Nasal nitric oxide measurement in children for the diagnosis of primary ciliary dyskinesia: European Respiratory Society technical standard

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Shareable abstract ([ERSpublications]


Abstract

Nasal nitric oxide (nNO) is extremely low in most people with primary ciliary dyskinesia (PCD) and its measurement is an important contributor to making the diagnosis. Existing guidelines and technical standards focus on nNO measurements in older, cooperative children using chemiluminescence analysers. However, measurements of nNO in pre-school-age children (age 2–5 years) may facilitate early diagnosis and electrochemical rather than chemiluminescence analysers are widely used. Pre-schoolers often need different methods to be employed when measuring nNO. Hence, a European Respiratory Society Task Force has developed this technical standard as the first step towards standardising sampling, analysis and reporting of nNO measured as part of the diagnostic testing for PCD in all age groups, including pre-school-age children. Furthermore, we considered both chemiluminescence and electrochemical analysers that are in use worldwide. There was a paucity of quality evidence for electrochemical analysers and sampling methods used in young children, and the Task Force proposes future research priorities to allow updates of this technical standard.
Background

Primary ciliary dyskinesia (PCD) is a genetically and clinically heterogeneous syndrome estimated to impact 1 in 7,500 people worldwide [1]. Impaired function of motile cilia causes failure of mucociliary clearance leading to symptoms of neonatal respiratory distress of unknown cause at term, daily wet cough from infancy, perennial rhinosinusitis, otitis media with effusion, chronic bronchitis and bronchiectasis [2, 3]. Approximately 40% of patients have situs inversus totalis and about 12% have heterotaxy [4]. Male and female subfertility is common [5]. Diagnosis requires access to a combination of specialised investigations which may include transmission electron microscopy, genotyping, high-speed video microscopic analysis of cilia function and immunofluorescence staining of ciliary proteins [6, 7]. Nasal nitric oxide (nNO) concentration measurements contribute to the diagnosis because many people with PCD have reproducibly low levels compared to healthy individuals and to people with other airway diseases [6–9]. Although nNO results cannot confirm or refute the diagnosis in isolation, the European Respiratory Society (ERS) diagnostic guidelines recognise its importance for determining the likelihood of PCD when used in conjunction with other tests or as a screening test [6]. Low nNO levels in PCD were first reported over 25 years ago, but the mechanisms underlying low nNO in PCD and its pathophysiological consequences are unknown [8, 10].

The recommended technique for measuring nNO requires the individual to exhale orally against resistance while gas is sampled from the nares and nasopharynx [11]. This exhalation against resistance (ER) technique ensures velum closure, thereby avoiding contamination and dilution of nasal gas with air from the lower airways. However, ER can only be achieved by older, cooperative children (usually age >5 years) and alternative sampling methods have evolved to facilitate measurements being obtained from infancy. Simple breath-hold (BH) may be possible with some children who cannot follow protocols that include manoeuvres involving ER. Alternatively, measurement of nNO during tidal breathing (TB) is feasible from early infancy [12–15]. Therefore, nNO measurements can technically be performed in all age groups, including infants. However, normal reference values are scarce and diagnostic cut-off values may be difficult to establish in younger children since nNO concentrations are inherently very low during infancy and increase with age during the first years of life [14, 16].

Chemiluminescence analysers are the standard device used to measure nNO levels, relying on the reaction between NO sampled from the patient’s nostril and ozone generated in the instrument. This instantaneous reaction results in emitted electromagnetic radiation in the form of light (photons), which is proportional to the continuously sampled NO molecules [17]. Electrochemical analysers use amperometric sensors to measure the quantity of NO accumulated in a chamber from the current generated by the chemical reaction between the sampled gas and the active sensing material [18].

While American Thoracic Society (ATS)/ERS guidelines recommend that nNO measurements are performed using chemiluminescence analysers [6, 7, 19], a recent worldwide survey reported that electrochemical devices are more frequently used, particularly outside North America [20]. Chemiluminescence analysers produce a real-time display of the NO signal, which is important for quality assurance, and there are standardised, validated methods using these analysers. However, the purchase and maintenance of these non-portable devices is expensive compared to electrochemical analysers. Discriminative nNO values are possible with electrochemical hand-held NO devices [21, 22], but these analysers do not have fully standardised operating procedures nor have they been tested against established diagnostic criteria in large multicentre trials. No electrochemical analysers have been validated in diagnostic settings and supporting data were originally derived from the NIOX MINO analyser [21, 22], which is no longer available (table 1). While electrochemical analysers are generally more affordable, they do not display real-time NO levels graphically and hence do not allow selection of optimal nNO measurement plateaus. Thus, while electrochemical analysers have benefits in low-resource settings or where portability is important [23], technical improvements as well as studies are still needed to achieve standardised, validated methods for nNO measurements using these devices.

Internationally, clinicians and researchers are using different equipment, breathing manoeuvres, diagnostic cut-off values and reporting standards. The PCD community has identified the need for standardised and validated methods for nNO measurements as a priority [20].

The aim of this ERS Task Force was to develop a technical standard for the sampling, analysis and reporting of nNO levels as part of the diagnostic testing for PCD in childhood, including in pre-school-age children and infants. We aimed to consider both chemiluminescence and electrochemical analysers that are in use worldwide, and to identify future research priorities.
Parental perspective
This verbatim contribution is from a parent representative of the Task Force (M.C.):

“When our child was having diagnostic tests, we had a little stress with the result of each test. We were correctly explained that the nNO measurement was a very important test because depending on its results, PCD would become very likely or unlikely. It was therefore a lot of stress when we learned that the child’s nose must not be obstructed in order to be able to do this test. Our child has chronic rhinitis (as part of her disease) and we did not know how to make it different for this important test.

