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Nasal Potential Difference Measurements to Assess CFTR Ion Channel Activity

Steven M. Rowe,

Departments of Medicine, Pediatrics, and Physiology and Biophysics MCLM, University of Alabama, 35294-0006, Birmingham, AL, USA

Jean-Paul Clancy, and

Departments of Medicine, Pediatrics, and Physiology and Biophysics MCLM, University of Alabama, 35294-0006, Birmingham, AL, USA

Michael Wilschanski

Respiratory Medicine and Cystic Fibrosis Center, Shaare Zedek Medical Center, 91031, Jerusalem, Israel

Steven M. Rowe: smrowe@uab.edu; Jean-Paul Clancy: john.clancy@cchmc.org

Abstract

Nasal potential difference is used to measure the voltage across the nasal epithelium, which results from transepithelial ion transport and reflects in part CFTR function. The electrophysiologic abnormality in cystic fibrosis was first described 30 years ago and correlates with features of the CF phenotype. NPD is an important in vivo research and diagnostic tool, and is used to assess the efficacy of new treatments such as gene therapy and ion transport modulators. This chapter will elaborate on the electrophysiological principles behind the test, the equipment required, the methods, and the analysis of the data.

Keywords

Nasal potential difference; CFTR; ENaC; amiloride; cAMP; isoproterenol; electrodes; ion transport

1. Introduction

The measurement of nasal potential difference (NPD) provides a direct and sensitive evaluation of sodium and chloride transport in secretory epithelial cells via assessment of transepithelial bioelectric properties (1–3). This serves as a diagnostic aid in difficult cases where abnormal CFTR function is suspected (4, 5). As the only direct in vivo measure capable of separating sodium and chloride transport, NPD has been used as an important endpoint in clinical trials evaluating therapeutic agents intended to replace dysfunctional CFTR with wild-type CFTR cDNA (including viral and nonviral gene transfer (6–8)), restore mutant CFTR function (9–14), or address other abnormalities in CF ion transport such as novel high-affinity ENaC blockers, channel-activating protease inhibitors, or activators of alternate Cl^- channels (15–18).

1.1. Principles Underlying Nasal Potential Difference Measurements

The premise behind NPD measurements is that the bioelectric abnormality of the CF nasal airway reflects transport abnormalities observed in the lower airways of CF patients. In non-CF patients, the potential is maintained by a balance of sodium absorption and chloride transport, resulting in the tight regulation of the airway surface liquid volume and ionic content, both of which are important for maintaining normal mucociliary clearance. The nasal cavity is accessible which makes it a good site to examine the ion transport characteristics of airway epithelia. Because respiratory epithelia form a tight monolayer harboring a stable and sufficient transepithelial resistance, the active secretion or absorption of charged salts such as sodium (Na^+) and chloride (Cl^-) ions induces a potential difference, measured as a voltage across the epithelial surface (19). The bioelectric potential can be measured by using a high-impedance voltmeter between two electrodes, with one in continuity with each side of the epithelial surface. The electrode on the airway surface (the exploring electrode) rests against the surface of the target epithelium. The internal electrode (the reference electrode) can theoretically be placed in any interstitial compartment of the body, although generally the subcutaneous tissue of the forearm is used. Due to the importance of appropriate placement within the nasal cavity, and the need for an electrically quiet environment, some training and experience are required to achieve accuracy and reproducibility with the method.

