Are you using any other methods (not mentioned above) to measure nNO?	text
13	Do you have a standardised operating procedure (SOP) for each technique?	YES / NO
	If YES, please share it with us by emailing it to Lynn (L.E.Reeves@soton.ac.uk) and to Nicole (nicole.beydon@aphp.fr)	

	Sampling Line	
14	Do you know the sample flow you are using for measurements in children?	YES / NO
	If Yes, which ranges of flows do you use?	≥ 1 answer
		0,25 - 0,40 L/min
		0,40 - 0,60 L/min
		0,60 - 0,80 L/min
		≥ 1 L/min
	Measurements (number, side)	
15	Do you measure NO from each nostril?	1 answer
		always
		often
		sometimes
		never
16	How many measurements do you routinely perform on each nostril?	1 answer
		1
		2
		≥3
	If you compare two measurements how many ppb or % would you consider as reproducible nasal NO results?	1 answer
17	in ppb	number
18	in %	number
	Do you report?	≥1 answer
19	nasal NO value in ppb?	YES / NO
20	nasal NO value in nL/min?	YES / NO
21	the sample flow?	YES / NO
22	the ambient NO?	YES / NO
23	practical problems (crying, nose busy...)?	YES / NO
24	Do you have a standardised report for nNO results?	YES / NO
	If YES, please share it with us by emailing it to Lynn (L.E.Reeves@soton.ac.uk) and to Nicole (nicole.beydon@aphp.fr)	
25	Which cut-off values do you use (1 per method, state the units at the beginning)	
	Units	ppb or nL/min
	blow against resistor or party-favour	number

	breath hold	number
	hum	number
	tidal breathing	number
	other	number
	<i>Training staff and environmental considerations</i>	
26	Do you have a training programme for measuring nNO?	YES / NO
	If YES, please share it with us by emailing it to Lynn (L.E.Reeves@soton.ac.uk) and to Nicole (nicole.beydon@aphp.fr)	
	If Yes, select who is training your staff?	≥1 answer
		A designated member of your team
		A representative from the analyser company
		A designated trainer from another institution from the PCD foundation / a reference centre
		Training workshops (e.g. BEATPCD, consortia workshops)
		Other (detail)
27	Do you perform machine calibration?	YES / NO
28	Do you measure ambient NO?	1 answer
		before each measurement
		daily
		often
		sometimes
		never
	If not "never" above which value in ppb, do you consider ambient NO high (ie. with possible consequence on nasal NO result)?	number
29	If ambient NO is high, do you take it into account when interpreting nNO?	YES / NO
	<i>Free text box for additional comments</i>	