### TABLE 1 Advantages and disadvantages of chemiluminescence and electrochemical analysers for nasal nitric oxide (nNO)

<table>
<thead>
<tr>
<th>Examples</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td><strong>Chemiluminescence analysers</strong></td>
<td>Higher accuracy (e.g. &lt;1 ppb with 1% linearity from 0.1 to 5000 ppb; CLD 88) Continuous measurement and real-time display of NO test sample Allows for identification of the measurement end-point: stable plateau (ER and BH) or regular peaks (TB) without a fixed sample collection minimum time requirement Online, real-time display allows identification and recording of unreliable measurements (nares leak, contamination from lower airways), aiding the validation of the result Ambient NO can be measured and recorded prior to each test occasion Rigorous testing in young people ⩾5 years of age with published, validated diagnostic or screening cut-off values</td>
<td>Expensive to purchase and maintain (high cost per test for centres performing a limited number of measurements) Need for regular calibration and preventative maintenance Requirement for increased operator training and expertise Difficult to transport and although an “offline method” of measurement allows for remote sampling, it loses the advantage of the real-time trace</td>
</tr>
<tr>
<td>CLD 88 sp (Eco Physics/Eco Medics, Switzerland) Sievers/Zysense NOA 280 (General Electric Analytical Instruments, USA) NIOX FLEX (Aerocrine AB, Sweden) LR2000 (Logan Research Ltd, UK) EVA 4000 (SERES, France)</td>
<td></td>
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</tr>
<tr>
<td><strong>Electrochemical analysers</strong></td>
<td>Simple to use and requires no calibration Cost-effective solution for low-volume sites Smaller, portable device allowing nNO measurement at different sites</td>
<td>Difficult to detect unreliable measurements (leak, lower airway contamination) without a real-time sample display NIOX VERO relies on a cost per test model with the analyser requiring replacement after a set number of tests or a set timeframe; discourages recording of ambient NO levels and repeated measurements Lower accuracy (e.g. ±5 ppb for values &lt;50 ppb and 10% for values &gt;50 ppb; NIOX VERO) Inconsistent training and standard operating procedures provided by manufacturers</td>
</tr>
<tr>
<td>NIOX MINO (discontinued 2018) and NIOX VERO (Circassia; formerly Aerocrine AB, Sweden)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


https://doi.org/10.1183/13993003.02031-2022
Another point is that we were very keen to have the results of the diagnostic tests quickly to continue our child’s assessment. I have spoken to parents who have told me that on the occasion of some tests they have been quite harsh/severe (and angry??) with their child who was not cooperative (because of fear or lack of good will). This can be the case for any test, including nNO test if too much importance is given to it.

In order to decrease the stress related to this specific test, I would suggest to explain to parents that if the result of nNO measurement is not undoubtedly normal (high above the cut-off) it will be necessary to check it on a second occasion, because it is a delicate test and local/nasal disease may alter the results. Also it would be good to specify that 1) deciding to go on with other more invasive tests will also rely on the clinical probability of the disease assessed by a panel of experts, 2) the final diagnosis will not rely only on nasal NO but on a range of tests, 3) in the meantime, the care of the child would be similarly adapted regardless of the final diagnosis (after the exclusion of differential diagnoses).

If there are technical considerations about the reliability of results depending on the technique used (chemiluminescence or electrochemical), it is very difficult for parents to capture them, and to report them if questioned. Therefore, I suggest that all relevant technical issues be flagged in the nNO measurement report.”

**Methods**

The membership and roles of the Task Force panel and methods are detailed in the supplementary material (supplementary tables E1 and E2 and supplementary text). In brief, to inform the development of the technical standard, we relied on evidence collected through a systematic literature review (January 1994 to December 2021). Each work group reviewed all of the titles, and if relevant the abstracts and then full text to utilise the information relevant to their area of focus. Work groups discussed the manuscripts and then drafted the text which was reviewed by the full Task Force. Iterative changes were made in a series of virtual meetings until agreement was reached.

**Outcomes from the Task Force**

**Considerations for analysers**

The advantages and disadvantages of chemiluminescence and electrochemical analysers are summarised in table 1, and although electrochemical analysers can produce reproducible measurements, their results are considered as less accurate than reproducible measurements obtained using chemiluminescence analysers (box 1).

**Sampling rates**

nNO is constantly produced within the nasal cavity. During nNO measurements, air is aspirated from one nostril using an olive attached to the device’s vacuum pump, while the other nostril remains open to the

### BOX 1 Grading of nasal nitric oxide (nNO) measurements: Grade A is the most acceptable and Grade F is the least acceptable (explained in the supplementary material)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Chemiluminescence analyser</th>
<th>Electrochemical analyser</th>
<th>ER or BH</th>
<th>TB§</th>
<th>Inter-nostril repeatability (%)</th>
<th>Intra-nostril repeatability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td>B</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td>&gt;10§</td>
<td>&gt;10§</td>
</tr>
<tr>
<td>C</td>
<td>✓</td>
<td>✓</td>
<td>&lt;10</td>
<td></td>
<td>&gt;10</td>
<td>&gt;20</td>
</tr>
<tr>
<td>D</td>
<td>✓</td>
<td>✓</td>
<td>&gt;30♀</td>
<td></td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>E</td>
<td>✓</td>
<td>✓</td>
<td>&lt;10♀</td>
<td></td>
<td>&gt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>F</td>
<td>✓</td>
<td>✓</td>
<td>&gt;10♀</td>
<td></td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
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</table>

ER: exhalation against resistance; BH: breath-hold; TB: tidal breathing. Repeatability: difference between two measurements. Measurements can be the mean of five peaks of NO (chemiluminescence) or the mean NO over a period of sampling (electrochemical). §: TB in an awake or sleeping child not moving, vocalising or crying with visually a regular breathing pattern; ♀: if only one repeatability >10% or undetermined, grade is B; ♀: if only inter-nostril repeatability >30% or undetermined, or intra-nostril repeatability >20% or undetermined, grade is E; ♀: if only one repeatability >10% or undetermined, grade is F.
atmosphere (figure 1). The collected air is analysed by the NO gas analyser. The rate at which the air is aspirated can vary depending on the type of device and its settings. Knowledge of this rate is vital for interpretation of the nNO measurements.

