The Diagnosis of CF in 2017

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Israel Cystic Fibrosis Society Conference
Dan Cesaria , October 2017

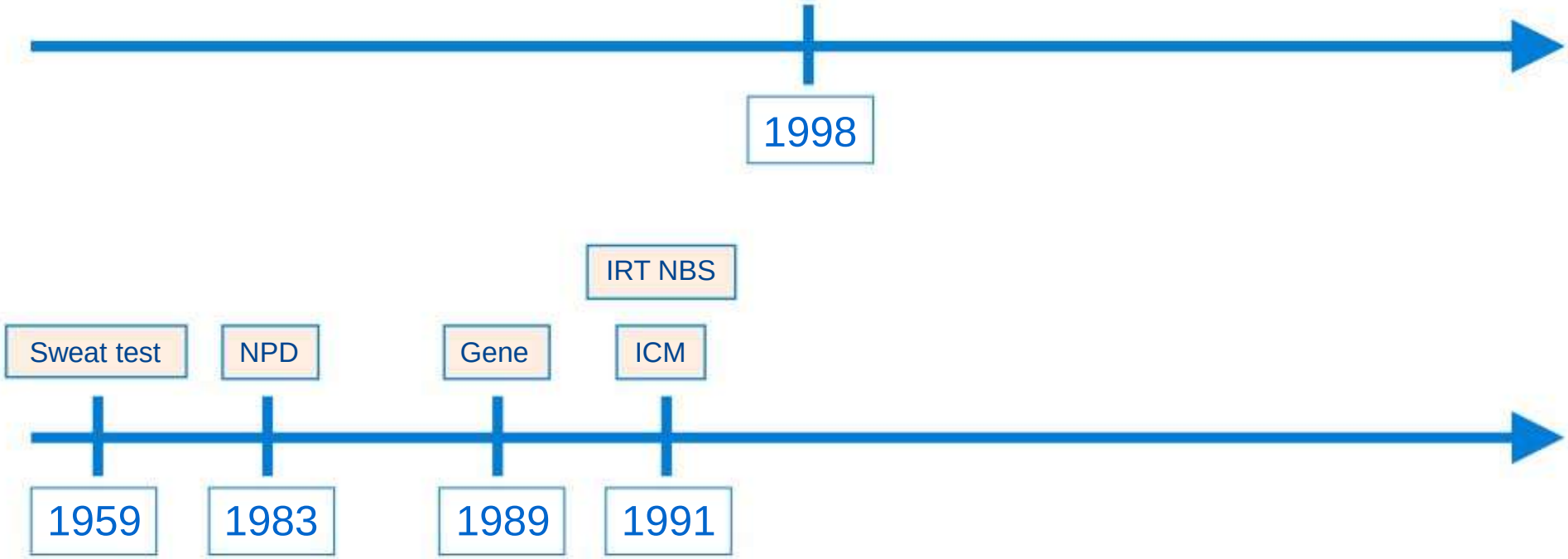
CF diagnostic consensus timeline

CFF diagnosis consensus
Rosenstein et al.

