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This manuscript has recently been accepted for publication in the European Respiratory Journal. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.
Nasal nitric oxide measurement in children for the diagnosis of primary ciliary dyskinesia: European Respiratory Society technical standard

Short Title: PCD and nNO: a technical standard

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“Take home” message
ERS technical standard for measuring nasal nitric oxide in children with suspected primary ciliary dyskinesia.

158/ 250 word abstract
6571/ 8000 words main text;
11/15 tables/ figures/ boxes;
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 chemiluminescent analysers. However, measurements of nNO in pre-school children (2-5 years) may facilitate early diagnosis, and electrochemical rather than chemiluminescence analysers are widely used. Preschoolers 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 preschool-age children. Furthermore, we considered both chemiluminescence and electrochemical analysers that are in use worldwide. There was paucity of quality evidence for electrochemical analysers and sampling methods used in young children, and this manuscript 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 7500 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 ERS Diagnostic Guidelines recognises 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 >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 whilst gas is sampled from the nares and nasopharynx [11]. This technique ensures velum closure, thereby avoiding contamination and dilution of nasal gas with air from the lower airways. However, exhaling against resistance (ER) can only be achieved by older, cooperative children (usually >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/European Respiratory 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]. Chemiluminescent 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 was originally derived from the NIOX MINO [21, 22], which is no longer available. 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, whilst 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 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 preschool 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 (MC):

“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 to 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.

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 Supplementary files (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**

Nasal NO 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 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 breath-hold technique, Qian et al. 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 sec) [25]. 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 these recommendations, further evidence for optimal aspiration flow has been sparse. Struben et al. compared three aspiration flow rates of 0.28, 0.7 and 1.2 L.min⁻¹ using the NIOX chemiluminescent analyser and a breath-hold method in adult subjects [26]. 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 seconds and the procedure was well tolerated by adult study participants. In children, using the velum closure method, Beydon et al. 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 tidal breathing technique because higher flow disturbed young children [12].

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 “Interpretation of results - general”).

**Licensed 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 chemiluminescent 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 CLD88 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 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 (EcoPhysics CLD88sp). In both cases, as the equipment mouthpiece is used, an additional microbiological filter is required [29].

**Calibration, maintenance, and environmental nitric oxide**

Despite the importance of obtaining accurate and reproducible data, most manuscripts 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 below 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, this 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.
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 and 0.6 L.min\(^{-1}\)) [27].

Regular calibration of chemiluminescent analysers with standardised NO gas with two (high and low) certified calibration gases is recommended by some manufacturers, whilst 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 (Aerocrine AB) (no longer available) required calibration every 14-days, but other chemiluminescent 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, inline 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 preschool children (6-months - 5-years) with increasing operator experience [13]. As with other

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 x 0.33 L.min\(^{-1}\) = 93 nL.min\(^{-1}\) (> cut-off of 77 nL.min\(^{-1}\))

nNO output ‘corrected’ for ambient NO = (281-49)ppb x 0.33 L.min\(^{-1}\) = 232 ppb x 0.33 L.min\(^{-1}\) = 76 nL.min\(^{-1}\) (< cut-off of 77 nL.min\(^{-1}\))

*In this case the result should be evaluated on another day when ambient level is low because the ‘corrected’ output is close to the diagnostic cut-off value.*
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. BEATPCD, 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 authors, 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. reported significantly reduced nNO measurements with increasing size of adenoids in non-allergic non-PCD patients between the ages of 5-18 years; these measurements normalised following adenoidectomy [37]. Therefore, members of the Task Force consider referring children for ENT 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.

Nasal NO 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 measures.

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.

**Special considerations when measuring in different age groups**

The ERS Task Force agrees with the recently published North American Technical Paper, which focused on individuals > 5 years of age [27]. Briefly, that paper concluded that ER is the preferred sampling method, ideally using a mouth resistor or party favour (blow out toy) partially occluded at the distal end. The breath-hold (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-aged and older children, it is often not feasible in pre-school aged children. Piacentini and co-workers reported that only 14% of healthy children between the ages of 2-5 years could actively cooperate [45]. 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 whilst tidal breathing, a non-velum closure method, shows greater variability [46].

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

Feasibility and success rates for infant nNO measurements using the TB method have been described in two studies [14, 15]. Buechel et al. 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 [15]. A similar high success rate (99.6%) was reported by Marthin et al. in 44 infants 2-weeks to 2-years old using a chemiluminescence analyser [14].

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 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 older than 12-months, 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 under 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 (SOP) for performing a measurement using different respiratory manoeuvres**

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.

**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 1).
- 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 the room’s 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 1).

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 seconds 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 files).

**Expiration against resistance manoeuvre**

The methods for measuring nNO using an ER manoeuvre 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 above. 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 their mouth with a targeted expiratory mouth pressure of at least 10 cm H$_2$O in adults and 5 cm H$_2$O in children [19]. Alternatively, a party favour (blowing toy taped closed at the distal end to prevent too rapid an exhalation) 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 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 at least 3 seconds).

The technician should manually choose the plateau rather than using the automated software [27]. Acceptable nNO plateaus are 3-seconds or longer in duration and there should be less than 10% variation (between the minimum and maximum), using the maximum attainable mean plateau values [27] (Figure 4). 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 chemiluminescent 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 not be less than 10-seconds [49]. The plateau duration 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 manufacturers stated that for the NIOX MINO set at 5 mL.s$^{-1}$, the last 30-seconds over the 45-second 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 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].
Breath-hold manoeuvre

This manoeuvre is an alternative to the preferred method of exhalation against resistance and may be used for patients who cannot perform ER. Details are similar to 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 chemiluminescent analyser, the BH manoeuvre should be maintained to achieve a plateau of > 3 seconds with < 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-sec at 2 mL.s⁻¹ and 45-sec at 5 mL.s⁻¹) [21], while in the newer versions (e.g. NIOX VERO) the sampling time is 30-seconds at 5 mL.s⁻¹.

Tidal breathing method

Nasal NO measured during tidal breathing 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 files 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 [14-16, 42, 50] (Figure 1). During tidal breathing, which can be performed with open or closed mouth, 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 from the tracing curve that displays regular breathing [12, 16, 50] (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 3 to 5 maximum observed peaks is reported during a period of regular breathing over 30 seconds. If the child is uncomfortable and breathes irregularly, then a break before a new trial is preferable than the 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 the greatest [46]. (Figure 5) 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 such as humming, slow nasal or oral expiration without velum closed

Other techniques, such as 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 Online Supplemental Material text and Supplementary Figures E2-5.

Reporting and interpretation of results

The minimum information to include in a report is summarised in Box 4, and in a sample proforma for data collection. (Supplementary Figure E6). Interpretation of the result is summarised in Box 5.

Reporting and interpretation of results - general

Normative data are generally lacking, particularly for young children, electrochemical devices, and TB manoeuvres (Supplementary files 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 calculating 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 around 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 tidal breathing [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).
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:

**Standardised nNO value (nL.min⁻¹) = nNO concentration (ppb) x flow sampling rate of analyser (L.min⁻¹)**

For example, using a CLD88sp analyser (EcoPhysics) 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 x 0.33 = 165 nL.min⁻¹.

The sampling rate should be recorded as a subscript of nNO to allow between-result comparisons, e.g., TB nNO₀.₃₃ = 500 ppb; or TB nNO₀.₃₃ = 165 nL.min⁻¹.

The standardised nNO from the highest result from the nostril with highest reading, should then be compared to reference data or cut-off as described below, 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 is as 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 - exhalation against resistance*

Several single centre studies have evaluated reference data and nNO values obtained using ER in diagnostic settings (Supplementary files Tables E3 and E4). In a multicentre study, Leigh et al. reported that for children 5-years and older, nNO measured using chemiluminescence is typically >250-300 nL.min⁻¹ in healthy controls and <77 nL.min⁻¹ in PCD [11]. This 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 [9, 22, 35, 40, 42, 43, 51, 57-60] (Supplementary files Table E4). There is a strong agreement between repeated ER measurements made 1 to 4 months apart (ICC 0.80; 95% CI 0.61 to 0.89) [43].

To be consistent with the North American Technical standard, for nNO measurements performed during exhalation against resistance 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 workup. This assumes that most cooperative children will be 5-years or older.

*Interpreting results - breath hold*
Several studies have evaluated nNO measurements obtained through the BH method in PCD (Supplementary files 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. 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 to 4 months apart (ICC 0.85; 95%CI 0.70 to 0.92) [43].

We recommend a cut-off of 77nL.min$^{-1}$ for nNO measurements during breath hold (sampling rate 0.3 or 0.33 L.min$^{-1}$, and assuming around 5-years or older). If the level is lower than 77nL.min$^{-1}$, we recommend that a tidal breathing manoeuvre additionally be performed to exclude a false low reading (e.g., if velum closure was not attained).

**Interpreting results - tidal breathing**

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 age 7-years; IQR 4.7-11.0) undergoing PCD diagnostic testing reported that nNO levels measured during TB were two-thirds of the ER values with excellent correlation between methods, as was also found by Boon et al. [50, 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 under 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. also reported lower specificity when measuring nNO during TB compared to ER [51]. 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 less than 1-year of age, Adams et al. reported low mean nNO levels (< 15 nL.min$^{-1}$) in neonates, that increased to approximately 60 nL.min$^{-1}$ by 12-months of age [16]. Similarly, Marthin et al. 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 [14] 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 (ages 5-18 years), Mateos-Corral et al. found very strong agreement of TB measurements when repeated after 1 to 4 months (ICC 0.88; 95%CI 0.76 to 0.94) [43]. Gupta et al. showed similar reproducibility over 24 hours in 21 children under 5-years of age (ICC 0.88; 95%CI 0.71 to 0.95) [13].

Small, single centre studies measuring the mean of 3 to 5 peaks with a sampling rate close to 0.3 L.min⁻¹ suggest a cut-off of 30 nL.min⁻¹ (ie. lower limit of normal) when measuring nNO during TB in children between the ages of 1-2 years, and for children ≥2-years, we suggest 44 nL.min⁻¹ cut-off (ie. best cut-off established in children 4 years and older) [14, 16, 50]. For the mean of 30 s of TB in children 4 years and older, 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 measures over time are needed to confirm low results in young children.