Historically, the aspiration (or sampling) rate used to measure nNO has varied widely (from 0.2 to 6.2 L·min\(^{-1}\)) [24]. Using the BH technique, Qian et al. [25] demonstrated that the measured NO output is flow dependent. Slower aspiration flow results in lower NO outputs, possibly because lower, laminar flow does not reach the deeper nasal cavities. A slower aspiration rate takes a longer time for the nNO output to plateau (>10 s). In contrast, turbulent, higher aspiration flow results in significantly higher NO outputs and a shorter time to reach a plateau; high flow rate can cause discomfort (>1.2 L·min\(^{-1}\)) or nostril collapse (>5.2 L·min\(^{-1}\)) [25]. For these reasons, previous ATS/ERS guidelines (2005) recommended limiting the sampling rate to between 0.25 and 3 L·min\(^{-1}\), and documenting the aspiration flow [19]. Since the publication of the 2005 recommendations, further evidence for optimal aspiration flow has been sparse. Struben et al. [26] compared three aspiration flow rates of 0.28, 0.7 and 1.2 L·min\(^{-1}\) using a NIOX chemiluminescence analyser and a BH method in adult subjects. They reported that the time to plateau and the resulting mean nNO measurement were significantly different when using these differing aspiration flow rates. These investigators recommended a preferred flow of 0.7 L·min\(^{-1}\) since the NO plateau was reached within 7 s and the procedure was well tolerated by adult study participants. In children, using the velum closure method, Beydon et al. [12] reported a significant effect of aspiration flow (0.3 versus 1 L·min\(^{-1}\)) on the measured NO output, but could not perform the same comparison using the TB technique because higher flow disturbed young children.

Commercially available devices provide a range of default flows between 0.12 and 0.33 L·min\(^{-1}\) [11, 21, 22, 27]. We suggest that the sampling rate be set at 0.3 or 0.33 L·min\(^{-1}\). Users should directly measure and record their device’s sampling rate to calculate the output of NO (nL·min\(^{-1}\)) (see section “Reporting and interpretation of results: general”).

**Licensing and regulatory approvals**

Another consideration in device selection may depend on licensing and regulatory approval. Both chemiluminescence and electrochemical devices are licensed for nNO measurements in Europe. Neither chemiluminescence nor electrochemical devices are approved by the US Food and Drug Administration, and the former are used at PCD Foundation-accredited clinical centres, Genetic Disorders of Mucociliary Clearance Consortium sites or in research settings. Only two chemiluminescence NO analysers suitable for clinical testing (Eco Physics CLD 88 device and Zysense NOA 280i) are available for purchase in North America.
Considerations for consumables

Measurement of nNO requires single-patient-use nasal probes with fixed (plastic) or compressible (foam) olives that can be inserted tightly into the nostril to prevent air leakage [28]. Commercially available nasal probes are provided in several sizes, ranging from neonatal to large adult. To prevent contamination of the sampling line with humidity, infectious agents, mucus or other debris, it should be single-use or the nasal probe should have appropriate built-in or in-line filters [29].

For ER manoeuvres, disposable mouth restrictors are required, which can be inexpensive cardboard cylinders with a 1-mm opening at the distal end or a party favour (a blow-out toy partially occluded at the distal end; figure 1c) to provide similar resistance [11]. For some commercially available analysers the restriction is achieved by adding a paper restrictor with a 1-mm hole at the back side of the standard bacterial filter that is part of the equipment’s mouthpiece (NIOX VERO). In others, closure of the palate is achieved through exhalation flow control supported by an incentive screen that provides feedback (Eco Physics CLD 88 sp). In both cases, as the equipment mouthpiece is used, an additional microbiological filter is required [29].

Calibration, maintenance and environmental NO (supplementary video 1)

Despite the importance of obtaining accurate and reproducible data, most articles provide minimal information on calibration of devices used in their studies.

Ideally, nNO should be measured in a clinic or hospital space that has low ambient NO levels to avoid potentially artefactual results [30, 31], but a recent survey showed that not all users measure ambient levels [20]. Environmental NO levels <20 ppb are generally considered acceptable to allow testing to proceed. The 2020 North American Technical Paper on testing suggests that inhalation of NO-free air through the nostril open to the air may help if ambient levels are high [27]; however, this method has not been validated and may complicate testing of young children. An alternative option is to subtract the ambient NO level from the nNO result [32]. In the absence of any validated method, the Task Force suggests that an estimate of the effect of ambient nNO on the outcome can be made by deducting the ambient nNO from the child’s nNO result. If the “corrected” value is clearly above the diagnostic cut-off value the result can pragmatically be accepted. If the result is close to (potentially impacting the outcome) or below the cut-off the measurement should be confirmed on another day.

Example to estimate effect of ambient NO

nNO measured with 0.33 L·min$^{-1}$ sampling flow and an ambient NO of 49 ppb was 281 ppb.

- nNO output=281 ppb×0.33 L·min$^{-1}$=93 nL·min$^{-1}$ (above cut-off of 77 nL·min$^{-1}$)
- nNO output “corrected” for ambient NO=(281–49) ppb×0.33 L·min$^{-1}$=232 ppb×0.33 L·min$^{-1}$= 76 nL·min$^{-1}$ (below cut-off of 77 nL·min$^{-1}$)

In this case the result should be evaluated on another day when the ambient level is low because the “corrected” output is close to the diagnostic cut-off value.