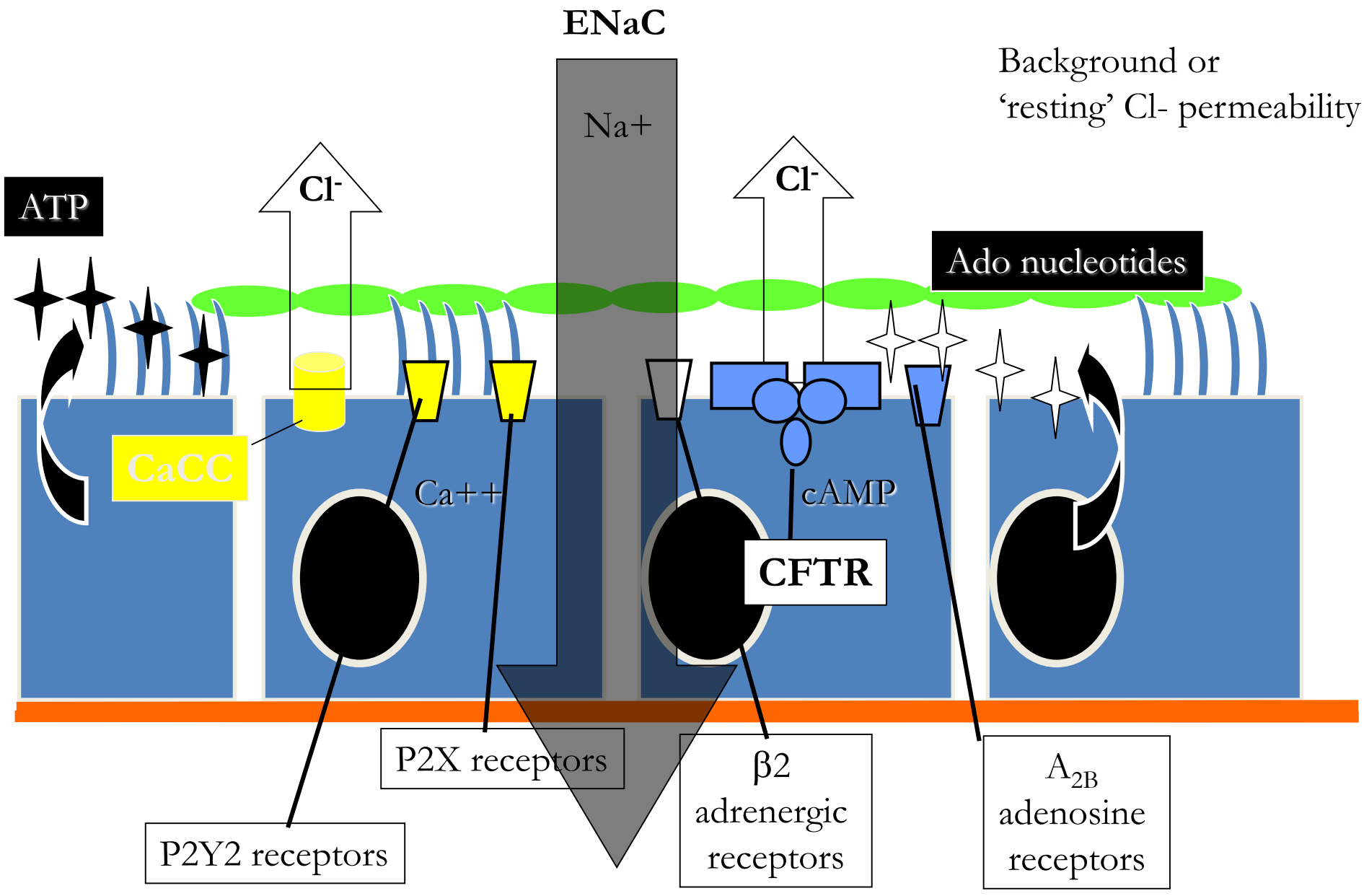
Diagnostic criteria 1998:
- phenotypic features, CF in a sibling, or positive NBS
- laboratory evidence of CFTR abnormality: sweat Cl^- > 60mmol/l
- or: identification of mutations in each CFTR gene
- or NPD CF typical