1.2. General Methodology

Less than a centimeter into the nose, the squamous ("skin type") epithelium becomes ciliated pseudocolumnar epithelium under the inferior turbinate, sharing many characteristics with the more distal airways (20–22). Under basal conditions, Na^+ absorption is the primary ion transport activity in normal airway epithelia (1). The resulting (or basal) PD is negative or polarized (viewed from the epithelial surface) and in normal subjects, it is usually between -15 and -25 mV. The measurement continues by the sequential perfusion of compounds that block inwardly directed sodium transport through inhibition of ENaC (amiloride), followed by augmentation of chloride transport through CFTR. During perfusion of amiloride, the potential difference depolarizes as ENaC transport is reduced, and the PD typically approaches a low polarizing value (typically between 0 and -10 mV). Perfusion of a chloride-free solution induces a chloride diffusion PD through CFTR channels, resulting in a rapid and often large hyperpolarization of the PD. CFTR-mediated Cl^- transport is further enhanced pharmacologically by the addition of agents known to activate CFTR, such as isoproterenol (which increases intracellular cyclic AMP as a β_2 receptor agonist) (23, 24). It is not unusual to observe transient hyperpolarization that is believed to represent Cl^- transport through calcium-activated chloride channels (CaCCs) during chloride-free perfusion (with or without isoproterenol). These transient polarizations typically resolve within 1–2 min and are not CFTR dependent. Finally, ATP is perfused, which activates chloride secretion through alternative (non-CFTR) CaCCs and serves as a marker of epithelium integrity.

1.3. Healthy and CF Nasal PD Measurements

Example tracings in normal and CF subjects are shown in Fig. 6.1. In CF, this ion transport profile is abnormal and the nasal PD measurement has a number of features that distinguish the PD signature. At the beginning of the measurement with buffered Ringer's, the basal PD in CF is much more negative due to increased ENaC activity, thought to be due to the absence of regulation of ENaC function by CFTR. As expected, the depolarizing response to amiloride is also enhanced. Although the sodium abnormality is usually readily apparent, the most consistent abnormality in CF is the absence of hyperpolarization following perfusion with chloride-free solution and isoproterenol. In CF, the addition of ATP (or other purines) leads to a large hyperpolarization and occurs through non-CFTR-mediated chloride

secretory pathways, including stimulation of P2Y2 receptors which activate CaCCs (an approach now being tested as a potential CF therapy through long-acting derivatives of UTP (25)). In aggregate, the differences in Na and Cl transport are sufficient to discriminate CF from non-CF subjects, and also detect individuals with intermediate phenotypes (Table 6.1) (26, 27).

2. Materials

There are numerous choices in regard to equipment and supplies that can be employed to successfully perform the NPD measurement and capture the data generated. There are advantages and disadvantages to various setup choices, and often the final choice at a given NPD site is a balance of the desire to standardize performance across sites, feasibility, and cost. The equipment described below is based upon experience developed through sites participating in clinical trials through the CFF-TDN. The use of the electronic data capture, sequence, solutions, and electrodes has been validated through comparative testing across a number of NPD sites in CF and non-CF subjects. Equipment that is felt to be highly important for standardization across study sites participating in therapeutic clinical trials is noted by an asterisk.

2.1. Electronic Data Capture (EDC) Equipment

Hardware and software employed for NPD measurements that have been standardized across study sites for use in therapeutic clinical trials have been based on pilot testing of performance, manufacturer support, and good laboratory practice (GLP) compatibility (21CFR part 11 compliance). This equipment is available from ADInstruments (www.adinstruments.com):

- *PowerLab 4/30 data acquisition system.
- *BMA-200 AC/DC portable bioamplifier.
- *IS0-Z isolation headstage for BMA-200.
- *ADInstruments software: LabChart Pro (GLP Client V6 (win) or higher).

2.2. Perfusion Equipment and Supplies

Ensuring that the NPD perfusates are delivered at 5 ml/min typically requires the use of programmable syringe pumps (capable of holding 60-ml syringes) and relatively large diameter perfusion tubing. The larger bore also helps to ensure that target temperatures are achieved for perfusates (by passing the tubing through a water bath). Specific equipment (pumps and disposables) are listed below:

- Infusion pump for 60-ml syringes (Medfusion 3,500 Syringe Pump or equivalent).
- Isotemp 210 water bath (Fisher Scientific, Cat. # 15-462-10).
- *Electrodes – mini-calomel reference (Fisher Scientific, Cat. # 13-620-79).
- *PE50 tubing (0.023 in. i.d., 1 × 100 ft; Becton Dickinson, Cat # 427411).
- *PE90 tubing (0.034 in. i.d., 1 × 100 ft; Becton Dickinson, Cat. # 427421).
- *Silastic tubing (0.058 in. i.d., 0.077 in. o.d.; Dow Corning, Cat. # 508-006).
- IV extension tubing (30 in., 50/Box; International Limited, Cat. # IMN30).
- Three-way stopcock (50/Box; Medex, Cat # MX5311L).
- Intramedic luer stub adapter (20 G; Becton Dickinson, Cat. # 427564).