Gaps in knowledge and future directions

Despite longstanding 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 tidal breathing 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, 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 preschool 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
Nasal nitric oxide is a relatively quick and inexpensive test which contributes to the diagnosis of PCD. Previous technical guidelines have focussed 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 use of electrochemical analysers, and the role of nNO measurements in the diagnostic work-up of PCD in pre-school children. A 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.

Acknowledgements: We are grateful to the Guidelines Working Group for their advice and support. Thank you to Lynn Reeves for the administrative support for the Task Force. Members of the Task Force are members of BEAT-PCD, an ERS Clinical Research Collaboration, and the European Reference Network for Rare Diseases (ERN-Lung).
**Table 1:** The advantages and disadvantages of chemiluminescence and electrochemical analysers

<table>
<thead>
<tr>
<th>Chemiluminescent Analyser</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g., CLD88sp (EcoPhysics/EcoMedics, Switzerland) Sievers/Zysense NOA-280i (General Electric Analytical Instruments, USA)), NIOX Flex (Aerocrine AB, Sweden), LR2000 (Logan Research Ltd, UK) EVA 4000 (SERES, France)</td>
<td>Higher accuracy (eg. &lt; 1 ppb with 1% linearity from 0.1 to 5000 ppb, CLD 88® Eco Medics)</td>
<td>Expensive to purchase and maintain (high cost per test for centres performing a limited number of measurements)</td>
</tr>
<tr>
<td>Continuous measurement and real-time display of NO test sample</td>
<td>Need for regular calibration and preventative maintenance</td>
<td></td>
</tr>
<tr>
<td>Allows for identification of the measurement endpoint – stable plateau (ER, BH) or regular peaks (TB) without a fixed sample collection minimum time requirement</td>
<td>Requirement for increased operator training and expertise</td>
<td></td>
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<tr>
<td>Online, real-time display allows identification and recording of unreliable measurements (nares leak, contamination from lower airways), aiding the validation of the result</td>
<td>Difficult to transport, and although an “offline method” of measurement allows for remote sampling, it loses the advantage of the real-time trace</td>
<td></td>
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<tr>
<td>Ambient NO can be measured and recorded prior to each test occasion</td>
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<td></td>
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<tr>
<td>Rigorous testing in young people 5-years and older with published, validated diagnostic or screening cut-off values</td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Electrochemical Analyser</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g., Circassia (formerly Aerocrine AB) NIOX MINO (discontinued 2018) and NIOX VERO models</td>
<td>Simple to use and requires no calibration</td>
<td>Output parameter of mean nNO lacks controlled, multicentre studies validating reference ranges for interpretation (mean values acquired during TB are significantly lower than the peaks values) diagnostic or screening cut-off value.</td>
</tr>
<tr>
<td>Cost effective solution for low volume sites</td>
<td>Requires uninterrupted sampling for a fixed time that can be problematic in young children due to interrupted flow (sniffing, crying) or difficulty maintaining the desired technique for the fixed duration of the test.</td>
<td></td>
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<tr>
<td>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</td>
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<tr>
<td>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.</td>
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<td></td>
</tr>
<tr>
<td>Lower accuracy e.g., ± 5 ppb for values &lt; 50 ppb and 10% for values &gt; 50 ppb, (Niox Vero®, Circassia*)</td>
<td>Inconsistent training and standard operating procedures provided by manufacturers</td>
<td></td>
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</tbody>
</table>

ER: Expiration against resistance; BH: Breath-hold; TB: Tidal breathing; ppb: parts per billion

Box 1: Grading of measurements. Grade A is the most acceptable and F the least acceptable measurements (explained in supplementary file).

<table>
<thead>
<tr>
<th>Grade</th>
<th>chemilum</th>
<th>electrochem</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>≤ 10%</td>
<td>≤ 10%</td>
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<tr>
<td>B</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>&gt; 10%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt; 10%&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>C</td>
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<td></td>
<td>≤ 10%</td>
<td>≤ 10%</td>
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<tr>
<td>D</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>≤ 30%</td>
<td>≤ 20%</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&gt; 30%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&gt; 20%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>&gt; 10%&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt; 10%&lt;sup&gt;d&lt;/sup&gt;</td>
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</tbody>
</table>

Repeatability: difference between two measurements

<sup>a</sup>: Tidal breathing in an awake or sleeping child not moving, vocalizing or crying with visually a regular breathing pattern.

* Measures can be the mean of 5 peaks of NO (chemiluminescence) or the mean NO over a period of sampling (electrochemical)

<sup>b</sup>: if only one repeatability > 10% or undetermined, grade is B

<sup>c</sup>: if only inter-nostril repeatability > 30% or undetermined, or intranostril repeatability > 20% or undetermined, grade is E

<sup>d</sup>: if only one repeatability > 10% or undetermined, grade is F

Box 2: Considerations for nNO 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 PCD centre if in doubt:
  - Levels in healthy children < 5 years are lower than older healthy children
  - Limited normative data
- To choose manoeuvre:
  - ER if compliant (usually > 5-year)
  - Breath hold if compliant but unable to achieve ER
  - TB if non-compliant or unable to achieve ER/BH
Box 3: Measuring nasal nitric oxide

General
1. Check equipment and calibrate if needed.
2. Assess the child to determine likely attainable breathing method and olive size.
3. Explain the procedure and practice.
4. Attach flow sampling line to olive with attached filter.
5. Position olive in nostril with no leak; other nostril open.
6. Obtain x2 nNO levels from first nostril (ER/BH: ideally within 10%; TB 20%)
7. Repeat in second nostril (ER/BH ideally within 10%; TB 30%)

Exhaled against resistance
- Resistor mouthpiece 5-10 cm H₂O; or party favour (blow out toy taped closed at the distal end)
- Ideally, with chemiluminescence device:
  - Slow oral exhalation against resistor until a plateau > 3 seconds with ≤ 10% variation (min/max)
  - View real-time and manually determine optimal plateau
- Alternatively with electrochemical device:
  - Slow oral exhalation for duration set by machine (must be > 10 seconds)
  - If available, the NO tracing is not real-time but can be used to manually select the plateau (> 3 seconds with ≤ 10% variability). Alternatively accept the machine’s result.

Breath hold
As for ER except:
- Instruct child to inhale to total lung capacity
- Achieve velum closure whilst holding breath by closing glottis and performing Valsalva manoeuvre
- If measured, CO₂ at the open/free nostril should be zero

Tidal Breathing
- During > 30 seconds of stable tidal breathing:
  - Ideally, with chemiluminescence device, manually choose the mean of 3 to 5 peaks (can be non-consecutive and should be ≤ 20% variability or 10 ppb, whichever is greater).
  - With electrochemical, if possible, select the peaks from a tracing. Alternatively report the result calculated by the analyser (this is the average nNO sampled, not the mean of peak values and is therefore lower than the chemiluminescence result).
Box 4: Reporting
As a minimum the following should be included in a report (sample proforma Supplementary figure E6)

- analyser model
- sampling rate
- ambient NO
- testing method used
- x2 repeatable nNO results from right nostril
- x2 repeatable nNO results from left
- Intra-nasal repeatability (ideally ER/BH < 10% variation; TB < 20%)
- Inter-nostril variability (Ideally ER/BH < 10%; TB ≤ 30%)
- Any technical or other noteworthy comments
- **Final result in ppb** = highest result from highest nostril (MINUS ambient level if >20 ppb)
- **Final standardised value (nL.min⁻¹)** = final result in ppb x sampling rate of analyser (L.min⁻¹)

Box 5: Interpretation of results

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

**Exhaled against resistance and breath hold (chemiluminescence or electrochemical devices)**
- Cut off is 77nL.min⁻¹ with sampling rate close to 0.3 L.min⁻¹
- If < 77nL.min⁻¹ ideally perform tidal breathing to exclude false positive result
- If < 77nL.min⁻¹, further PCD diagnostic testing is indicated (consider repeating nNO first)

**Tidal breathing**

Reference data is limited. In the experience of Task Force experts
- 1-2 years cut off 30 nL.min⁻¹
- > 2-years cut off 44 nL.min⁻¹ for mean of peaks (chemiluminescence) or 40 nL.min⁻¹ for mean of 30 s of tidal breathing (all types of device)
References


Figure 1: Nasal nitric oxide measurement in children, a) a 1-year-old sleeping in his mother arms with a nasal olive (blue) inserted in the left nostril to sample nasal air during tidal breathing; b) a 4-year-old performing tidal breathing nasal NO measurement while sitting on her mother’s lap. The mother maintains the olive (blue) in the right nostril while keeping a hand behind the girl’s head and neck to prevent her from moving backward and causing leaks around the olive; c) an 8-year-old performing expiration against a resistance (party-favour) while the technician places the olive (white in the left nostril) and keeps a hand at the back of the head. (Parental consent obtained for use of images).
**Figure 2** Suggested approach for measuring 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. nNO= nasal nitric oxide; ER= exhaled against resistance; BH= breath hold; TB= tidal breathing.