The diagnosis of cystic fibrosis: A consensus statement

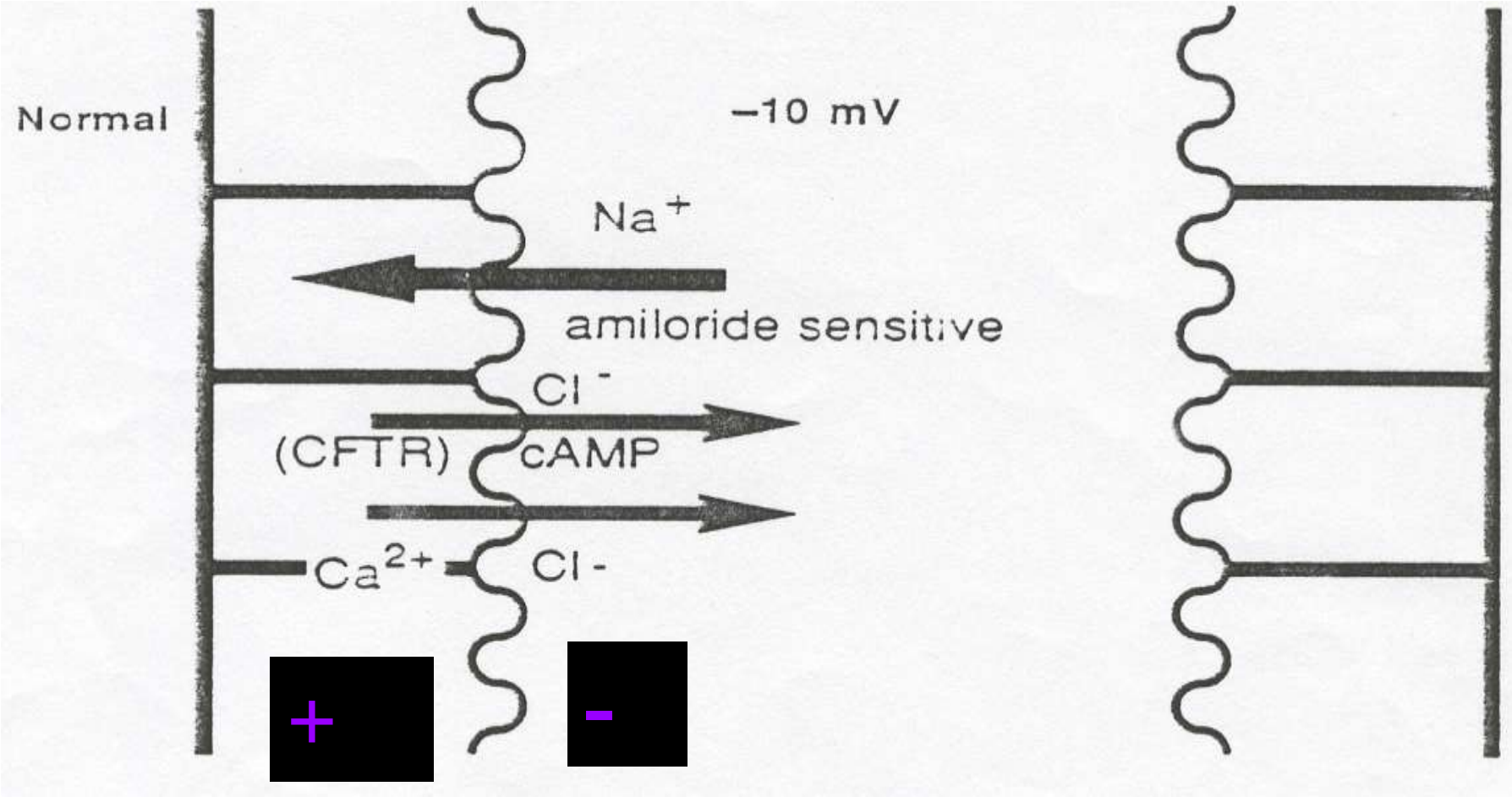
Beryl J. Rosenstein, MD, and Garry R. Cutting, MD, for the Cystic Fibrosis Foundation Consensus Panel