- Syringe 60 cm³ w/o needle luer slip (30/Box; Becton Dickenson, Cat. # 309653).
- 23G.3/4 vacutainer needles (0.6 mm × 19 mm, 50 U/Box; Becton Dickenson, Cat. # 367283).
- Difco Laboratories agar (Fisher Scientific, Noble 100 g, Cat. # 0142-15-2; or equivalent).
- Welch Allyn rhinoscope 71000-C; 3.5 V convertible handle battery 72300; nasal illuminator 26530.

2.3. Solutions and Reagents

There are three isotonic base NPD solutions used in most NPD protocols:

Solution #1 = Ringer's + phosphates.

Solution #2 = Ringer's + phosphates + amiloride (100 μM).

Solution #3 = 0[Cl⁻] + phosphates + amiloride.

Isoproterenol (final concentration = 10 μM) and ATP (final concentration = 100 μM) are added to solution #3 to make solutions #4 and #5, respectively. While there is uncertainty regarding the optimal salt concentrations to maximize detection of CFTR activity, the following recipes for base solutions (when stored in sterile, single-use glass bottles) have been tested for stability of osmolality, sodium, chloride, calcium, potassium, and amiloride concentrations. Repeated measurements indicate that these concentrations vary by 15% over 18 months (28). In efforts to reduce variability in NPD performance during multicenter trials, base solutions have been centrally produced through partnerships with academic or industry pharmacies. Since NPD solutions are not routinely available outside of sponsored clinical trials, it is often necessary for sites to have the capability to mix solutions on-site. The CFF-TDN recommends the use of USPgrade agents when available and storage at 4°C in single-use, 50–100-ml bottles or vials following sterile filtration at 4 °C. The instructions for mixing solutions and 3% agar dissolved in Ringer's (for bridges and nasal non-perfusion catheter probes) provided below are based on CFF-TDN Standard Operation Procedures (SOPs; 2009) and have been successfully used to discriminate between CF and non-CF subjects, and to detect biologic activity of CFTR modulators (29):

- *Ringer's (solution #1)*: Commercial Ringer's injection (Baxter Healthcare) + phosphates (pH 5.5, 147.5 meq Na, 4 meq K, 4.5 meq Ca, 156 meq Cl, 309 mOsm)

	Salts	MW	mM/L	G/L
	NaCl	58	148	8.58
	CaCl ₂ •2H ₂ O	147	2.25	0.33
	KCl	74	4.05	0.30
Add	K ₂ HPO ₄	174	2.4	0.42
Add	KH ₂ PO ₄	136	0.4	0.05
Add	MgCl ₂ •6H ₂ O	203	1.2	0.24

- *Amiloride (solution #2)*: 30 mg/L of amilorideHCl (MW= 302) is added to the Ringer's + phosphates described above. Warming of the solution to 35–37°C and stirring can help to dissolve the amiloride. The base solution used is double distilled H₂O.

- *Zero Cl⁻ (solution #3):* 0 [Cl⁻] solution (to otherwise match Ringer's injection): (pH 5.5, 147.5 meq Na, 4 meq K, 4.5 meq Ca, 156 meq Cl, 309 mOsm). The base solution used is ddH₂O.

	Salts	MW	mM/L	G/L
Add	Na gluconate	218	148	32.26
Add	Ca gluconate	430	2.25	0.97
Add	K gluconate	234	4.05	0.95
Add	K ₂ HPO ₄	174	2.4	0.42
Add	KH ₂ PO ₄	136	0.4	0.05
Add	MgSO ₄ •7H ₂ O	246	1.2	0.30

The pH of solutions is adjusted to 7.4 (1 N NaOH), followed by sterile filtering (0.22 µm) and storage in single-use, 50–100-ml bottles or polystyrene tubes. Solutions should be stored at 4°C and warmed immediately prior to use.