In general, users should follow detailed directions for airflow and NO calibrations provided by the manufacturer, although some commercially available electrochemical devices do not provide such information.

When recommended, the flowmeter of the device is calibrated daily, usually using a syringe appropriate to the flow and volume expected during testing. Sites should have an external flowmeter capable of measuring the sampling flow of the NO analyser within a sampling range (0.2–0.6 L·min$^{-1}$) [27].

Regular calibration of chemiluminescence analysers with standardised NO gas with two (high and low) certified calibration gases is recommended by some manufacturers, while others require calibration with a high NO standard and with a gas-zero NO (ambient air screened for NO by a module connected to the machine) for low-level calibration. The NIOX FLEX (no longer available) required calibration every 14 days, but other chemiluminescence analysers are thought to stay accurate for a longer time and a monthly calibration is appropriate [33], therefore manufacturer’s guidance should be followed. If the nNO values obtained during calibration are unrealistic, a leak should be considered and calibration or measurement should be repeated. Additional calibration should be conducted following any change in the sampling line, in-line resistor or other component of the testing system.

It is not possible to calibrate electrochemical analysers using certified gas, but users can consider biological control testing using a healthy employee with known normal and stable nNO levels.

Maintenance schedules should follow the manufacturer’s guidelines.
Training of personnel

While there have been no studies to investigate the impact of operator training, it is evident that operators must understand the performance and limitations of the device used at their centre, as well as the relative accuracy of the result depending on the manoeuvre and the repeatability for a given child (box 1). One study reported improving success rates measuring nNO during TB in pre-school-age children (age 6 months to 5 years) with increasing operator experience [13]. As with other physiological measures, operators and technicians must receive formal training in the maintenance and calibration of equipment; to recognise equipment malfunction, select the appropriate manoeuvre for the patient (box 2), calculate nNO production from acceptable tracings and interpret the result based on standardised protocols. Training should be conducted by experienced personnel (e.g. BEAT-PCD, ERN-LUNG PCD core or PCD Foundation) and knowledge should be regularly updated. Training by manufacturers may not be adequate, as in the experience of the Task Force the manufacturers may not have a good understanding of the testing requirements.

Assessment and preparation of the patient before testing

A clinical history suggestive of PCD [34, 35] must be confirmed before testing, because a low pre-test probability of PCD will lead to an unacceptably high false-positive rate [36]. The protocol for measuring nNO is summarised in box 3.

Information about assessing and preparing patients before nNO measurements is limited. Knowledge gaps exist in the literature regarding the assessment of the nasal passages. Nasal obstruction for any reason (e.g. nasal polyposis) might hamper measurement and result in repeatedly low nNO levels or large inter-nostril differences. RYBNIKAR et al. [37] reported significantly reduced nNO measurements with increasing size of adenoids in non-allergic non-PCD patients between the ages of 5 and 18 years; these measurements normalised following adenoidectomy. Therefore, members of the Task Force consider referring children for ENT (ear, nose and throat) assessment if an obstruction of any cause is suspected, particularly if the nNO level is low.

Patients should be asked to blow their nose before testing. Gentle saline lavage could be helpful in those unable to adequately clear their nasal passages, taking care not to injure the mucosa. nNO measurements can be performed after a few minutes to allow NO to reaccumulate after the lavage.

nNO should be measured before nasal brushing or biopsy to avoid falsely low readings caused by bleeding, since haemoglobin avidly binds NO and could theoretically reduce measured nNO levels.

Several reports have described reduced nNO measurements in people with primary immunodeficiencies, diffuse panbronchiolitis and cystic fibrosis [38–42], and although levels are not usually as low as in PCD, these differential diagnoses should be considered (supplementary table E3). Indeed, at most specialist sites cystic fibrosis must be excluded before performing nNO measurements.

There is limited data regarding the effect of infections on nNO. Respiratory tract infections in otherwise normal infants temporarily suppress nNO levels by ~80% [14]. While children requiring PCD diagnostic testing typically have chronic upper and lower airway symptoms, these data strongly indicate that nNO measurements should not be measured during infective exacerbations. Many centres delay testing for 2–4 weeks following infection [20], but there is no evidence concerning the appropriate duration. In the absence of clear evidence, we suggest testing should be delayed 2–4 weeks after exacerbation symptoms have resolved and if doubt exists concerning a low nNO measurement the test should be repeated on a separate day.

BOX 2 Considerations for measuring nasal nitric oxide in different age groups

<12 months: extremely low in healthy infants, therefore research tool only, not diagnostic
<5 years: interpret with caution and refer to primary ciliary dyskinesia centre if in doubt:
  • Levels in healthy children <5 years of age are lower than in older healthy children
  • Limited normative data
To choose manoeuvre:
  • ER if compliant (usually age >5 years)
  • BH if compliant but unable to achieve ER
  • TB if non-compliant or unable to achieve ER/BH

ER: exhalation against resistance; BH: breath-hold; TB: tidal breathing.
Special considerations when measuring in different age groups

The Task Force agrees with the recently published North American Technical Paper, which focused on individuals $\geq 5$ years of age [27]. Briefly, that paper concluded that ER is the preferred sampling method, ideally using a mouth resistor or party favour. The BH technique can serve as an alternative in children who cannot perform ER, but it requires subject cooperation to voluntarily close the velum [42–44].

Although ER or any other velum closure method is the preferred method of sampling in school-age and older children, it is often not feasible in pre-school-age children. PIACENTINI et al. [45] reported that only 14% of healthy children between the ages of 2 and 5 years could actively cooperate. The TB method is the most feasible method for infants and younger children. If velum closure is achieved, BH and ER show similar repeatability in children, whereas TB, a non-velum closure method, shows greater variability [46].