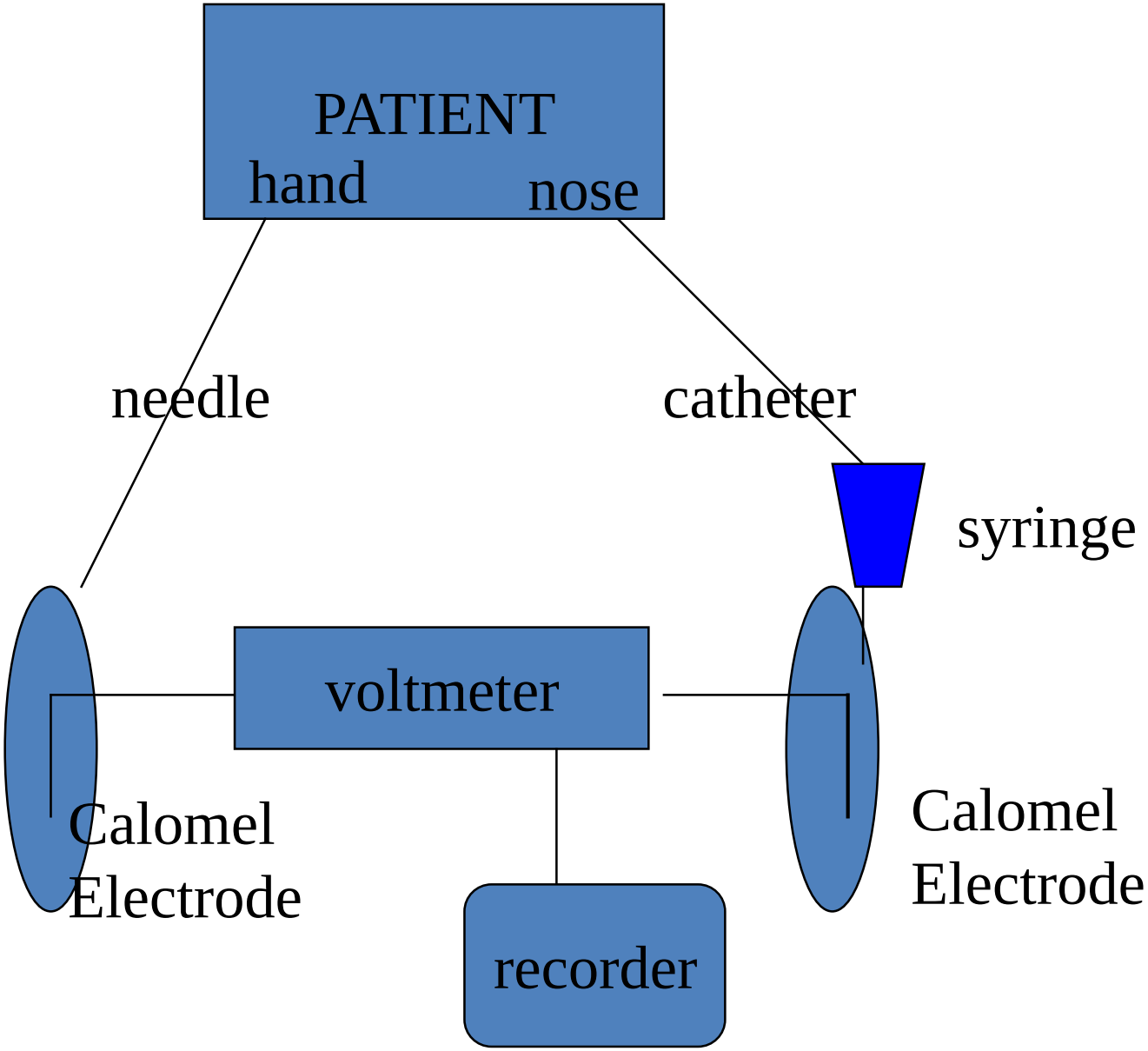
Nasal Potential Difference



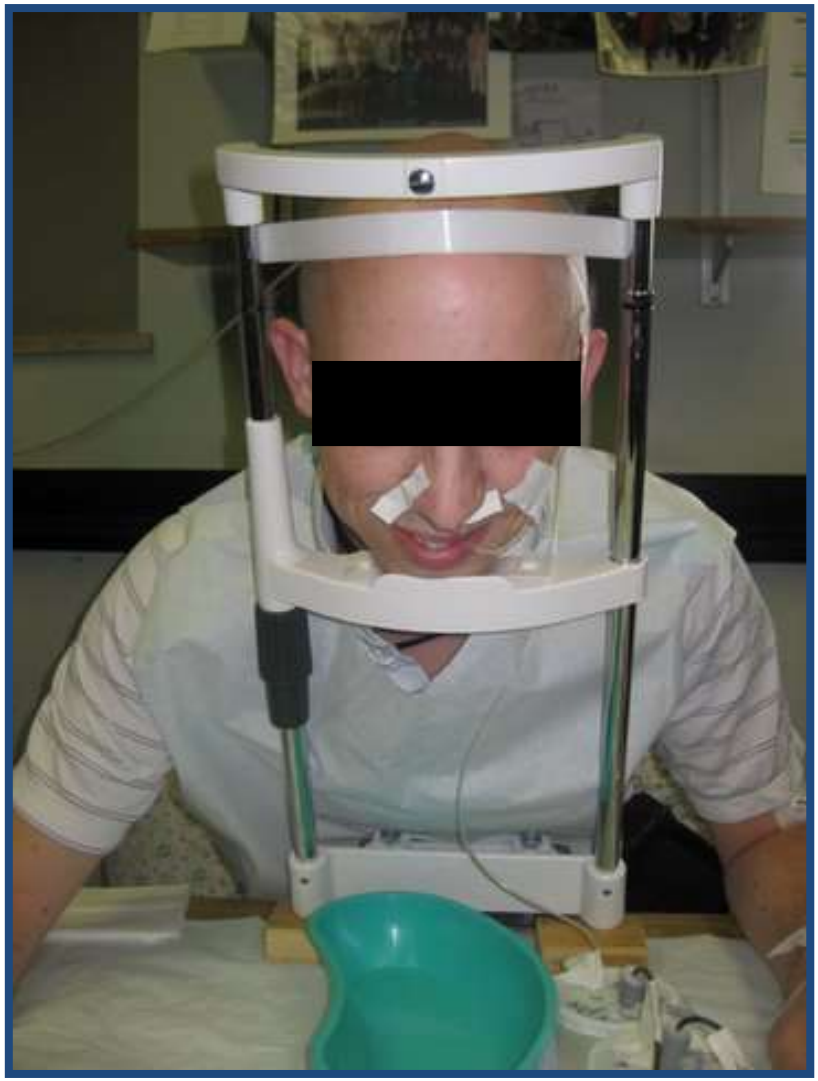
Mechanisms of Cl^- and Na^+ flux across epithelial cells- normal cells