**Prepare:** Check no symptoms of infections in recent weeks, explain procedure, blow nose, measure ambient nNO.

**Measure nNO in both nostrils**

- **Child < 5 years**
  - Measure TB-nNO

- **> 5 years**
  - Measure ER-nNO
    - Failure
    - Measure BH-nNO
      - Failure
      - Success

  - ER or BH nNO NO < 77nL.min⁻¹ at 0.3 L.min⁻¹ sampling flow in both nostrils
  - ER or BH nNO minus ambient NO > 77nL.min⁻¹ at 0.3 L.min⁻¹

**Report**
- all nNO results and manoeuvres
- highest nNO in ‘highest’ nostril
- ambient NO
- sampling flow rate

**Interpret results**
Figure 3: Training of a 4.9-year-old to achieve nasal NO measurements during expiration against a resistance using a chemiluminescent analyser a) 2 unsuccessful trials. The plateau (circled) is high, between 700 – 800 ppb but the duration is too short (< 3s); b) after training, the child succeeds in a stable (variation min-max of 3.6%, ie. < 10%) plateau of 4.7s (> 3s, between vertical lines) showing a mean value of 768 ppb = 253 nL.min⁻¹, far above the cut-off of 77 nL.min⁻¹. Note the low ambient NO (green arrows) measured between trials and after the end of the plateau.
Figure 4: Using an electrochemical device a 5.3-year-old performed nNO-ER measurements, 2 in each nostril, with the following results: right side: no result, 230 ppb/76 nL.min⁻¹, left side 262 ppb/86 nL.min⁻¹, and 223 ppb/74 nL.min⁻¹. This is a Grade F result (>10% variation between 2 left, and between right and best left results with electrochemical technique). Since the result is poorly reliable and alternately higher or lower than the cut-off (77 nL.min⁻¹), without assessment of ambient NO, tidal breathing measurements were performed, which showed a mean nNO-TB of 328 ppb/109 nL.min⁻¹ right side and 350 ppb/115 nL.min⁻¹ left side. This is a Grade E result (because intra-nostril repeatability is undetermined). The result is largely higher than the cut-off (44 nL.min⁻¹) in favour of a high NO level in this child. However, this ER measurement is unreliable because the nNO-ER levels were lower than nNO-TB which is not possible. If performed using a chemiluminescence analyser, the same results would be Grade B for nNO-ER (>10% intra- and inter-nostril variability), and Grade E for nNO-TB (because intra-nostril repeatability is undetermined and inter-nostril > 30%). However, regular high peaks (> cut-off, dotted purple lines) with low ambient NO levels (green arrows) for the left nNO-TB measure can confidently be interpreted as a normal result (For Grades see Box 1).
Figure 5: A 6.5-year-old failed to exhale against a resistance or perform breath hold for a long enough time. They were able to perform regular, repeatable TB measurements from the left nostril (<20% variation) a) 5 reproducible (within 20%) maximal peaks (purple arrows) during a regular NO tracing are not consecutive, but acceptable, showing a mean of 485 ppb = 160 nL.min⁻¹; b) on the second attempt, 5 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)
Nasal nitric oxide measurement in children for the diagnosis of primary ciliary dyskinesia: a technical standard

Nicole Beydon, Panayotis Kouis, June K. Marthin, Philipp Latzin, Murielle Colas, Stephanie D. Davis, Eric Haarman, Amanda Lea Harris, Claire Hogg, Emma Kilbride, Claudia E. Kuehni, Diana Marangu, Kim G. Nielsen, Catherine Pendergrast, Phil Robinson, Nisreen Rumman, Matthew Rutter, Woolf T. Walker, Thomas Ferkol, Jane S. Lucas

Online Supplemental Material

Task Force and Work Group Composition

The membership and roles of the Task Force panel are summarised in Supplementary Tables E1 and E2. Jane Lucas and Nicole Beydon (Chairs) were responsible for the governance and integrity of the work conducted in this TF, and coordinated the activities including writing the manuscript. Panel members disclosed potential conflicts of interest according to ERS policies at the start of the Task Force and prior to the publication of this manuscript. Following review of these statements, the Chairs and Guidelines Working Group considered it unnecessary for any panel member to abstain from decisions for any of the recommendations. Work Groups (WG) leaders were proposed and agreed upon at the task force’s second meeting, based on their expertise.

The TF panel was comprised of experts and trainees in the field of paediatric PCD from multidisciplinary backgrounds including pulmonologists, PCD Nurse Specialists, and diagnostic scientists. We aimed to include clinicians from diverse geographical locations and backgrounds. Some members of the panel lead national diagnostic centres, and there were members from countries with limited diagnostic facilities. The panel members volunteered to participate in WG activities based on their expertise and interests. Our panel included the parent of a young child with PCD.

Due to the COVID pandemic, WG and whole panel meetings were held virtually.

Methods and results of systematic review

To inform the development of the technical standard, we relied on evidence collected through an updated systematic literature review that was originally carried out in a previous ERS Task Force (PCD Diagnostic testing TF-2014-04)(1). In more detail, the systematic review answered the PICO question “In patients suspected of having PCD, should nasal NO measurement be used as a diagnostic tool?” and covered the period January 1994 to March 2015. For the purposes of the current TF, we updated the literature search to extend the covered period to December 2021 and assessed the combined recovered manuscripts from 1994 to 2021 independently of the previous Guideline’s review to determine whether they contribute to the technical standard.

The updated literature search was carried out in Medline (accessed via PubMed) and Embase and the employed search algorithms and PRISMA diagram are presented in Table E5 and Figure E1.
In total, the employed search algorithm recovered 390 items (181 items in Medline and 209 additional items in EMBASE). After screening the title and abstract, 166 items were considered relevant and were reviewed in full text for inclusion. Eventually, 41 items contributed to the technical standard.

Methods to develop the practice standard

Each work group considered all the manuscripts identified by the systematic search and decided if they contained information relevant to their area of focus (Table E2). Although the search was systematic, the review of each document included careful reading by several group members, but not a systematic data extraction following the GRADE approach as would be required for an ERS Guideline. Meetings of each WG discussed the relevant details from each paper, and then reported back to the panel for further discussion. The four groups then drafted text for the manuscript. The draft text was reviewed by the full panel by email, and in a series of meetings. The full panel agreed on the final text.

Advantages and pit-falls of nasal nitric oxide (nNO) measurements according to techniques

Chemiluminescence analysers continuously measure nNO and provide an online, real-time display of the test sample. The absence of a fixed sample time ensures the test continues until the child has achieved the required respiratory manoeuvre. The real-time nNO trace allows the identification of a successful measurement – stable plateau (exhalation against resistance, breath-hold) or regular peaks (tidal breathing) – which can then be individually selected to determine the final nNO result. The tracing can help detect unreliable measurements (nares leak, contamination from lower airways), aiding the validation of the result. Ambient NO can also be measured and recorded prior to each test. These analysers have been rigorously tested in people 5 years and older with published, validated diagnostic or screen cut-off values (2).

Chemiluminescence analysers are expensive to purchase and operate, requiring regular calibration and maintenance and it is recommended that operators be trained in approved operating protocols (3). This type of measurement may not be feasible for centres performing a limited number of measurements without the qualified personnel to perform the technique (4). The analysers are stationary devices and while an “offline method” of measurement allows for remote sampling, the advantage of the real-time tracings is lost (5).

Electrochemical analysers are simple to use, require no calibration, little maintenance and offer a more cost-effective option for the measurement of nNO. They are smaller devices and can be portable allowing nNO measurements at different sites. However, there are significant limitations to these devices. Electrochemical analysers require complete, uninterrupted sampling throughout a fixed sample time, which can be problematic in young children due to interrupted flow (sniffing, crying) or difficulty maintaining the desired technique for the fixed duration of the test (6, 7). The nNO result generated is the mean nNO over a fixed sample time, meaning individual, distinct peaks cannot be selected. As a result, mean values acquired during tidal breathing are significantly lower than the peaks values and must be interpreted accordingly (8). Electrochemical analysers do not provide a real-time
display of the sampling, which makes it difficult to detect unreliable measurements (leak, lower airway contamination).

To overcome some of these limitations, a recent device computes a NO curve, which is displayed after the end of the analysis (Niox Vero®, CircassiaLTD). The ability of this device to discriminate healthy subjects from patients with PCD has been described in a publication as an abstract (9). Finally, the sensor in this electrochemical device has a limited test capacity and must be replaced once this limit is reached. Repeated measurements in an individual due to low results limits the lifetime of these sensors, as does the measurement of ambient NO levels, which may make users less likely to monitor or record (4).

Perhaps most importantly, controlled, multicentre studies validating electrochemical devices are lacking, and diagnostic or screening cut-off values have not been established. In addition, based on our survey results, many sites that use electrochemical analysers do not have a standardised operating procedure, and training is inconsistent (4).

Standard operating procedures (SOP) for performing a measurement using different respiratory manoeuvres

Order of different methods as adapted to the respiratory manoeuvre

For infants, toddlers and preschoolers, the tidal breathing method is the most feasible method with acceptability of nNO levels of 95.2% in infants down to 14-days or 100% during the first week of life. This highly exceeds acceptability of nNO measurements performed during expiration against a resistance (ER) where only 25.8% of toddlers between the ages of 2.5 to 6-years had acceptable manoeuvers. For nNO measurements using the breath-hold (BH) method the acceptability was as low as 3.2% between the ages of 4.1 and 6-years (10-12).

Other manoeuvers such as humming, slow nasal or oral expiration without velum closed

Inconsistent or incomplete velum closure can lead to intake of exhaled air from the pulmonary and oropharyngeal space, where nitric oxide concentrations are much lower than those found in the nasopharyngeal cavities, thus diluting the nasal sample, and artifactually reducing the value, and potentially leading to false positive results. Low nNO values must be interpreted cautiously and should be confirmed, possibly using a different method.

Results of the nNO measurement using different sampling methods

Examples of measurements

A single-tidal breathing nNO measurement

A single-tidal breathing nNO measurement has most often has been reported as the mean of three (10, 13-15), or five peaks from a tracing curve that displays regular breathing (8, 16). Peaks were chosen for being the highest observed and sometimes a reproducibility criterion was used, such as consecutive breaths or tidal breaths between 5% to 15% of each other to guarantee regularity of tidal breathing. In other studies, the result was the average over 10, 5 or 3-second periods with steady plateaus (17), or a mean value through 10-seconds of regular tidal breathing, (8, 18), or a mean value through the last 30-seconds of the recording (8).

Causes of flawed nNO results according to methods of measurement
For measurements involving a plateau of nNO that can be seen from the displayed NO curve, the mean of at least a 3-second plateau is reported. In opposition to recommendations given for orally expired NO measured in children less than 12 years old (19), it is probable that a 2-second plateau is unreliable to report nNO (possible underestimation of the value), which is particularly true when this short plateau is reached early during the manoeuvre or when the NO tracing is constantly increasing during the procedure. The much higher upper airway concentration of NO as compared to that of the lower airways, along with the lower sampling flow rate used to measure NO in the nares as compared to bronchial NO, explain the longer time warranted to achieve a plateau and the unreliability of the measure when the manoeuvre is aborted early (20).

nNO during tidal breathing must be recorded while the child’s breathing is calm and regular. Cries or shouts, as forced expiratory manoeuvres, can increase (at first) or decrease the nNO output. Therefore, measurements performed during cries or shouts cannot be reported confidently. It is possible to perform the measurement 5 to 10-minutes later after the child has resumed a calm and regular breathing pattern. Infants have been measured while sleeping but no comparison between awake or sleeping infants has been performed; this also applies to the positioning of the infant (supine or lying position) (10-12, 16, 21).