- *Isoproterenol (solution #4):* It is made by adding sterile isoproterenol (e.g., Hospira, Inc., Lake Forest, IL; 0.2 mg/ml; MW = 248) to solution #3 to a final concentration of 10 µM. Isoproterenol should be added immediately prior to use and protected from light. It can be frequently ordered through the institutional pharmacy.
- *ATP (solution #5):* It is made by adding ATP to solution #4 to a final concentration of 100 µM. A stock solution of concentrated ATP can be made by adding 55 mg to 2 ml of ddH₂O, sterile filtering, and storing as 0.10 ml aliquots at –20°C. About 0.10 ml is then added to 50 ml of solution #4.
- *3% agar in Ringer's* is used to make the skin bridge and the nasal non-perfusion probe catheter. To make stock, 6 g of agar (Difco or equivalent) is dissolved in 200 ml of solution #1 in a large-mouth, autoclave-tolerant bottle with a screw top. The sealed bottle is then autoclaved (160°C, 30 min) and allowed to cool. This process fully dissolves the agar and removes all bubbles, which helps to ensure that the single-use bridges and catheters are free from bubbles when made immediately prior to NPD performance.
- *3 M KCl* dissolved in H₂O is used to store the calomel electrodes. The glass end of the two calomels should be submerged in the 3 M KCl, and the two calomel solutions should be connected by a 3% agar bridge when not in use to ensure that no offsets develop between the calomels. Periodically check to ensure that the 3 M KCl solutions are full and the calomels are submerged. The internal chamber of the calomels is also filled with 3 M KCl and should be periodically checked to ensure that the solution has not been depleted/evaporated. Excess KCl crystals on the surfaces of the calomels and calomel solutions should be removed.

3. Methods

3.1. Preparation and Setup of the Potential Difference Apparatus

- Warm stock solutions #1, #2, and #3 prior to use. Make solution #4 by adding aliquoted isoproterenol to solution #3. Make solution #5 by adding aliquoted isoproterenol and ATP to solution #3.
- Place all solutions in syringes labeled #1, #2, #3, #4, and #5.

- Attach infusion tubing to syringes, and place three-way stopcocks on the free ends of the infusion sets as shown in Fig. 6.2. The stopcocks can then be connected and taped to the side of the warmer bath.
- Pass the infusion tubing through the warming water bath (typically set at approximately 40°C to achieve probe temperature of 32–37°C). Check the temperature of the solution exiting the probe catheter to ensure that it is in the appropriate range.
- To make the skin bridge, loosen the lid of the agar bottle and warm the 3% agar for 10–30 s in a microwave. Carefully remove approximately 5 ml with a 10-ml sterile syringe and remove bubbles. Attach the syringe to a 23-gauge butterfly needle luer lock. Slowly inject the agar until the agar escapes from the needle end of the butterfly and backfill the luer lock to ensure that no bubbles are entrained. Allow the bridge to cool (~5 min).
- To make the non-perfusion nasal catheter probe, measure approximately 3 ft of PE90 and PE50 tubing. Bring the end of both tubes together and place a 1-cm silastic sleeve over the end of the tubes (helped by dipping the tip in 70% EtOH). Cut the end so that the sleeved end is flushed. Attach a 23-gauge needle to the free end of the PE50, and attach the luer lock end to the first stopcock. For the non-perfusion probe, place a 20-gauge luer stub adapter on the free end of the PE90 tube. Slowly inject the warmed 3% agar through the PE90 until the agar is seen exiting the sleeved end. Backfill the luer lock to ensure that no bubbles are entrained and allow it to cool (~5 min).
- Backflush the five perfusates through the probe electrode catheter, including perfusion of solutions #5, #4, #3, #2, and #1 for approximately 30 s each. Turn the stopcocks in reverse order during this procedure to ensure that later solutions are cleared from the nasal probe. Solution #1 (Ringer's) should be the solution filling the tubing at the end of this procedure.