GUPTA et al. [13] reported successful TB measurements in 69% of a non-PCD cohort of children between the ages of 6 months and 5 years. MARTHIN and NIELSEN [42] found that nNO was reliably measured during TB in 95.2% of children $<6$ years of age, which highly exceeded the success of prolonged BH velum closure for 30–40 s or short intervals of velum closure obtained by blowing repeatedly in a party favour. Similarly, BEYDON et al. [12] found that the ability to perform velum closure nNO manoeuvres increased with age, with a 20% success rate in children $<3$ years of age, increasing to 83% in those 3–6 years of age and 98% in those $>6$ years of age.

Feasibility and success rates for infant nNO measurements using the TB method have been described in two studies [14, 15]. BUECHEL et al. [15] successfully obtained nNO measurements from 100% of 62 neonates during natural sleep using chemiluminescence assays, whereas the success rate was lower (85.5%) when they used an electrochemical device. A similar high success rate (99.6%) was reported by MARTHIN et al. [14] in 44 infants 2 weeks to 2 years old using a chemiluminescence analyser.

All studies reported extremely low nNO concentrations in healthy infants [14–16, 47, 48], with levels increasing rapidly during the first 18 months [14, 16, 48], then more gradually until 12 years of age when...
they reach adult levels [30]. Since healthy infants and young children can have low nNO levels, reference and cut-off values are needed at different ages to discriminate children with PCD from those without. Normative data remain limited, largely based on small, single-centre studies usually involving healthy infants [14, 16, 48] and neonates [15].

Because of extremely low levels of nNO in healthy infants <12 months of age and the paucity of normative data, Task Force members do not measure nNO diagnostically, but some use it as a research tool in this age group until age-related testing standards and diagnostic cut-off values are established. For children ≥12 months of age, an experienced technician should assess the likelihood of a child’s ability to achieve a manoeuvre based on age and cognition. Again, assessment of results in children <5 years of age is limited by the lack of normative and cut-off values for disease and should be interpreted with caution (supplementary tables E3 and E4), referring to a PCD centre and repeating the measure when the child is older is recommended if doubt exists.

**Standard operating procedure for performing a measurement using different respiratory manoeuvres**

(supplementary videos 2–5)

The order in which different nNO test manoeuvres are undertaken to determine feasibility for a specific child does not impact the result and the technician should decide which manoeuvre to use based on the likelihood of success (figure 2). The preparation and measurement of nNO are summarised in box 3.

![Flowchart](https://doi.org/10.1183/13993003.02031-2022)

**FIGURE 2** Suggested approach for measuring nasal nitric oxide (nNO) in children. The ages are provided as guidance and the technician should decide which manoeuvre to attempt based on their assessment of the child. ER: exhalation against resistance; BH: breath-hold; TB: tidal breathing.
**General considerations for measuring nNO in all age groups**

For the measurement:

- Children should be comfortably seated on a chair or a parent’s lap. Some infants will be calmer staying in their pushchairs or they might sleep during the procedure (figure 1a).
- The technician should explain the procedure to the patient. Demonstrating the procedure and practising (e.g. positioning of the olive, breathing manoeuvre) before the actual testing may avoid repetitive testing (figure 3).
- A nasal olive appropriate for the child’s size should be inserted into one nostril to form an airtight seal, avoiding sampling of room air. The olive is maintained in place by the patient whenever possible, or by the technician or a parent in the case of young children. To avoid leaks when testing young children, it might be beneficial to support the child’s head by placing a hand behind the head or to sit the child on the parent’s lap, with their back resting on the parent’s torso. The other nostril should be unobstructed (figure 1b).

Further measurements should be undertaken from the same nostril until two nNO levels within 10% (20% for TB measurements) of each other are obtained, with 30 s of rest between measurements [11, 27, 46]. The same procedure is repeated using the opposite nostril aiming for 10% inter-nostril repeatability (30% for TB measurements) [11, 27, 46]. Single measurements or assessing a single nostril can be prone to error [46].

It may be impossible to attain “ideal measurements” in children, e.g. the child may be too young to achieve ER, only a single measure may be attained in one nostril and chemiluminescence may not be available. The Task Force has therefore developed a scoring system to denote acceptability, where Grade A is the ideal nNO test and Grade F is the least reliable (box 1 and explained in supplementary material).

**ER manoeuvre**

The methods for measuring nNO using an ER manoeuvre (box 3) have been described in a recent technical standard for older children and adults [27]. In brief, testing is ideally performed using an analyser that allows the technician to view the nNO concentration curve in real-time and manually determine the plateau value following the measurement, but this is only possible using a chemiluminescence analyser [27, 49].

The technician should first measure and record the sampling flow as explained earlier. After checking the equipment and assessing the ambient NO levels, the technician attaches the flow sampling line to the nasal olive probe and filter, and inserts the probe into the patient’s nostril. A resistor mouthpiece is then held in the patient’s mouth with a targeted expiratory mouth pressure of at least 10 cmH2O in adults and 5 cmH2O in children [19]. Alternatively, a party favour can be used to create resistance (figure 1c). The individual should then inhale to near-total lung capacity before beginning a prolonged exhalation manoeuvre, sealing

![FIGURE 3](https://doi.org/10.1183/13993003.02031-2022)
the resistor tight with their lips, blowing in a steady, low-flow manner until they are directed to stop by the technician (i.e. when the curve shows an acceptable plateau of $\geq 3$ s).

The technician should manually choose the plateau rather than using the automated software [27]. Acceptable nNO plateaus are $\geq 3$ s in duration and there should be $\leq 10\%$ variation (between the minimum and maximum values), using the maximum attainable mean plateau values (figure 4) [27]. A low nNO level should ideally be confirmed by testing during TB (figure 4).