Irregular NO tracings might show peaks of NO above or under the threshold, especially in young children. In this case, repeated measurements usually lead to more regular NO tracings due to the child’s acclimatisation to the procedure. If the tracing remains with peaks on both sides of the threshold, it is important to compute the mean of the reproducible (20% or 10 ppb, whichever greater) consecutive peaks which may better reflect the true NO output rather than averaging the highest peaks from different parts of the irregular breathing recording. Using electrochemical devices, the criteria for calm and regular breathing are only from observing the child while performing the measurement.

Using both chemiluminescence and electrochemical techniques, the NO value achieved while the velum is closed (expiration against a resistance or breath-hold methods) should be higher than that measured at peaks of tidal breathing. Indeed, taking into account the low sampling flow rate usually used to measure nasal NO (< 0.40 L.min⁻¹) (4) there is no way that during tidal breathing with the velum open the nNO output equals that present after the subject has closed the velum for several seconds in the absence of other nasal airflow other than the sampling flow rate. The literature confirms that tidal breathing nNO values are between 50% and 77% of the values measured with a closed velum in the same sick or healthy patients (6, 10, 14-16), except for two PCD populations in whom tidal breathing and velum closure NO values were both very low (10, 15). Therefore, when the result of nNO measured during tidal breathing is higher than that of nNO measured during expiration against resistance or through breath-hold manoeuvres, a cause of falsely low NO values should be investigated, which may reveal partial nasal obstruction. The erroneously low result should not be reported.

**Reporting and interpretation of results - general**

*Grading system to interpret nasal NO results (Box 1 in main document)*

As the respiratory manoeuvres and techniques used to measure the amount of nNO have different accuracy and repeatability, it is proposed to score the nNO results against these objective factors, assuming perfect performance.
of the measurement to the extent that the investigator can judge (inspection of the child and NO tracing if available). The score should be understood as an indicator of the degree of confidence that a person interpreting the results of a nNO value can have that the value actually reflects the child’s nNO production relative to the method and technique used. This is distinct from the ultimate clinical interpretation. For example, four (two in each nostril) non-repeatable nNO measurements performed during TB using an electrochemical analyser of 80 and 98 nL.min⁻¹ right side, 105 and 131 nL.min⁻¹ left side have low accuracy (Grade F) but can help exclude PCD in a child with a low clinical suspicion. Alternatively, repeatable (within 10%) low nNO values using the ER method measured by a chemiluminescence analyser has high accuracy (Grade A) but would be of poor help in the diagnostic work-up if performed in a child during an acute upper airway infection. In this respect, the grading system cannot be used alone to affirm the reliability of the measurement. The entire SOP developed in this Technical Standard document must be followed for proper testing.

The most reliable result (Grade A) would be a well-performed manoeuvre, using a chemiluminescence analyser, with the velum closure and the sampled nNO having a ≤10% intra- and inter-nostril repeatability between 2 measurements. When the repeatability of either the same nostril and/or both nostrils is >10%, again using a chemiluminescence analyser, the measurement could still be considered as highly reliable (Grade B) since the NO tracings show at least 3 second plateaus during correct respiratory manoeuvres, and the highest value is reported. Next, correct ER or BH manoeuvres resulting in repeatable (≤10%) results using an electrochemical analyser may be accurate enough (Grade C) to be clinically useful. As nNO measurements performed using the TB method are more variable than methods involving velum closure and reference values are scarce, its accuracy in distinguishing PCD from non-PCD patients is considered moderate. However, when the regular TB pattern result in reproducible NO peaks (≤20%) displayed on the NO tracings (chemiluminescence analyser) and repeatable TB measurements (≤20% same nostril, ≤30% both nostrils) are displayed, the highest value can be reported and clinically useful (Grade D). Lastly, some measurements with poor repeatability measured either during TB with chemiluminescence or electrochemical analysers (Grade E) or during ER or BH using an electrochemical analyser (Grade F) would not be acceptable. Even when not repeatable, TB measurements might be slightly more accurate than measurements performed with the velum closed with no NO tracings available because it is easier to evaluate the correct (regular breathing without moving, speaking, crying...) sampling of nNO during TB than a correct, constant expiration or breath-hold supposedly resulting in a stable plateau without visual feed back.
Table E1: Task force composition, presented in alphabetical order.

<table>
<thead>
<tr>
<th>Task Force member</th>
<th>Speciality/ expertise</th>
<th>Role/ (Work Group (WG) membership)</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beydon, Nicole</td>
<td>Respiratory Paediatrician and Physiologist</td>
<td>Co-chair; Work package lead WG2</td>
<td>France</td>
</tr>
<tr>
<td>Colas, Murielle</td>
<td>Parent of a child with PCD</td>
<td>Parent representative</td>
<td>France</td>
</tr>
<tr>
<td>Davis, Stephanie</td>
<td>Respiratory Paediatrician</td>
<td>WG4</td>
<td>USA</td>
</tr>
<tr>
<td>Ferkol, Thomas</td>
<td>Respiratory Paediatrician</td>
<td>WG2</td>
<td>USA</td>
</tr>
<tr>
<td>Haarman, Eric</td>
<td>Respiratory Paediatrician</td>
<td>WG3</td>
<td>The Netherlands</td>
</tr>
<tr>
<td>Harris, Amanda</td>
<td>PCD &amp; Children's Respiratory Nurse Specialist</td>
<td>WG2</td>
<td>UK</td>
</tr>
<tr>
<td>Hogg, Claire</td>
<td>Respiratory Paediatrician</td>
<td>WG3</td>
<td>UK</td>
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<tr>
<td>Kilbride, Emma</td>
<td>Respiratory Physiologist</td>
<td>WG2</td>
<td>Ireland</td>
</tr>
<tr>
<td>Kouis, Panayiotis</td>
<td>Biologist, PCD diagnostics</td>
<td>Lead systemic literature search; WG1</td>
<td>Cyprus</td>
</tr>
<tr>
<td>Kuehni, Claudia</td>
<td>Respiratory Paediatrician and Epidemiologist</td>
<td>WG3</td>
<td>Switzerland</td>
</tr>
<tr>
<td>Lucas, Jane</td>
<td>Respiratory Paediatrician</td>
<td>Co-Chair; Work package lead WG4</td>
<td>UK</td>
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<tr>
<td>Nielsen, Kim</td>
<td>Respiratory Paediatrician, Lung Function Testing in Young and School-age Children</td>
<td>WG2</td>
<td>Denmark</td>
</tr>
<tr>
<td>Latzin, Philipp</td>
<td>Respiratory Paediatrician, Physiology and Epidemiology</td>
<td>Work package lead WG1</td>
<td>Switzerland</td>
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<tr>
<td>Marthin, June</td>
<td>Respiratory Paediatrician. PhD in PCD diagnostics including nasal NO</td>
<td>Work package lead WG3</td>
<td>Denmark</td>
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<tr>
<td>Marangu, Diana</td>
<td>Paediatric Pulmonologist and Global Health Specialist</td>
<td>Junior member; systemic literature search; WG1</td>
<td>Kenya</td>
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<td>Pendergrast, Catherine</td>
<td>Respiratory physiologist</td>
<td>WG1</td>
<td>Australia</td>
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<td>Robinson, Phil</td>
<td>Respiratory Paediatrician</td>
<td>WG1</td>
<td>Australia</td>
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<td>Rumman, Nisreen</td>
<td>Respiratory Paediatrician</td>
<td>Diagnostics in resource-limited countries; systematic literature search, WG2</td>
<td>Palestine</td>
</tr>
<tr>
<td>Name</td>
<td>Profession</td>
<td>WG</td>
<td>Country</td>
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<td>Rutter, Matthew</td>
<td>Respiratory Physiologist</td>
<td>WG1</td>
<td>UK</td>
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<td>Walker, Woolf</td>
<td>Respiratory Paediatrician</td>
<td>WG3</td>
<td>UK</td>
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<tr>
<td>Work Group (WG)</td>
<td>Key topics</td>
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<tr>
<td><strong>WG1</strong></td>
<td>Which technical standards should the equipment meet?</td>
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<tr>
<td>1. Equipment requirements (inclusive of the variety of commercially available equipment)</td>
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<tr>
<td>2. Equipment calibration and quality control</td>
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<tr>
<td>3. Sampling rates</td>
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<tr>
<td>4. Infection control</td>
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<tr>
<td>5. Knowledge and data gaps</td>
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<tr>
<td><strong>WG2</strong></td>
<td>How to perform an acceptable nNO measurements using different methods?</td>
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</tr>
<tr>
<td>1. Different methods to measure nNO (e.g. velum closure, tidal breathing) in:</td>
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<tr>
<td>a. Infants</td>
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<td>b. Pre-school children</td>
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<tr>
<td>c. Older children and adults</td>
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<tr>
<td>2. What is the standard procedure for making a measurement using each manoeuvre (e.g. velum closure, tidal breathing)</td>
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<tr>
<td>3. What is the final reported result from different samplings</td>
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<tr>
<td>4. Knowledge and data gaps</td>
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<tr>
<td><strong>WG3</strong></td>
<td>Which patient-related or environmental components influence nNO measurement?</td>
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<tr>
<td>1. Pretest assessment of patient</td>
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<tr>
<td>2. Choosing correct olive and other consumables</td>
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<tr>
<td>3. Patient considerations for testing (e.g. infection free)</td>
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<tr>
<td>4. Ambient NO (mitigation for high levels)</td>
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<tr>
<td>5. Knowledge and data gaps</td>
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<tr>
<td><strong>WG4</strong></td>
<td>How to report results? How to train personnel?</td>
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<td></td>
</tr>
<tr>
<td>1. Training and competencies of staff undertaking, reporting and interpreting measurements</td>
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<tr>
<td>2. Interpreting the NO concentration for different manoeuvres; reliability etc</td>
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<td></td>
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</tr>
<tr>
<td>3. Reporting standards; reference standards</td>
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</tbody>
</table>
4. Knowledge and data gaps
<table>
<thead>
<tr>
<th>Study</th>
<th>Technique and equipment used</th>
<th>Sampling rate (L.min⁻¹)</th>
<th>Population, health status and age (years or specified)</th>
<th>Method of nNO measurement</th>
<th>nNO results (nL.min⁻¹)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karadag B. Eur Respir J. 1999</td>
<td>Chemiluminescence LR 2000¹</td>
<td>0.25</td>
<td>21 PCD: 10.8 (3.2) 20 HC: 10.8 (3.5)</td>
<td>Breath hold</td>
<td>PCD: 13.8 [0.8- to 239.8] HC: 138.3 [29.0-359.3]</td>
<td>Nasal CO₂ monitored</td>
</tr>
<tr>
<td>Narang I. Thorax. 2002</td>
<td>Chemiluminescence LR 2000²</td>
<td>0.25</td>
<td>31 PCD: 11.0 [5.5 to 17.3] 21 Bx: 11.6 [7.2 to 17.0] 17 CF: 13.2 [7.2 to 17.0] 53 HC: 10.7 [5.5 to 19.0]</td>
<td>Breath hold</td>
<td>PCD: 15.1 [0.8 to 230] Bx: 133.4 [2 to 513.3] CF: 122.8 [7.8 to 287.5] HC: 179.0 [99.5 to 359.3]</td>
<td>Nasal CO₂ monitored</td>
</tr>
<tr>
<td>Csoma Z. Chest. 2003</td>
<td>Chemiluminescence LR 2000¹</td>
<td>0.25</td>
<td>15 PCD: 10.3 (0.7) 14 HC: 11.5 (0.4)</td>
<td>Velum closure method</td>
<td>PCD: 14.9 (3.1) HC: 126.4 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Corbelli R. Chest. 2004</td>
<td>Chemiluminescence CLD88³</td>
<td>1.20</td>
<td>34 PCD: 11.4 [1.2] of whom -17 biopsy proven PCD -17 non-biopsy proven PCD 24 HC: 12.4 (1)</td>
<td>Breath hold or exhalation against resistance</td>
<td>PCD biopsy proven: 16.4 [8.2 – 33.4] PCD non-biopsy proven: 159.2 (95CI: 91.8 – 368.3) HC: 268.4 (210.6 – 342.2)</td>
<td>Nasal CO₂ monitored</td>
</tr>
<tr>
<td>Struben VMD. Eur Respir J. 2005</td>
<td>Chemiluminescence NIOX Flex⁴</td>
<td>0.70</td>
<td>289 HC: 11.6 (6 to 17)</td>
<td>Breath hold</td>
<td>HC: 314.3 (80.5)</td>
<td></td>
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<tr>
<td>Pifferi M. Chest. 2007</td>
<td>Not documented</td>
<td>0.108</td>
<td>64 children with recurrent pneumonia of whom -12 PCD: age not documented -50 &quot;Secondary&quot; ciliary dyskinesia (SCD), age not documented, except for 4 with &quot;atypical PCD&quot;: 0.7, 0.7, 8.5, and 4.1 -2 Non-PCD: age not documented 30 HC: age not documented</td>
<td>Velum closure or tidal breathing</td>
<td>nNO = highest peak value</td>
<td>PCD: 14.0 (5.1) 46 SCD: 82.1 (23.9); in 3 to 5 months old 84.0 (21.3) 4 Atypical PCD: 13.8 (7.4). in 3 to 5 months old 14.3 (6.0) HC: not documented but similar to SCD group</td>
</tr>
</tbody>
</table>