3.2. The Nasal Potential Difference Procedure

3.2.1. Offsets and Calibration—The next steps are to ensure that there are no offsets in the entire setup and that the skin bridge and the nasal probe are functional. Remove the connecting storage bridge between the two 3 M KCl calomel solutions. The electrodes are then attached to the appropriate connectors of the ISO-Z headstage (+ (red) for nasal electrode, – (black) for skin electrode). First, measure the potential between the two calomels by placing both in a single 3 M KCl calomel solution. Dial any offset out by adjusting the bioamplifier. Second, place the two calomels in their separate 3 M KCl baths and connect the two solutions with the skin bridge. The PD should be near zero and confirms that the skin bridge is functional. Third, remove the skin bridge and replace with the nasal probe electrode catheter. Again, the PD should be near zero. Fourth, place the luer end of the skin bridge in the skin 3 M KCl solution and the luer end of the probe catheter in the nasal 3 M KCl solution. Place the free ends of the skin bridge and the probe catheter (the needle end of the skin bridge and the sleeved end of the nasal probe) in a single 50-ml tube of Ringer's and measure the PD. This is the “closed-loop offset,” and ensures that all components of the PD apparatus are functional. The PD should be near zero (± 3 mV). Dial the bioamplifier to remove any remaining offsets. If the offset is larger or the circuit is open, either the probe catheter or the skin bridge is dysfunctional and should be replaced.

3.2.2. Skin, Anterior Tip, and Basal PDs—Using sterile technique, place the skin bridge needle in the subcutaneous space of the forearm of the study subject and gently pinch the probe catheter tip between their index finger and thumb. Dipping the end of the probe

catheter in the Ringer's solution may be necessary to make a stable connection. The skin PD is typically between -35 and -75 mV. Record the "skin PD." Place the probe catheter in the right nares under direct visualization with the otoscope and nasal illuminator, and advance the probe catheter into the inferior meatus (under the inferior turbinate) to 3 cm to measure the PD. Record the PD at 3, 2, 1.5, 1.0, and 0.5 cm for approximately 5 s each. Place the catheter on the anterior tip of the inferior turbinate (AT) at the completion of the basal measurements at the anterior tip of the inferior turbinate. This is the AT measurement and it is generally between 0 and -15 mV. This value is similar in CF and non-CF subjects, as the AT surface is typically squamous epithelium (and not respiratory epithelium) and serves as a reference value (along with the skin PD) to be rechecked throughout the procedure. Record the AT measurement for 5 s. Remove the catheter and repeat the procedure for the left nostril. These steps complete the basal PD measurements.