If an electrochemical device is used, the manoeuvre is similar to a chemiluminescence device, except that reliable feedback showing the real-time NO curve is not possible. With the electrochemical device, the child exhales for the duration set by the machine, which should be $\geq 10$ s [49]. The plateau durations for electrochemical devices displaying nNO tracings (e.g. NIOX VERO) have not yet been established. Moreover, electrochemical analysers without a display of a NO tracing do not allow manual selection of a technically acceptable plateau, but provide the mean NO measured from part of or from the entire sample. For instance, the manufacturer stated that for the NIOX MINO set at 5 mL·s$^{-1}$, the last 30 s over the 45 s sampling time was measured.

Typically, the measured nNO values obtained from the two nostrils should be similar. Greater variation may indicate nasal obstruction, e.g. polyps [12]. Alternatively, the sampling line or an in-line resistor may have become obstructed. When variation occurs, the technician should remeasure the sampling flow.

![Figure 4](https://doi.org/10.1183/13993003.02031-2022)
Following testing to determine whether the value has increased compared to the pre-test value. The test should be repeated after secretions are cleared or the element replaced [27].

**BH manoeuvre**
The BH manoeuvre is an alternative to the preferred method of ER and may be used for patients who cannot perform ER. Details are similar to the ER method, except for the breathing manoeuvre itself.

The patient is instructed to take a deep breath to total lung capacity. They then ideally perform velum closure, in which they close the glottis and perform a Valsalva manoeuvre to elevate the hypopharynx and close the soft palate. If measured, nasal CO₂ from the opposite (free) nostril can confirm velum closure has been achieved (CO₂=0%) [19]. Failure to achieve velum closure can falsely reduce nNO measurements due to contamination with exhaled air from the lungs and oropharynx, diluting NO in the nasal sample.

When using a chemiluminescence analyser, the BH manoeuvre should be maintained to achieve a plateau of \( \geq 3 \text{ s} \) with \( \leq 10\% \) variation between minimum and maximum values of the plateau. In contrast, electrochemical analysers have a predetermined duration for the sampling, and patients need to hold their breath until sampling is completed, which may be difficult for some. Using older electrochemical devices (e.g. NIOX MINO), the sampling duration depended on flow (90 s at 2 mL·s\(^{-1}\) and 45 s at 5 mL·s\(^{-1}\)) [21], while in the newer versions (e.g. NIOX VERO) the sampling time is 30 s at 5 mL·s\(^{-1}\).

**TB manoeuvre**

nNO measured during TB has been described in more than 15 study reports or reviews [9, 12–16, 22, 32, 42–44, 48, 50–52] where the method has been used among infants, children and adults, whether healthy or patients with suspected PCD, definite PCD or other respiratory conditions (supplementary tables E3 and E4). Measurements have been made predominantly in an upright sitting position or, in the case of infants, lying down and possibly during sleep (figure 1) [14–16, 42, 50]. During TB, which can be performed with the mouth open or closed, the sampling described in the literature varies widely from a few seconds to a full minute [9, 12–16, 22, 32, 42–44, 48, 50–53].

TB nNO results have most often been reported as the mean of either three [14, 22, 42, 43, 51, 53] or five peaks [12, 16, 50] from the tracing curve that displays regular breathing (supplementary table E3). Peaks were chosen based on the highest values, sometimes needing to be consecutive (reflecting the regular respiration) or sometimes based on reproducibility criterion. In other studies, the result was the average over the time of the sampling [12, 13, 44, 45, 48].

To standardise the method for measuring TB nNO using a chemiluminescence analyser, we recommend that the mean of three to five maximum observed peaks is reported during a period of regular breathing over 30 s. If the child is uncomfortable and breathes irregularly, then a break before a new trial is preferable rather than continuation of the measurement. If, for no clear reason (calm child breathing regularly), successive peaks are still not regular in height before 30 s the recording can be extended after 30 s. The peaks should be within 20% or 10 ppb, whichever is greater (figure 5) [46]. It is a limitation of electrochemical devices that you can only report the average nNO sampled during the time set by the machine, which is always lower than the peak values and requires specific cut-off values or algorithms [12].

**Other manoeuvres**

Other techniques, e.g. humming and slow nasal or oral expiration without velum closure, have been tested in smaller, single-site studies [43, 44, 54]. These approaches require subject cooperation and reproducibility, which may be difficult for some patients [42]. The reproducibility and accuracy of these measures have not been determined and large-scale validation studies would be needed.

**Causes of flawed nNO results according to the methods of measurement**

Considerations about flawed results and troubleshooting are provided in the supplementary material and supplementary figures E2–E5.

**Reporting and interpretation of results (supplementary videos 2–5)**

The minimum information to include in a report is summarised in box 4 and in a sample proforma for data collection in supplementary figure E6. Interpretation of the results is summarised in box 5.

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Reporting and interpretation of results: general

Normative data are generally lacking, particularly for young children, electrochemical devices and TB manoeuvres (supplementary tables E3 and E4).

Evidence is based on studies using chemiluminescence analysers and further research is needed to confirm whether electrochemical devices are equivalent.

The difference between ER or BH nNO values obtained using one or the other analyser should be small and only due to the difference in the chemical process involved in the calculation of NO molecules. However, the difference between TB nNO measured using chemiluminescence or electrochemical devices is additionally altered by the difference in the part of the sampling used to calculate the result (mean of peaks versus mean of a period of TB).

The highest of two repeatable nNO measurements for each nostril should be recorded. In ~75% of cases, nNO variability in the same nostril is expected to be ≤10% for measurements performed with the velum closed and ≤20% for measurements performed during TB [46] (figure 4). The nNO levels in parts per billion (ppb) obtained from each nostril should then be reported along with inter-nostril repeatability, since the result reliability will decrease with increasing variability, especially with electrochemical analysers where no NO curve is displayed (box 1).