*Prediction equations nNO (ppb)*

<12y = 314.6 + (11.5 x age) – (57.5 x history adenoidectomy) + (0.5 x ambient NO)

>12y = 452.6 – (2.9 x (age - 12)) – (16.0 x history adenoidectomy) + (0.5 x ambient NO)

Age in years, history of adenoidectomy yes = 1, no = 0
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gupta et al. (2010)</td>
<td>57 patients: 34 (1.1) of whom 43 non-atopic, 14 atopic</td>
<td>Immunol, Allergy, Pediatr GL. Pediatr</td>
<td>Tidal breathing nNO = mean value of 3 periods of 10 s, 5 s and 3 s duration</td>
</tr>
<tr>
<td>Piacentini et al. (2008)</td>
<td>10 PCD (2 uncooperative): 177 HC of whom 50 uncooperative aged &lt; 6 months, aged 6-12 months: 27, cooperative: 7</td>
<td>Immunol, Allergy, Pediatr GL. Pediatr</td>
<td>Breath hold (BH) in cooperative children nNO = mean plateau value Tidal breathing (TB) in uncooperative children + 15 cooperative children nNO = mean value of a TB period</td>
</tr>
<tr>
<td>Santamaria et al. (2008)</td>
<td>14 PCD: 15 (7 to 27) 14 HC: 16 (7 to 27)</td>
<td>Immunol, Allergy, Pediatr GL. Pediatr</td>
<td>Each subjects performed triplicate measurements of the 3 different methods Breath hold Mean plateau value Oral &amp; nasal exhalation Mean plateau nasal NO minus mean plateau orally exhaled NO Humming exhalation Mean last 80% exhalation</td>
</tr>
<tr>
<td>Gehring et al. (2009)</td>
<td>719 HC and asthma (11.5%) all 8 y, distributed across 3 centres: 257 Groningen: ambient NO 2.1 (0 to 109.3) ppb 144 Rotterdam: ambient NO 4.8 (0 to 335.7) ppb 318 Utrecht: ambient NO 4.4 (0 to 210.5) ppb</td>
<td>Immunol, Allergy, Pediatr GL. Pediatr</td>
<td>Breath hold Mean plateau value nNO = mean of 3 measures</td>
</tr>
<tr>
<td>Moreno et al. (2010)</td>
<td>9 PCD 36 asthma 31 CF 8 post infectious bronchiectasis (Bx) 37 HC</td>
<td>Immunol, Allergy, Pediatr GL. Pediatr</td>
<td>Measurement method in Spanish PCD: 22.0 (2.4-41.5) Asthma: 255.8 (227.8-284.3) CF: 109.5 (91.8-127.0) Bx: 90.3 (63.0-117.5) HC: 224.5 (200.3-248.8)</td>
</tr>
<tr>
<td>Piacentini et al. (2010)</td>
<td>293/300 HC of whom 250 uncooperative &lt;3: 8 (3.1%) 3-4: 43 (16.7%) 4-5: 73 (28.4%) 5-6: 92 (35.8%) &gt;6: 41 (16.0%) -43 cooperative &lt;3: 0 (0.0%) 4: 3 (6.7%) 5: 16 (37.2%)</td>
<td>Immunol, Allergy, Pediatr GL. Pediatr</td>
<td>Breath hold (BH) in cooperative children nNO = mean plateau value Tidal breathing (TB) in uncooperative children and cooperative children nNO = mean value of a TB period</td>
</tr>
<tr>
<td>Study</td>
<td>Method</td>
<td>Subjects</td>
<td>Success Rate</td>
</tr>
<tr>
<td>-------</td>
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</tr>
<tr>
<td>Torretta S.</td>
<td>Chemiluminescence CLD88&lt;sup&gt;®&lt;/sup&gt;</td>
<td>35 with grade of adenoid hypertrophy (GAH), no allergy, no asthma, no recent (30 days) infection: 8 [4 to 17]</td>
<td>35/81 (43%) (age of children with unsuccessful measurements 5 [3-9])</td>
</tr>
<tr>
<td>Marthin JK.</td>
<td>Chemiluminescence NIOX Flex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>282 subjects: 12.3 [0.0 to 65.8] with 165 (58.5%) &lt; 16 years</td>
<td>236.4 (12.3)</td>
</tr>
<tr>
<td>Mateos-Corral D.</td>
<td>Chemiluminescence CLD88&lt;sup&gt;®&lt;/sup&gt;</td>
<td>85 children: 11.5 (5 to 18)</td>
<td>85/85 (100%) for all 5 methods</td>
</tr>
</tbody>
</table>
| Study | Chemiluminescence/Exhalation method | Subjects | NO result | Exhalation against resistance 
Mean value of 2 or 3 reproducible plateaus (< 10% difference) for each nostril. nNO = mean of the right and left nostril values |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Leigh MW. Ann Am Thorac Soc. 2013</td>
<td>Chemiluminescence 280i NOA(^1), CLD88sp(^1) NIOX Flex(^1)</td>
<td>140 PCD: 19.1 (14.8) (5.1 to 73.0) 78 HC: 20.9 (15.7) (5 to 73.6) 37 Asthma: 14.8 (11.5) (5.4 to 53.5) 77 CF: 16.0 (9.4) (5.5 - 56.0) 32 COPD: 61.1 (8.9) (43.2 to 77.8)</td>
<td>Exhalation against resistance \nPCD: 11.32 (9.4) CF: 132.76 (210.4) Non-CF Bx: 543.64 (389.6) HC: 1001.52 (503.3)</td>
<td>Measurements on different analyzers at the same visit in a subset of participants had comparable values after adjusting for different sampling flow rates. Longitudinal nNO data from 42 PCD patients (5.1 – 16.7 years of age) demonstrated in all but one subject excellent reproducibility with repeated values &lt; cut-off value of 77 nl/min</td>
</tr>
<tr>
<td>Marthin JK. PLoS ONE. 2013</td>
<td>Chemiluminescence CLD88sp(^1), or NIOX Flex(^1), Electrochemical NIOX MINO(^1)</td>
<td>20 HC: 31 [15.6 to 58.4] 21 CF: 11.0 [3.9 to 23.2] 16 PCD: 25.9 [8.4 to 60.9]</td>
<td>Exhalation against resistance: CLD88sp Breath hold: NIOX Flex Mean plateau value Tidal breathing Mean of highest 3 peaks Electrochemical Breath hold: MINOS: Mean over 45 s sampling Tidal breathing: MINOS and MINO2 Mean of NO over 45 s and 2 min sampling, respectively</td>
<td>CLD88sp, ER 20 HC: 263.7 (18.8) 16 CF: 131.7 (11.9) 15 PCD: 23.8 (6.9) NIOX Flex, BH 20 HC: 267.0 (18.6) 14 CF: 150.3 (14.7) 15 PCD: 23.7 (5.7) MINOS, BH 20 HC: 180.9 (12.6) 8 CF: 97.2 (14.7) 12 PCD: 19.2 (5.4) CLD88sp, TB 20 HC: 164.7 (11.6) 21 CF: 107.6 (12.5) 16 PCD: 15.8 (4.0) NIOX Flex, TB 20 HC: 145.8 (10.2) 21 CF: 81.9 (9.6) 16 PCD: 17.7 (4.2) MINOS, TB 20 HC: 102.0 (6.9) 21 CF: 67.8 (7.5) 16 PCD: 11.4 (10.2) MINO2, TB 20 HC: 90.2 (7.1) 19 CF: 68.8 (8.8) 16 PCD: 8.9 (2.8)</td>
</tr>
<tr>
<td>Boon M. Eur J Clin Invest. 2014</td>
<td>Chemiluminescence CLD88sp(^1)</td>
<td>226 Subjects 49 HC: 14.9 [10.8;20.4] 38 PCD: 14.3 [8.8;18.1] 46 CF: 14.0 [9.2;17.9] 45 Asthma: 12.1 [9.8;16.5] 48 Humoral immunodeficiency: 10.7 [8.2;15.6]</td>
<td>Exhalation against resistance Mean plateau value nNO result: mean of 3 plateaus Tidal breathing: CLD88sp ER 20 HC: 263.7 (18.8) 16 CF: 131.7 (11.9) 15 PCD: 23.8 (6.9) MINOS BH 20 HC: 267.0 (18.6) 14 CF: 150.3 (14.7) 15 PCD: 23.7 (5.7) MINO2, TB 20 HC: 164.7 (11.6) 21 CF: 107.6 (12.5) 16 PCD: 15.8 (4.0) MINOS, TB 20 HC: 145.8 (10.2) 21 CF: 81.9 (9.6) 16 PCD: 17.7 (4.2) MINO2, TB 20 HC: 90.2 (7.1) 19 CF: 58.8 (8.8) 16 PCD: 8.9 (2.8)</td>
<td>Exhalation against resistance: HC: 236.4 [198.3;295.8] PCD: 16.8 [8.1;35.7] CF: 109.5 [75.6;169.5] Asthma: 286.1 [175.5;357.0] Humoral immunodeficiency: 211.8 [147.6;294.9] Tidal breathing: HC: 150.0 [106.5;180] PCD: 8.4 [6.0;22.2] CF: 54.0 [36.0;87.0]</td>
</tr>
</tbody>
</table>
Adams PS.
Respiratory Medicine.
2015