3.2.3. Performing the NPD Tracings—Measure the PD of the right nostril AT and then remove the nasal illuminator. Place a small piece of water-resistant tape on the tip of the nose. Place the probe catheter in the right nares under the inferior turbinate. This can often be done "blind" based on knowledge of the values obtained during the "basal PDs" but can also be performed by directly visualizing with a otorhinoscope or a nasal dilator and illuminating headlamp. Attempt to locate the most polarized PD under the right inferior turbinate and secure the catheter in position with tape. Check that the catheter is in the inferior meatus using the otoscope and nasal illuminator, and note the distance within the inferior meatus (0.5–3 cm). Start perfusion with solution #1 (Ringer's) and have the subject assume a comfortable position (often with head slightly down so that the perfusate exits the nostril). Begin recording the "tracing PD" and indicate the start of perfusion with "Ringer's." Once Ringer's has been perfused for 1 min and a stable PD has been obtained ($\times 1$ mV) for a minimum of 30 s, turn off the solution #1 pump, turn the first stopcock "off" to solution #1, and open the solution #2 perfusate. Turn on the solution #2 (Ringer's + amiloride) pump, mark the initiation of "amiloride" perfusion, and record for a minimum of 3 min (maximum of 5 min). When a stable PD has been obtained, turn off the solution #2 pump, turn the second stopcock "off" to solution #2, and begin perfusion with solution #3 (zero Cl + amiloride). Mark the initiation of "zero Cl" perfusion and record for a minimum of 3 min (maximum of 5 min). When a stable PD has been obtained, turn off the solution #3 pump, turn the third stopcock "off" to solution #3, and begin perfusion with solution #4 (zero Cl + amiloride + isoproterenol). Mark the initiation of "Iso" perfusion and record for a minimum of 3 min (maximum of 5 min). When a stable PD has been obtained, turn off the solution #4 pump, turn the fourth stopcock "off" to solution #4, and begin perfusion with solution #5 (zero Cl + amiloride + isoproterenol + ATP). Mark the initiation of "ATP" perfusion and record for a minimum of 1 min (maximum of 5 min) until a peak value is obtained. Once completed, turn off solution #5 and stop recording. Remove the tape and probe catheter, and backflush solutions #4, #3, #2, and #1 in reverse order (30–60 s each), turning the stopcocks in reverse order. Using the nasal illuminator and otoscope, re-measure the right AT PD and record. Measure the skin PD and then repeat the "tracing PD" steps for the left nostril, including the left AT before and after the tracing. Re-measure the skin PD at the completion of the left tracing PD and then remove the skin bridge. Repeat the "closed-loop offset" measurement at the end of the procedure to ensure that no offsets have developed during the procedure (the value should again be near zero). This completes the entire NPD procedure.

3.3. Analysis

Nasal potential difference tracings should be reviewed by an expert familiar with the technique, as care must be taken to discriminate between tracings reflecting an accurate result and tracings that are unstable and therefore may not reflect the true potential

difference of the subject. In the setting of clinical research trials, blinded review is strongly recommended to prevent potential bias from affecting interpretation of the NPD or determination of the validity of particular tracings. Review by a single interpreter should also be considered, as the inter-reader agreement between blinded reviewers has not been evaluated in a rigorous fashion. The analysis discussed below is based upon experience during CFF-TDN studies.

3.3.1. Scoring the Potential Difference Tracing—The basal PD is measured at various distances within the inferior meatus in the right nostril (3.0, 2.0, 1.5, 1.0, and 0.5 cm), followed by the left nostril. For each of these measures, a plateau value is obtained for approximately 5 s, and the mean PD is determined during the stable plateau. A similar method is used for the finger, anterior tip of the inferior meatus, and offset measurements. Based on these results, both the maximally negative (most polarizing) value and the mean basal PD are calculated for each nostril. To score each NPD tracing, the PD for each superperfusion solution is measured once the PD reaches a stable value after the minimum perfusion time (*see Section 5.3* above). The mean PD for the final 10 s of perfusion solution is calculated using the LabChart software and should represent a stable area of the tracing (Fig. 6.3). For solution #5 (ATP), the peak (most polarizing) value is calculated as the mean value over 2 s at the peak (most negative) value. From these values, derived within tracings changes can be calculated. These include alternative measures of sodium transport (change in PD following amiloride and percentage change in PD following amiloride), measures of CFTR-dependent chloride transport (change in PD following zero-chloride perfusion, change in PD following isoproterenol perfusion, and the sum of these changes), and measures of CFTR-independent chloride transport (change in PD following ATP perfusion). In CF, the Ringer's PD, the derived parameter of sodium transport, and the derived measures of CFTR-dependent Cl^- transport each correlates with the severity of the CF genotype (Table 6.1). The change following zero chloride + isoproterenol is widely considered the most sensitive and specific indicator of CFTR-dependent anion transport, is included in diagnostic algorithms for CF, and is a frequent outcome measure in CF clinical trials of CFTR modulators. The total change in PD (end Cl^- -free isoproterenol – end Ringer's perfusion (26)) and the Wilschanski index [$e^{(\text{response to chloride-free isoproterenol} / \text{response to amiloride})}$, with a cut-off >0.70 to predict a CF diagnosis (30)] are alternative derived endpoints that incorporate both sodium and chloride transport and have also demonstrated reasonable diagnostic accuracy.