BOX 4 Reporting nasal nitric oxide (nNO) measurements

As a minimum the following should be included in a report (sample proforma in supplementary figure E6):

- Analyser model
- Sampling rate
- Ambient NO
- Testing method used
- ≥2 repeatable nNO results from right nostril
- ≥2 repeatable nNO results from left nostril
- Intra-nasal repeatability (ideally ER/BH ≤10% variation; TB ≤20%)
- Inter-nostril variability (ideally ER/BH ≤10% variation; TB ≤30%)
- Any technical or other noteworthy comments
- Final result (ppb)=highest result from highest nostril (MINUS ambient level if >20 ppb)
- Final standardised value (nL·min⁻¹)=final result (ppb)×sampling rate of analyser (L·min⁻¹)

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

FIGURE 5 A 6.5-year-old failed to perform exhalation against resistance or breath-hold for a long enough time. They were able to perform regular, repeatable tidal breathing measurements from the left nostril (<20% variation). a) Five reproducible (within 20%) maximal peaks (purple arrows) during a regular nitric oxide (NO) tracing are not consecutive, but acceptable, showing a mean of 485 ppb=160 nL·min⁻¹. b) On the second attempt, five reproducible consecutive peaks (purple arrows) were recorded showing a mean of 506 ppb=167 nL·min⁻¹. The result is Grade D and above the cut-off of 44 nL·min⁻¹. Note the low ambient NO levels (green arrows) measured after the end of the measurements. For grades, see box 1.
Having determined the final nNO result in parts per billion for each nostril, the highest nNO value from the two nostrils is converted to nanolitres per minute to standardise for the sampling rate using the equation:

$$\text{Standardised nNO value} = \frac{nNO \text{ concentration (ppb)}}{\text{flow sampling rate of analyser (L·min}^{-1})}$$

For example, using an Eco Physics CLD 88 sp analyser with a sampling rate of 0.33 L·min$^{-1}$, if the final averaged nNO concentration is 500 ppb, the final standardised value is $500 \times 0.33 = 165$ nL·min$^{-1}$. The sampling rate should be recorded as a subscript of nNO to allow between-result comparisons, e.g. $\text{TB}_nNO_{0.33}$=500 ppb or $\text{TB}_nNO_{0.33}$=165 nL·min$^{-1}$.

The standardised nNO from the highest result from the nostril with the highest reading should then be compared to reference data or cut-offs as described in the following subsections, taking into account age, breathing manoeuvre, ambient NO and analyser.

When interpreting the results, it is important to remember that although nNO is an excellent test during the PCD work-up, false-positive and false-negative results occur. There are an increasing number of PCD genes associated with nNO levels above the 77 nL·min$^{-1}$ cut-off, which will need to be revisited in multinational studies [55, 56]. We recommend that all low or doubtful results are confirmed on a different day.

### Interpreting results: ER manoeuvre

Several single-centre studies have evaluated reference data and nNO values obtained using ER in diagnostic settings (supplementary tables E3 and E4). In a multicentre study, LEIGH et al. [11] reported that for children $\geq$5 years of age, nNO measured using chemiluminescence is typically $>250$–$300$ nL·min$^{-1}$ in healthy controls and $<77$ nL·min$^{-1}$ in PCD. The study reported that with a cut-off of 77 nL·min$^{-1}$, the sensitivity was $>98$% and specificity was $>99.9$% across all age groups to identify PCD. Other studies, primarily using similar cut-off values, reported variable but generally good accuracy (supplementary table E4) [9, 22, 35, 40, 42, 43, 51, 57–60]. There is a strong agreement between repeated ER measurements made 1–4 months apart (intraclass correlation coefficient (ICC) 0.80, 95% CI 0.61–0.89) [43].

To be consistent with the North American Technical Paper [27], for nNO measurements performed during ER with a sampling rate of 0.3 or 0.33 L·min$^{-1}$, we currently suggest using a cut-off of 77 nL·min$^{-1}$ as part of the PCD diagnostic work-up. This assumes that most cooperative children will be $\geq$5 years of age.

### Interpreting results: BH manoeuvre

Several studies have evaluated nNO measurements obtained through the BH method in PCD (supplementary tables E3 and E4). Mean BH nNO values may be lower than ER nNO measurements, with larger standard deviations if the velum is not completely closed resulting in dilution from the lower airway.

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**BOX 5 Interpretation of nasal nitric oxide (nNO) results**

**General**
- If ambient NO $>20$ ppb, estimate its effect on the result by subtracting the ambient NO from the patient’s NO as described in the text
  - If the final result is well above the cut-off, it can be accepted
  - If the final result is close to the cut-off (based on local experience of variability) or an accurate result is needed, the measurement should be repeated on another day
- False-positive and false-negative results can occur

**ER and BH manoeuvres (chemiluminescence or electrochemical devices)**
- Cut-off is 77 nL·min$^{-1}$ with sampling rate close to 0.3 L·min$^{-1}$
- If $<77$ nL·min$^{-1}$, ideally perform TB to exclude false-positive result
- If $<77$ nL·min$^{-1}$, further primary ciliary dyskinesia diagnostic testing is indicated (consider repeating nNO first)

**TB manoeuvre**

Reference data is limited. In the experience of the Task Force experts:
- Age 1–2 years: cut-off 30 nL·min$^{-1}$
- Age $>2$ years: cut-off 44 nL·min$^{-1}$ for mean of peaks (chemiluminescence) or 40 nL·min$^{-1}$ for mean of 30 s of TB (all types of device)

ER: exhalation against resistance; BH: breath-hold; TB: tidal breathing.
Despite this, its discriminatory value is comparable to ER, and BH provides an adequate alternative to ER using the same cut-off values. BH nNO demonstrated very strong agreement between measurements repeated 1–4 months apart (ICC 0.85, 95% CI 0.70–0.92) [43].