| Asthma: 162.0 [126.0;222.0] |
| Humoral immunodeficiency: 114.0 [75.0;180.0] |

Humoral immunodeficiency: 114.0 [75.0;180.0]
Adams PS.
Respiratory Medicine.
2015

Regression equation nNO (ppb) in the first year of life
nNO = exp(2.6511 + (0.2324 x age) – (0.00829 x age^2))
Age in months

Beydon N.
Pediatr Pulmonol.
2015

| Chemiluminescence
NIOX Flex^4 |
| Endono8000^5 |
| 0.30 |
| 0.30 |
| 142 children: 8.9 [5.7-12.8] |
| 49 PCD: 11.4 [IQR: 7-13.9] |
| 37 Non-PCD: 7.9 [IQR: 4.9-11.6] |
| 56 Inconclusive |

Exhalation against resistance or breath hold
(velum closure: VC)
Mean plateau value from each nostril (the highest if >1 measurement per nostril)

Tidal breathing
Mean of 5 peaks (TB5)
Mean over 10 s of regular breathing (TB10)
Mean of last 30 s of recording (TB30)

Success rate for VC methods
< 3 years: 20%
3 to 6 years: 83%
> 6 years: 98%
Comparisons between methods
VC-nNO significantly > than 3 TB-nNO methods
TB10-nNO not significantly different from TB30-nNO
The long-term (median interval time of 1.7 year) between-occasion reproducibility
in 19 children with variable levels of nNO showed a good agreement

Marthin JK.
Eur Respir J. 2018

| Chemiluminescence
CLD88^3 |
| 0.33 |
| 44 full term HC: 5 [1-14] days at first nNO measurement |
| 39 at 4M (3.6 to 6.3 months) |
| 31 at 8M (7.6 to 9.8 months) |
| 21at 12M (10.4 to 14.2 months) |
| 12 at 18M (15.2 to 21.0 months) |
| 16 at 24M (22.2 to 27.1 months) |
| 7 PCD: 3.8 [0.5 to 17]/M at first measurement |

Tidal breathing
Mean of 3 highest peaks

HC number age: nNO
44 < 8 days: 15.2 [9.6,22.8]
39 4M: 32.3 [18.5,42.9]
31 8M: 42.6 [29.8,66.0]
21 12M: 56.4 [36.3,75.2]
12 18M: 58.7 [45.2,86.1]
16 24M: 93.4 [67.0,128.4]
7 PCD age: nNO and follow-up
n1 1M: 11.6 and 7M 7.6
n2 4M: 13.2 and 4.5M 9.9
n3 3.5M: 5.0 and 71M 1.3
n4 17M: 2.0
n5 12.5M: 26.4 and 145M 26.4
n6 2.5M: 3.6 and 134M 9.9
n7 0.5M: 2.6 and 25M 35.6

In 27/ 44 HC with acute respiratory tract infection nasal NO was suppressed by 78.9%
(95% CI 73.4–83.2%)

Rybnikar T.
Int J Pediatr
2018

| Chemiluminescence
CLD88^3 |
| 0.33 |
| 48 children with GAH: (5 to 18 y) |

Exhalation against resistance
Mean plateau
nNO = mean of 3 plateaus

Before adenoidectomy
Grade 1: 303.6 [183.2,405.9]
Grade 2: 218.8 [154.1,282.2]
Grade 3: 162.0 [130.7,233.0]

Number (%) of children with positive skin prick test to 12 common aeroallergens
Grade 1: 5 (42%)
Grade 2: 6 (29%)
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>nNO</th>
<th>Exhalation against resistance</th>
<th>Success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otorhinolaryngol. 2018</td>
<td>12 Grade 1 (&lt;1/3): 11.1 (6.0 to 17.9) 21 Grade 2 (1/3 to 2/3): 8.9 (5.1 to 16.2) 15 Grade 3 (&gt;2/3): 9.3 (6.1 to 17.3)</td>
<td>276.9 (129.4;341.6)</td>
<td>Asthma: 186.3 (59.0) BO: 143.5 (49.7) CF: 90.90 (43.2) Non-PCD/non-CF Bx: 173.1 (63.8) PCD: 25.7 (13.8;60.6)</td>
<td>At least one nostril: 62/62 (100%) Both nostrils: 53/62 (85.5%) NIOX MINO At least one nostril: 53/62 (85.5%) Both nostrils: 33/62 (53.2%)</td>
</tr>
<tr>
<td>Zhang X. Pediatr Invest. 2019</td>
<td>45 Asthma: 9.3 (2.6) 41 Post-infectious bronchiolitis Obliterans (BO): 8.1 (2.8) 20 CF: 9.1 (3.2) 32 Non-PCD/non-CF Bx: 10.2 (2.7) 36 PCD: 8.9 (2.5)</td>
<td>Exhalation against resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemiluminescence CLD88§</td>
<td></td>
<td>0.30</td>
<td>nNO = higher value between right and left side measurement</td>
<td>At least one nostril: 62/62 (100%) Both nostrils: 53/62 (85.5%) NIOX MINO At least one nostril: 53/62 (85.5%) Both nostrils: 33/62 (53.2%)</td>
</tr>
<tr>
<td>Zhang X. Pediatr Invest. 2019</td>
<td>0.33</td>
<td>Exhalation against resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zysman-Colman ZN. J Clin Immunol. 2019</td>
<td>0.33</td>
<td>Exhalation against resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemiluminescence CLD88§</td>
<td></td>
<td>0.33*</td>
<td>Mean of right and left side</td>
<td>At least one nostril: 53/62 (85.5%) Both nostrils: 33/62 (53.2%)</td>
</tr>
<tr>
<td>Electrochemical NIOX MINO§</td>
<td></td>
<td>62 HC: 3 [2.3] [1 to 6] days 6 PCD: 6 [4 to 8] weeks</td>
<td>Tidal breathing Chemiluminescence</td>
<td></td>
</tr>
<tr>
<td>Buechel F. Pediatr Pulmonol. 2020</td>
<td></td>
<td>0.30*</td>
<td>CLD88sp: 12.5 (8.9;18.2) [3.0 to 33.0] PCD: 11.9 [3.0 to 20.5]</td>
<td>At least one nostril: 62/62 (100%) Both nostrils: 53/62 (85.5%) NIOX MINO At least one nostril: 53/62 (85.5%) Both nostrils: 33/62 (53.2%)</td>
</tr>
<tr>
<td>Buechel F. Pediatr Pulmonol. 2020</td>
<td></td>
<td>0.30*</td>
<td>für beide Geräte</td>
<td></td>
</tr>
</tbody>
</table>

PCD: primary ciliary dyskinesia; CF: cystic fibrosis; HC: healthy control; Bx: bronchiectasis

*: opposite to the sampling rates reported in this reference in the absence of answer to our letter to the Editor (Beydon N, Jucas J. Letter to the Editor on “Feasibility of nasal NO screening in healthy newborns”. Pediatr Pulmonol 2022;57:768–769. DOI: 10.1002/ppul.25784). Results of nasal NO output reported in the table have been recalculated from the concentrations of NO given in the text time the sampling rate we think was the right one according to the technique (device) used.
Table E4: Studies reporting the discriminatory ability of nNO measurement