4. Notes

4.1. Alternative Methods

A number of alternative methods to measure the NPD have been developed since its first description, as the relative lack of standardization of the measure (until recently) encouraged a number of innovations to be developed at individual centers. While this section does not capture all of these alternative methods, a number of these are highlighted here.

4.1.1. Perfusion Catheter—Rather than using an exploring probe filled with agar gel, the exploring probe can be filled with Ringer's solution that continuously perfuses at a slow rate (12 ml/h) so that continuity with the nasal surface is maintained. This method was utilized in the original description by Knowles et al. and in previous standard operating procedures adopted by the CFF-TDN (2). A disadvantage with this method is that bubbles can form in the continuous perfusion line (a process predicted to occur by Bernoulli's principle when pumping solution with dissolved gas through a narrow caliber line), which results in artifact and breaks in the tracing, and can also induce an electrical resistance in the probe electrode.

This method also detects greater electrical interference from the ambient environment which can include electrical noise from the continuous perfusion pumps used in the NPD setup.

4.1.2. Single Lumen Catheter—To reduce the size of the nasal probe, the perfusing line and the exploring probe can be united by a T connector (or equivalent device) proximal to the nasal interface (2). This has the advantage of allowing placement in infants and young children, or individuals with a small inferior meatus. The principal disadvantage is that the method faces the same challenges that perfusion catheter faces. The perfusion line is the distal portion of the exploring probe and can transmit microbubbles and electrical noise into the NPD signal.

4.1.3. Nasal Floor Placement—Instead of placement within the inferior meatus, the nasal catheter can be placed in the floor of the medial nasal cavity (3). This is facilitated by the use of a larger diameter catheter (such as a pediatric Foley catheter) and one that utilizes a side port to allow directional orientation to the exploring interface. Placement of the catheter with this method allows blinded placement, but may be more uncomfortable for some patients (as perfusion is more likely to run into the pharyngeal space rather than out of the nostril), and is a limitation in individuals with small nasal orifices.

4.1.4. Ag–AgCl Electrodes—Ag–AgCl electrodes are frequently used for medical applications and have been successfully employed for NPD measurements in lieu of KCl calomel electrodes. Care must be taken to maintain the working surface of the Ag–AgCl electrode and offsets measured between paired electrodes on a regular basis. Interface with the metal surface of the electrode is typically made through Ringer's solution or a mixture of Ringer's and ECG cream (2, 3). Common Ag–AgCl electrodes used for this purpose include AgCl microelectrode holder half cell (Item No. Ref-2L, 64-1302; Warner Instruments) or pellet-type electrodes (Cat. # E-22X, 10-02060; CWE, Inc.).

4.1.5. Abrasion Subcutaneous Bridge—The reference bridge can connect with the subcutaneous space using a small needle placed and secured into position as described above, or by inducing a small dermal abrasion with a sterile rotary tool, permitting access to the subcutaneous compartment. The dermal abrasion interface is typically made by placing ECG conducting gel and a Ag–AgCl electrode over the abrasion, and securing with an adherent dressing (3, 31, 32). This method is particularly advantageous for individuals with needle anxiety but necessitates use of Ag–AgCl electrodes and ECG conducting gel to maintain symmetry between electrodes. The method also requires careful attention to maintain the sterility of the rotary tool between subjects.

4.1.6. Strip-chart Recorder—Strip-chart recorder can be used instead of the electronic data capture device to obtain NPD results (12). While providing the essential information, analog capture and low-pass filters intrinsic to strip-chart recorders do not allow monitoring of ambient electrical noise. Moreover, handwritten notes that accompany the strip-chart tracings may need special handling to ensure blinding. A typical strip chart utilized for NPD include flatbed recorders (Item No. BD11E; Kipp and Zoe).