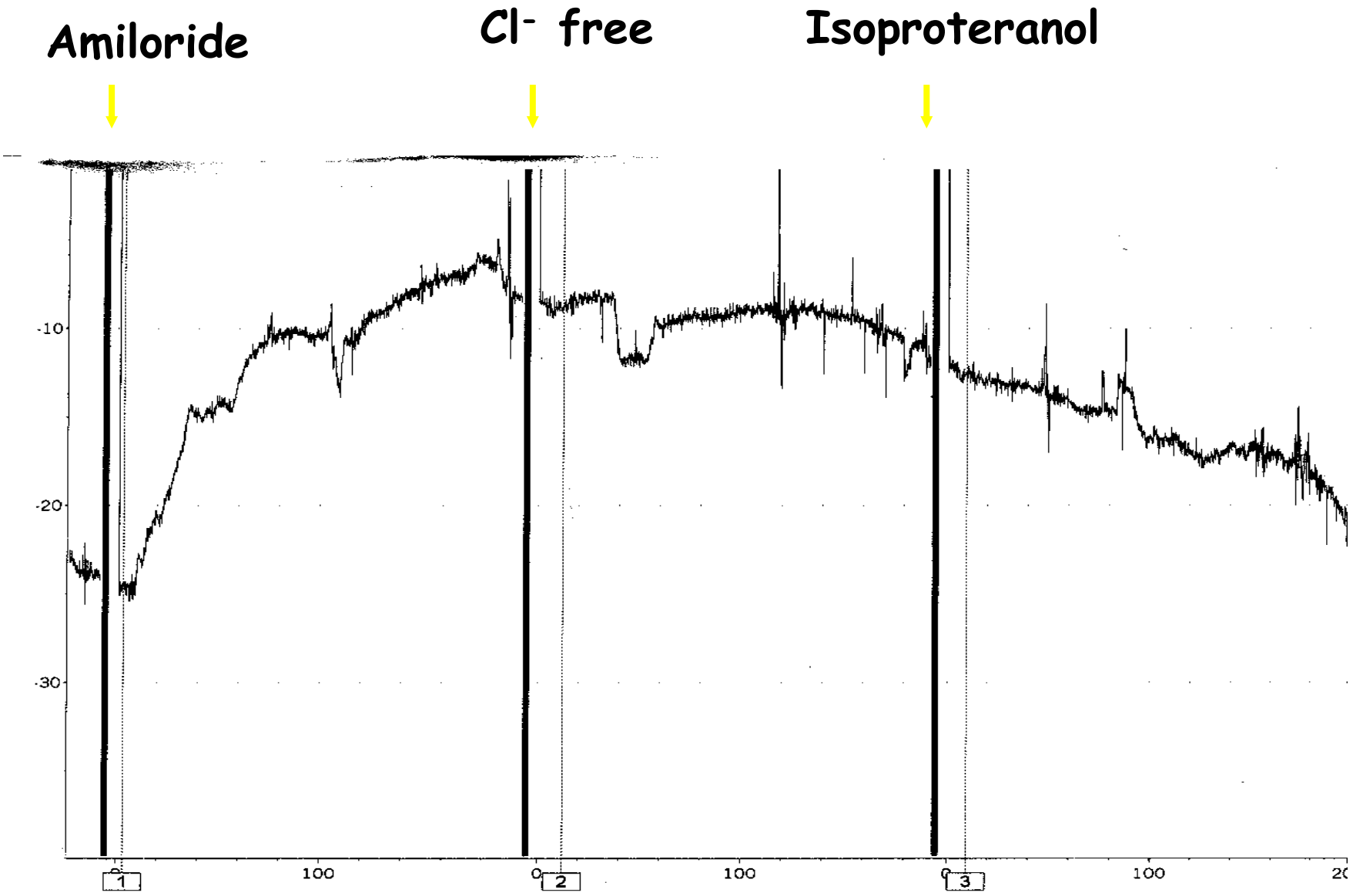
Nasal Potential Difference (NPD)