<table>
<thead>
<tr>
<th>Study</th>
<th>Equipment Used</th>
<th>Samplin g Rate (L/min)</th>
<th>PCD Subject Ages (years)</th>
<th>nNO Ability to Distinguish PCD vs. Healthy†</th>
<th>PCD Patients N, (Diagnosis Confirmed by)</th>
<th>Results Displayed</th>
<th>PCD Results in (nL•min⁻¹)</th>
<th>Other Disease Results (nL•min⁻¹ or ppb italics)</th>
<th>Healthy (nL•min⁻¹) or ppb (italics)</th>
<th>N: Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abitbul R, Respir Med. 2016</td>
<td>CLD88 SP¹</td>
<td>Not Specified</td>
<td>0.1-60</td>
<td>-UTC* / 233</td>
<td>84.0%</td>
<td>Not Specified</td>
<td>150 (HSVM, TEM, IF, Genetics)</td>
<td>nNO results unavailable</td>
<td>Non-PCD: 53; nNO results unavailable</td>
<td></td>
</tr>
<tr>
<td>Collins SA, Thorax. 2016</td>
<td>NIOX Flex²</td>
<td>0.3</td>
<td>6-79</td>
<td>77 / 257</td>
<td>93.6%</td>
<td>84.1%</td>
<td>31 (HSVM, TEM)</td>
<td>nNO results unavailable</td>
<td>Non-PCD: 251; nNO results unavailable</td>
<td></td>
</tr>
<tr>
<td>Harris A, BMC Pulmonary Medicine 2014</td>
<td>NIOX Flex²</td>
<td>0.3</td>
<td>5-71</td>
<td>38 / 127</td>
<td>100.0%</td>
<td>95.0%</td>
<td>13 (HSVM, TEM)</td>
<td>nNO results unavailable</td>
<td>Non-PCD: 0.0; non-CF BeX: 20: 170 (77.5-250) CF: 20: 85.8 (7.5-249.3)</td>
<td>15 nNO results unavailable</td>
</tr>
<tr>
<td>Horváth I, Thorax. 2003</td>
<td>LR 2000³</td>
<td>0.25</td>
<td>35 (4.6)</td>
<td>47 / 187</td>
<td>93.0%</td>
<td>95.0%</td>
<td>14 (Saccharin, HSVM, TEM)</td>
<td>Median (Range)</td>
<td>13.6 (12.5-67.25)</td>
<td>17, 165.8 (80.5-335.8)</td>
</tr>
<tr>
<td>Jackson CL, Eur Respir J. 2016</td>
<td>NIOX Flex²</td>
<td>0.3</td>
<td>5-79</td>
<td>30 / 100</td>
<td>91.0%</td>
<td>96.0%</td>
<td>34 (HSVM, TEM)</td>
<td>Mean (SD)</td>
<td>17 (20)</td>
<td>non-PCD: 267: 172 (94)</td>
</tr>
<tr>
<td>Marinth JK, Eur Respir J. 2011</td>
<td>NIOX²</td>
<td>0.3</td>
<td>4-66</td>
<td>53 / 175</td>
<td>91.1%</td>
<td>100%</td>
<td>45 (HSVM, TEM, MCC)</td>
<td>Mean (SE)</td>
<td>42.6 (12.6)</td>
<td>30: 272.4 (9.9)</td>
</tr>
<tr>
<td>Marinth JK, PLoS ONE. 2013</td>
<td>NIOX MINO² Nasal; NIOX Flex²</td>
<td>0.3</td>
<td>8-4-61</td>
<td>MINO: 64.2 / 214; FLEX: 78.6 / 262; MINO: 100% / FLEX: 100%</td>
<td>MINO: 95.2% / FLEX: 100%</td>
<td>92.3%</td>
<td>16 (HSVM, TEM, MCC)</td>
<td>Mean (SE)</td>
<td>MINO (n=12) / FLEX (N=15) 19.2 (5.4)/23.7 (5.7)</td>
<td>MINO (n=8) / FLEX (N=14) 97.2 (14.7) / 150.3 (14.7)</td>
</tr>
<tr>
<td>Mateos-Corral D, J Pediatr. 2011</td>
<td>CLD88¹</td>
<td>0.33</td>
<td>11.4 (3.5)</td>
<td>60.8 / 184.3</td>
<td>100%</td>
<td>92.2%</td>
<td>20 (TEM)</td>
<td>Mean (SD)</td>
<td>19.0 (13.6)</td>
<td>non-PCD: 0.0; non-CF BeX: 14: 376.2 (346.4) CF: 32: 138.8 (84.1)</td>
</tr>
<tr>
<td>Rademacher J, Pneumologie. 2017</td>
<td>CLD88sp¹</td>
<td>Not Specified</td>
<td>32 (12)</td>
<td>77 / UTC*</td>
<td>93.8%</td>
<td>96.7%</td>
<td>24 (HSVM, TEM, genetics, IF): 8</td>
<td>Mean (SD)</td>
<td>25 (31)</td>
<td>non-PCD: 0.0; non-CF BeX: 153: 227 (112)</td>
</tr>
<tr>
<td>Santamaria F, Med Sci Mont. 2008</td>
<td>NIOX²</td>
<td>0.28</td>
<td>7-27</td>
<td>16.8 / 60</td>
<td>100%</td>
<td>100%</td>
<td>14 (TEM)</td>
<td>Median (95% CI)</td>
<td>3.2 (2-5.3)</td>
<td>13th: 90.1 (75.8-143)</td>
</tr>
<tr>
<td>Wodehouse T, Eur Respir J. 2003</td>
<td>LR 2000³</td>
<td>Not Specified</td>
<td>34.2 (10.9)</td>
<td>UTC* / 200</td>
<td>100%</td>
<td>100%</td>
<td>40 (TEM)</td>
<td>Mean (SD)</td>
<td>64 (36.6)</td>
<td>non-PCD: 0.0; non-CF BeX: 20: 733.6 (163.7) CF: 15: 445.5 (162.6)</td>
</tr>
<tr>
<td>Boon M, Eur J Clin Invest. 2014</td>
<td>Spirowire 3¹</td>
<td>0.3</td>
<td>14.3 [8.8-18.1]</td>
<td>90 / 300</td>
<td>89.5%</td>
<td>97.9%</td>
<td>38 (HSVM, TEM)</td>
<td>Mean (IQR)</td>
<td>16.8 (8.1-35.7)</td>
<td>CF: 46: 109.5 (75.6-169.5)</td>
</tr>
<tr>
<td>Leigh MW, Ann Am Thorac Soc. 2013</td>
<td>280 NOA¹</td>
<td>0.5, 0.33, &amp; 0.3⁰</td>
<td>5.1-73</td>
<td>76.9 / UTC*</td>
<td>98.0%</td>
<td>&gt;99.9%</td>
<td>149 (TEM, Genetics)</td>
<td>Mean (SD)</td>
<td>20.7 (24.1)</td>
<td>147: 294.9 (147.6-294.9)</td>
</tr>
<tr>
<td>Leigh MW, Ann Am Thorac Soc. 2016</td>
<td>CLD88sp¹, or NIOX Flex²</td>
<td>0.5, 0.33, &amp; 0.3⁰</td>
<td>6-18</td>
<td>76.9 / UTC*</td>
<td>97.0%</td>
<td>99.1%</td>
<td>121 (TEM, Genetics)</td>
<td>Mean (SD)</td>
<td>20.9 (21.8) n=121</td>
<td>non-PCD: 102: 258.3 (146.9)</td>
</tr>
<tr>
<td>Marinth JK, Eur Respir J. 2011</td>
<td>NIOX²</td>
<td>0.3</td>
<td>4-66</td>
<td>72.6 / 242</td>
<td>94.3%</td>
<td>100%</td>
<td>35 (HSVM, TEM, MCC)</td>
<td>Mean (SE)</td>
<td>33.9 (12.6)</td>
<td>CF: 10: 123.6 (22.8)</td>
</tr>
<tr>
<td>Marinth JK, PLoS ONE. 2013</td>
<td>CLD 88sp¹</td>
<td>0.33</td>
<td>8.4-61</td>
<td>100.0 / 303</td>
<td>100%</td>
<td>95.2%</td>
<td>15 (HSVM, TEM, MCC)</td>
<td>Mean (SE)</td>
<td>23.8 (6.9)</td>
<td>CF: 16: 131.7 (11.9)</td>
</tr>
<tr>
<td>Mateos-Corral D, J Pediatr. 2011</td>
<td>CLD88¹</td>
<td>0.33</td>
<td>11.4 (3.5)</td>
<td>58.5 / 177.3</td>
<td>100.0%</td>
<td>96.9%</td>
<td>20 (TEM)</td>
<td>Mean (SD)</td>
<td>19.5 (13.5)</td>
<td>370.4 (278.6-400)</td>
</tr>
<tr>
<td>Narang I, Thorax. 2002</td>
<td>LR 2000³</td>
<td>0.25</td>
<td>5.5-17</td>
<td>25 and 62.5 / 100 and 250</td>
<td>75% / 97% / 96% / 90%</td>
<td>31 (HSVM, TEM)</td>
<td>Median (Range)</td>
<td>15.1 (0.8-230)</td>
<td>CF: 17: 122.8 (7.8-235)</td>
<td>53: 179 (99.5-359.3)</td>
</tr>
<tr>
<td>Piffeler M, CHEST. 2007</td>
<td>NOT DIVULGED</td>
<td>0.108</td>
<td>children</td>
<td>21.6 / 200</td>
<td>100.0%</td>
<td>100.0%</td>
<td>12 (HSVM, TEM)</td>
<td>Median (IQ)</td>
<td>PCD (IQR)</td>
<td>14.0 (5.1)</td>
</tr>
<tr>
<td>Zhang X, Pediatr Invest. 2019</td>
<td>CLD88sp with DENOX B⁴</td>
<td>0.3</td>
<td>8.9 (5.2)</td>
<td>76 / 253</td>
<td>86.1%</td>
<td>93.9%</td>
<td>36 (TEM, Genetics)</td>
<td>Median (IQ)</td>
<td>25.7 (13.8-60.6)</td>
<td>CF: 41: 186.3 (59)</td>
</tr>
<tr>
<td>Zysman-Colman. J Clin Immunol. 2019 CLD88</td>
<td>0.33</td>
<td>18.6</td>
<td>77 / 233 (vs. PID)</td>
<td>97.0%</td>
<td>91.0%</td>
<td>27 (TEM, Genetics)</td>
<td>Median</td>
<td>19.7</td>
<td>Primary Immunodeficiency 32, 228.9</td>
<td>19: 269.4</td>
</tr>
<tr>
<td>------------------------------------------</td>
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<td>---------</td>
</tr>
</tbody>
</table>