We recommend a cut-off of 77 nL·min\(^{-1}\) for nNO measurements during BH (sampling rate 0.3 or 0.33 L·min\(^{-1}\) and assuming age \(\geq 5\) years). If the level is <77 nL·min\(^{-1}\), we recommend that a TB manoeuvre additionally be performed to exclude a false low reading (e.g. if velum closure was not attained).

Interpreting results: TB manoeuvre

Several studies have shown that nNO values are lower during TB than during ER or BH manoeuvres [9, 12, 21, 22, 42, 43, 45, 48] and specific cut-off values are needed to discriminate PCD from non-PCD patients when using this technique in the diagnostic setting (supplementary table E4). Furthermore, values are slightly lower during TB with the mouth closed than when the mouth is open [43]. A study of children (median (interquartile range) age 7.0 (4.7–11.0) years undergoing PCD diagnostic testing reported that nNO levels measured during TB were two-thirds of the ER values with excellent correlation between methods [50], as was also found by BOON et al. [51]. In the former study, a cut-off value of 44 nL·min\(^{-1}\) (sampling rate of 0.3 L·min\(^{-1}\)) was calculated to identify patients with PCD [50]. In children \(<5\) years of age (n=90), TB sensitivity and specificity were 76.9% (95% CI 54–99.8%) and 85.7% (95% CI 77.9–93.5%), respectively [50]. The low sensitivity was explained by nNO levels above the cut-off value in three children (mutations in RSPH1, CCDC103 and FOXJ1). BOON et al. [51] also reported lower specificity when measuring nNO during TB compared to ER. The variability of nNO measured during TB is larger than in manoeuvres ensuring velum closure for PCD and controls, reducing the discriminatory ability of the test [9].

As previously discussed, infant nNO levels are extremely low at birth and increase throughout the first few years of life, most rapidly during the first 6 months [14, 16]. In a cross-sectional study of 42 healthy infants \(<1\) year of age, ADAMS et al. [16] reported low mean nNO levels \(<15\) nL·min\(^{-1}\) in neonates, which increased to \(\sim 60\) nL·min\(^{-1}\) by 12 months of age. Similarly, MARTHIN et al. [14] reported median values of 15 nL·min\(^{-1}\) at 2 weeks, 42.6 nL·min\(^{-1}\) at 8 months, 58.7 nL·min\(^{-1}\) at 18 months and 93.4 nL·min\(^{-1}\) at 24 months in a longitudinal study of 44 healthy infants recruited at birth. As expected, limited data suggest that nNO levels are even lower in the first few months in infants with PCD and remain very low [14–16].

In older children and adolescents (age 5–18 years), MATEOS-CORRAL et al. [43] found very strong agreement of TB measurements when repeated after 1–4 months (ICC 0.88, 95% CI 0.76–0.94). GUPTA et al. [13] showed similar reproducibility over 24 h in 21 children \(<5\) years of age (ICC 0.88, 95% CI 0.71–0.95).

Small, single-centre studies measuring the mean of three to five peaks with a sampling rate close to 0.3 L·min\(^{-1}\) suggest a cut-off of 30 nL·min\(^{-1}\) \(i.e.\) lower limit of normal) when measuring nNO during TB in children between the ages of 1 and 2 years, and for children \(\geq 2\) years of age, we suggest 44 nL·min\(^{-1}\) cut-off \(i.e.\) best cut-off established in children \(\geq 4\) years of age) [14, 16, 50]. For the mean of 30 s of TB in children \(\geq 4\) years of age, the cut-off would be slightly lower (40 nL·min\(^{-1}\)) [12, 22]. However, it is important to remember that published, multicentre studies validating these cut-off values are lacking. Repeated measurements over time are needed to confirm low results in young children.

Gaps in knowledge and future directions

Despite long-standing use of nNO measurements in the diagnosis of PCD, many issues remain unresolved regarding these measurements and research is urgently required. In particular, although electrochemical analysers are the most commonly used devices in Europe, no research has been conducted in the diagnostic setting. The following research is urgently needed:

- Reference data is required, particularly:
  - In young children and infants
  - Using electrochemical devices
  - During TB manoeuvres.
- The accuracy of nNO measurements in diagnostic settings and the optimal cut-off values taking into account:
  - The ages of patients
  - The genetic cause of PCD
  - The analyser type (chemiluminescence or electrochemical)
  - The breathing manoeuvre (ER, BH or TB).
• The effect of ambient nNO on readings, and how best to manage high levels of ambient nNO levels when reporting and interpreting results.
• How long to delay measuring nNO after a respiratory tract infection? Current practice is 2–4 weeks.
• How long to wait between within-occasion repeated measurements?
• The extent that pre-school children with frequent adenoidal hypertrophy may have false low nNO values and how to “correct” for this in routine practice.
• Other necessary studies include: maintenance frequency, calibration frequency, the influence of sampling tube diameter on sampling rate, the long-term stability of NO sensors and whether biological control testing can be used for electrochemical sensors where standardised calibration is not possible.
• It is also unclear if nNO measurement should be repeated to confirm results and, if so, what is the best timing of this repeat measurement (e.g. 1 month later).

Conclusions
nNO is a relatively quick and inexpensive test that contributes to the diagnosis of PCD. Previous technical guidelines have focused on measurements using a chemiluminescence analyser during velum closure manoeuvres. A recent global survey demonstrated that many centres instead use electrochemical analysers, which are less expensive to purchase and maintain [20]. Also, non-velum closure manoeuvres are commonly used, particularly with young children. Despite widespread use, there are many gaps in our current knowledge regarding the use of electrochemical analysers and the role of nNO measurements in the diagnostic work-up of PCD in pre-school-age children. An ERS Task Force of experts therefore developed this technical standard, relying more on experience rather than extensive multicentre evidence. The Task Force highlighted where research is urgently needed to facilitate future evidence-based standards.

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