4.2. Interpretation of Potential Difference Results

Internal consistency of the tracing can be judged by repeated measures of the electrical offset, finger, and anterior tip of the inferior turbinate performed throughout the session. Tracings with frequent or sustained breaks in the tracing often indicate poor interface with the study subject, which can occur due to problems with the nasal catheter, reference bridge, or monitoring equipment. The most frequent cause is development of a bubble in the nasal

probe or poor contact with the nasal mucosa, and is particularly problematic with nasal probes based on the continuous perfusion of Ringer's solution.

4.3. Tracing Interpretability and Quality

It is helpful to provide objective criteria regarding the quality of NPD tracings, a process facilitated by central review. The CFFTDN Center for CFTR Detection at the University of Alabama at Birmingham assigns a “confidence score” to each tracing reviewed in a blinded fashion for research studies. Criteria that can contribute to a low confidence score include sustained breaks in the tracing (>30 s), abrupt changes in the PD (>5 mV over 1 s or less that do not rapidly return to baseline), or tracings that do not have a significant response (>3 mV change) to both amiloride and ATP (often due to disruptions in the nasal membrane). Additional criteria that indicate accurate NPD results include tracing stability assessed by the variance of the PD plateau voltage and the frequency of tracing artifacts (defined as PD values that shift >5 mV and are not sustained or otherwise considered reliable), which likely represent transient breaks in the PD circuit.

4.4. Reproducibility

NPD is a reproducible procedure within subject, but care must be taken to optimize results, particularly when used in the context of clinical trials (12, 33). Yaakov et al. (34) examined the within-patient repeatability of NPD at a single center. They examined on at least two occasions 68 CF patients, 25 with classic disease and 43 with non-classic disease (defined as patients with a CF phenotype in at least one organ system but normal or borderline sweat chloride measurements, two CF-causing mutations, and abnormal NPD). Using the paired *t*-test and Wilcoxon test, the repeated NPD measures were reproducible. In part to improve the reproducibility of NPD measurement in the context of multicenter trials, the standard operating procedure of the Cystic Fibrosis Therapeutics Development Network was devised. This has included several factors to improve between-center and between-operator variability, including use of warmed centrally produced perfusion solutions, standardized equipment, and technical training of NPD operators. Avoiding changes in nasal medication known to alter NPD, including nasal steroids in particular, is another caveat to consider.

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Fig. 6.1. Representative nasal potential difference tracings from a normal (*black*) and a CF (*red/gray*) subject. Contents and duration of each perfused solution is designated above.

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Fig. 6.2.
Schematic diagram of the nasal potential difference apparatus.

Fig. 6.3. Representative potential difference tracing from a CF subject demonstrating methodology used to calculate PD and quantify tracing stability. Open boxes represent the 10 s scoring interval used to quantify the PD and tracing stability.

Table 6.1

Relationship between NPD parameters, sweat chloride and CFTR genotype

Group	n	Age (yr)	Sweat chloride (mmol/l)	Nasal potential difference (mv)				
				Max PD	ΔAmil	ΔCl-free	ΔCl-free+Iso	ΔAmil+Cl-free-Iso
Control	25	30±6	20±11	-24±8	13±4	-15±9	-29±10	-16±12
Incidental heterozygote	6	24±2	34±16	-26±9	15±6	-12±4	-27±3	-12±5
CF heterozygote	21	37±10	26±13	-22±6	14±5	-11±10	-23±10	-9±9
CBAVD-0	6	33±3	22±9	-25±7	12±4	-8±6	-22±10	-9±8
CBAVD-1	18	37±6	44±23	-28±11	19±7	-5±5	-12±7	+7±10
CBAVD-2	36	34±5	54±23	-35±9	23±10	-2±3	-8±7	+15±11
CF-PS	24	32±10	73±21	-44±13	27±12	+3±5	+2±5	+28±13
CF-PI	26	20±9	102±14	-54±9	36±8	+3±4	+4±5	+40±7

Definition of abbreviations: Amil, amiloride; CBAVD, congenital bilateral absence of the vas deferens; CF, cystic fibrosis; CF-PI, pancreatic-insufficient cystic fibrosis; CF-PS, pancreatic-sufficient cystic fibrosis; Iso, isoproterenol; PD, potential difference.

Values are mean ± SD.

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