**Tidal breathing**

<table>
<thead>
<tr>
<th>Adams PS Respir Med 2015 CLD88</th>
<th>0.3</th>
<th>0-1.3</th>
<th>Age-adjusted algorithm</th>
<th>93.3%</th>
<th>12 (situs inversus and/or TEM/Genetics)</th>
<th>Range</th>
<th>3.7-18.9</th>
<th>42: 9.9-96.3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boon M, Eur J Clin Inv 2014 Spiroware 3</td>
<td>0.3</td>
<td>14.3 [8.8-18.1]</td>
<td>60 / 200</td>
<td>89.5%</td>
<td>63%</td>
<td>Asthma 44: 162 (126-222)</td>
<td>CF 45: 54 (36-87)</td>
<td>Humeral Immunodeficiencies 43: 114 (75-180)</td>
</tr>
<tr>
<td>Harris A. BMC Pulmonary Medicine 2014 NIOX Mino</td>
<td>0.3</td>
<td>5-71</td>
<td>30 /100</td>
<td>100%</td>
<td>95%</td>
<td>CF 17: 72.9 (11.7)</td>
<td>52: 160.2 (9)</td>
<td></td>
</tr>
<tr>
<td>Marthin JK Eur Respir J 2011 NIOX FLEX</td>
<td>0.3</td>
<td>3.6-66</td>
<td>47.4 / 158</td>
<td>100%</td>
<td>97%</td>
<td>15 nNO results unavailable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marthin JK. PLoS ONE. 2013 NIOX MINO^2; NIOX FLEX^2, CLD88sp</td>
<td>0.3</td>
<td>8.4-60</td>
<td>MINOS 42.6 / 142</td>
<td>100%</td>
<td>100%</td>
<td>Asthma, 9, CF, 5, chronic suppurative lung disease, 7, nNO results unavailable</td>
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<tr>
<td>Marthin JK Eur Respir J 2018 CLD88sp</td>
<td>0.3</td>
<td>0-12</td>
<td>52 / 158</td>
<td>100%</td>
<td>94%</td>
<td>16 at 2 years: 93.4 (67.0-128.4)</td>
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<tr>
<td>Mateos-Corral D. J Pediatr. 2011 CLD88</td>
<td>0.33</td>
<td>11.4 (3.5)</td>
<td>37.1 / 112.6 vs HV plus other respiratory</td>
<td>100%</td>
<td>95.4</td>
<td>20 (TEM)</td>
<td>Mean (SD)</td>
<td>13.3 (9.5)</td>
</tr>
</tbody>
</table>

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1Eco Physics (Duerrnten, Switzerland); 2Aerocrine (Solna, Sweden); 3Logan Research Ltd (Rochester, UK); 4Sievers (Boulder, CO) – All Chemiluminescence-based detection; $ ages are range, or mean (SD), or median [IQR] in PCD subjects unless otherwise specified; *UTC = Unable to Calculate; † respectively; ‡ Unless Otherwise Specified; CF – Cystic Fibrosis, BeX – Bronchiectasis, COPD – Chronic Obstructive Pulmonary Disease, Non-PCD – referred for PCD diagnostics, with negative outcome. HSVM - High Speed Videomicroscopy, TEM - Transmission Electron Microscopy, IF – Immunofluorescence, IQR – Interquartile Range, SD – Standard Deviation $ PCD includes ‘definite (24) plus probable (8)’ PCDs; @infants were not healthy but having evaluation for condition not related to airways or situs;
### Table E5: Algorithms used for Medline and Embase searches

<table>
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<tr>
<th>Database</th>
<th>Period</th>
<th>Search algorithm</th>
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<td>Embase</td>
<td>1994-2021</td>
<td>('ciliary dyskinesia'/exp OR 'ciliary motility disorders'/exp OR 'kartagener syndrome'/exp OR primary) AND ciliary AND dyskinesia AND ('nitric oxide'/exp OR 'nasal nitric oxide') NOT (editorial OR comment) AND [1994-2021]/py AND [embase]/lim NOT [medline]/lim</td>
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Records identified from: 2 databases

Medline and Embase

Titles +/- abstract screened (n = 390)

Records excluded (n = 224)

Full text sought for retrieval (n = 166)

Reports not retrieved (n = 0)

Full text assessed for eligibility (n = 166)

Reports excluded: n = 125
Most common reasons: did not include children, review article, only published as abstract, methods not adequately described.

Studies included in review (n = 41)
Figure E2 Inconsistent results from a 4.1-year-old measured twice in both nostrils during tidal breathing. Results are not reliable because only one low measurement in right nostril (in b) and two non reproducible results (>30% variation, one above the cut-off, one under) from left nostril (c and d). It is probable that the nose is obstructed. The result was not reported and the child was measured 8 days later, after seen by an ENT specialist who prescribed an antibiotic, with repeatable nNO-TB = 499 ppb = 165 nL.min⁻¹ and even successful ER manoeuvre which showed 798 / 784 ppb, 263 / 259 nL.min⁻¹. (a) irregular peaks (>20% variation) sampled during probable irregular breathing in the right nostril with low NO levels between 48 and 78 ppb (16 to 26 nL.min⁻¹) (purple arrows); b) regular (within 20%) low peaks (purple arrows) in right nostril showing a mean of 70 ppb = 23 nL.min⁻¹; c) in the left nostril, circled at the left of the tracing: 3 reproducible highest peaks (within 20%) above the cut-off (dotted purple line) with a mean of 156 ppb = 51 nL.min⁻¹, in the middle of the tracing: 10 reproducible, regular low peaks of NO (between 100 and 120 ppb, with a mean of 110 ppb = 36 nL.min⁻¹), at the right of the tracing: 5 reproducible, regular peaks of which the 3 maximum are equal to the cut-off; d) reproducible (within 20%), irregularly displayed low peaks (purple arrows) in the left nostril showing a mean of 30,2 ppb = 10 nL.min⁻¹).
Figure E3: Using an electrochemical device in a 14.5-year-old, you perform nNO-BH measurements after failure with ER method. She succeeds once with a measurement of a low result of 148 ppb/44 nL.min\(^{-1}\) (Grade F, only one measure). You check this low result with nNO-TB measures which show good intra- and inter-nasal repeatable results, the highest of 299 ppb/90 nL.min\(^{-1}\) (Grade D). Both manoeuvres were correct according to the visual inspection of the child during the measurement. However, nNO-BH cannot be lower than nNO-TB, and if you do not know the ambient NO or if it is > 43 ppb, you cannot interpret the nNO-TB result as high or low.

If measurements were made using a chemiluminescence analyser, it would be seen that the child was not able to breathe hold with velum close in figure a (no result), while TB was regular, as shown in figure b for the 3 last highest peaks (431 ppb/129 nL.min\(^{-1}\)) with low pre-test ambient NO levels (green arrow) (Grade D).

On another occasion one nNO-ER right (809 ppb/243 nL.min\(^{-1}\)) and one left (859 ppb/258 nL.min\(^{-1}\)) were high, Grade B (figure c) confirming the normal nNO. (for Grades, see Box 1)
Figure E4: Using an electrochemical analyser in a 6.9-year-old, BH and ER manoeuvres show repeatable very low results of 5 ppb (1.5 nL.min⁻¹) and 11 ppb (3.3 nL.min⁻¹), respectively (Grade C). But TB method is tested and shows mean NO for the last 30 s of the sampling of 20 and 21 ppb (6 and 6.3 nL.min⁻¹) on right and left side, respectively (Grade D). These results are not coherent since the mean of TB cannot exceed the plateau obtained with BH or ER methods. Results must not be released and a new measurement must be performed after consultation with an ENT. Two months later, repeatable measurements show TB nNO = 452 ppb (136 nL.min⁻¹) (Grade C) and ERnNO = 770 ppb (231 nL.min⁻¹) (Grade D). If all these measurements had been made using chemiluminescence with corresponding tracings showed in panels a to d. In a) and b), unexpected peaks during BH and ER measurements would have been in favour of abnormalities in the sampling of the nostrils and no result could be reported. However, in c) TB measurements were low but repeatable (maximum 5 peaks 49.8 ppb, 15 nl.min⁻¹) (Grade D), but because of the inconsistent nNO values on a) and b) tracings, the child must be considered with nasal obstruction and test again. Height days later, high TB nNO and ER nNO results, Grade D for TB and Grade B for ER (only one nostril sampled), are measured.
Figure E5: A 4.9-year-old is unable to perform BH and ER manoeuvres, TB method shows regular peaks and is repeatable in both nostrils showing a highest value for the mean of 5 peaks of 200 ppb (66 nL.min\(^{-1}\)) (Grade D), above the cut-off (44 nL.min\(^{-1}\)). However, ambient NO is high (green arrow) = 48 ppb, therefore, it is difficult to know whether the true nasal NO output is higher or lower than the cut-off.
### NASAL NITRIC OXIDE REPORT

<table>
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<th>Measure 1</th>
<th>Measure 2</th>
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<tr>
<td>Method</td>
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<td>nNO - Left nare</td>
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<td>nNO - Right nare</td>
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<td>Inter-nasal Diff</td>
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<td>Ambient NO</td>
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<td>Sampling rate</td>
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<td>Highest nNO (highest nNO reported minus ambient NO if &gt; 20 ppb)</td>
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<td>nL/min</td>
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<td>PICIDAR Score</td>
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**Clinical comments:**
For example: RTI status, current medications at time of testing

**Technical Comments:**
For example: deviations from SOP, client cooperation

**Physician Report:**
References