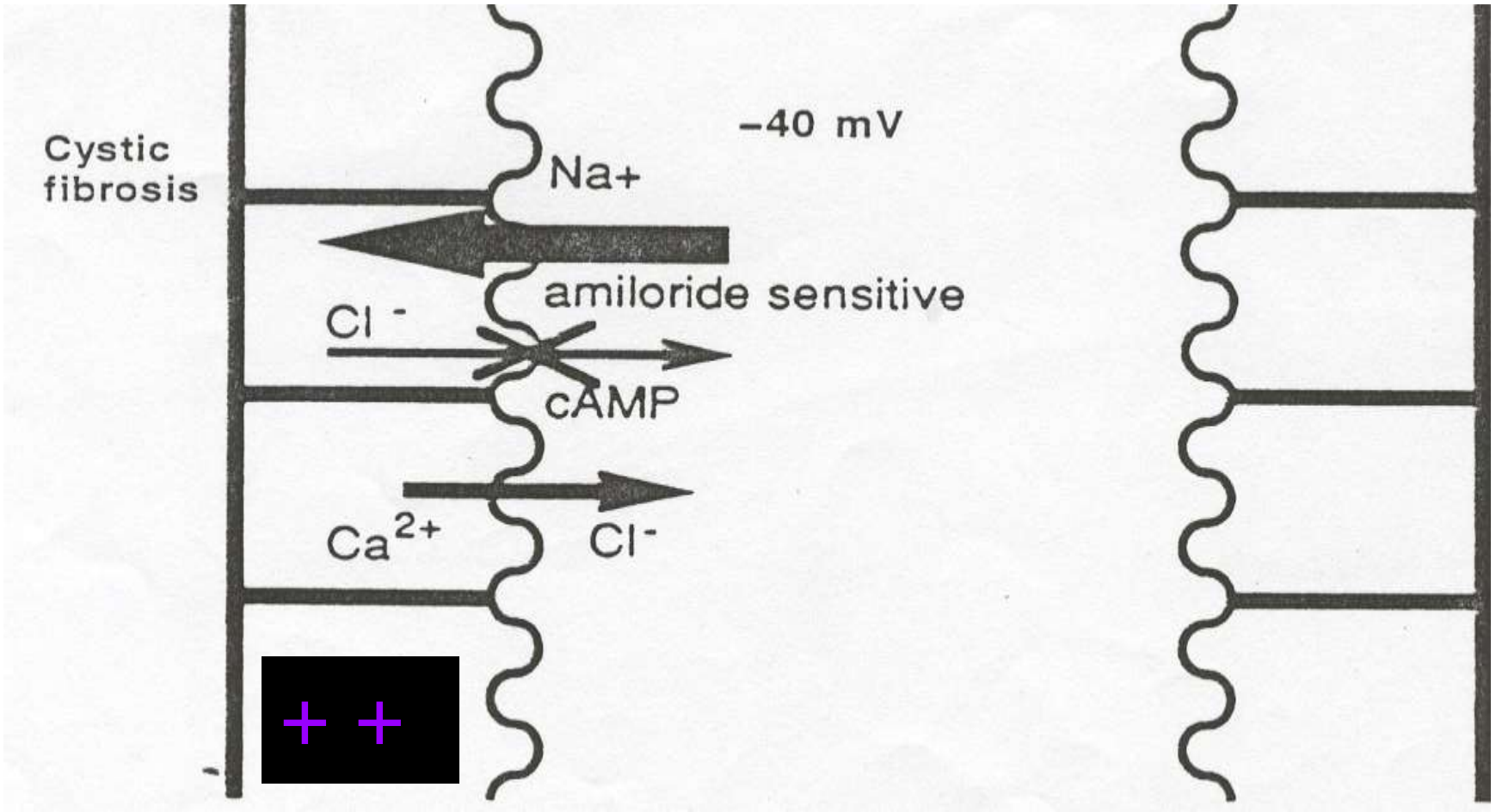
NPD Technique



NPD- non CF patient:



Mechanisms of Cl^- and Na^+ flux across epithelial cells- CF cells

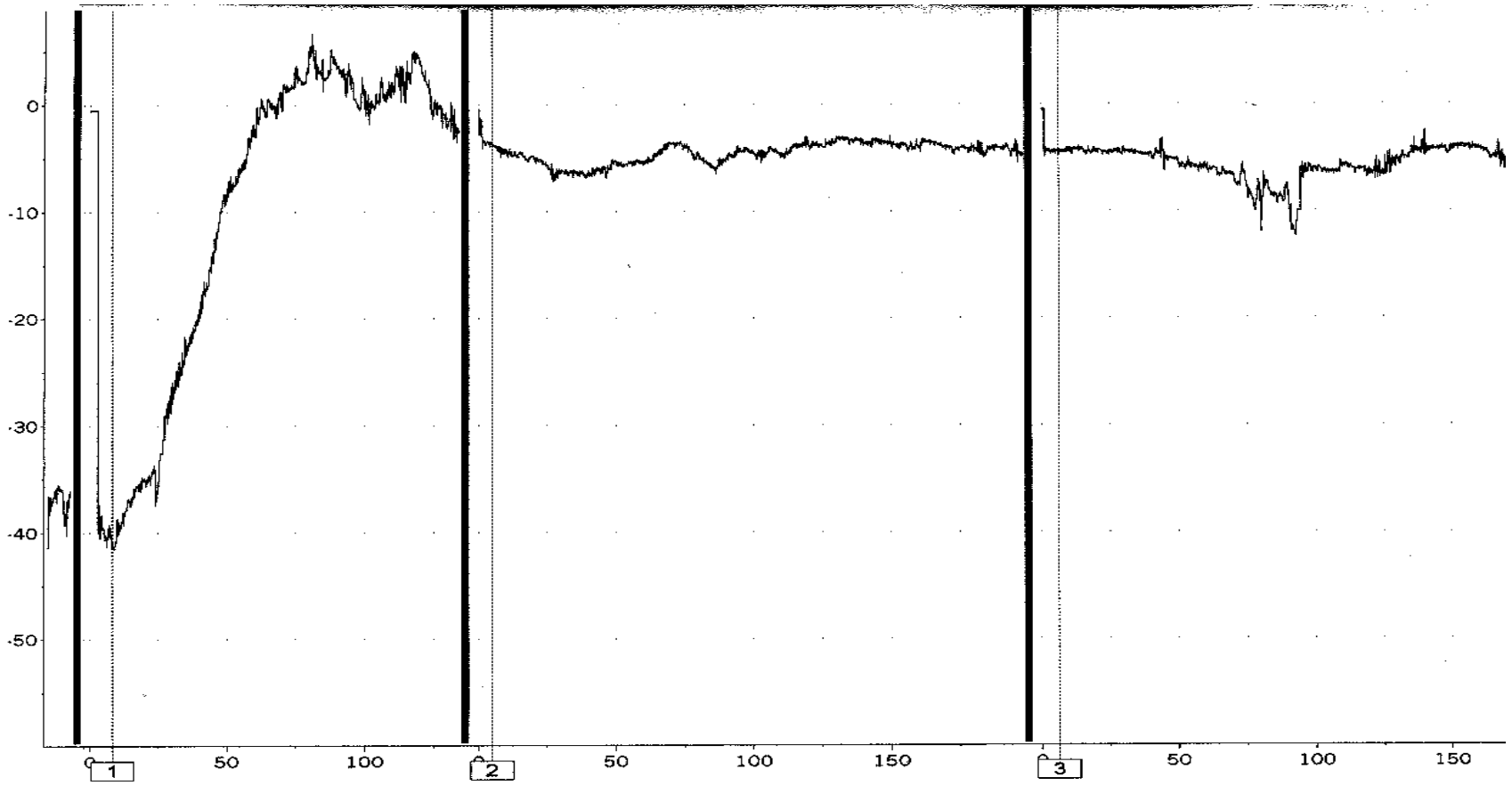


NPD- CF patient:

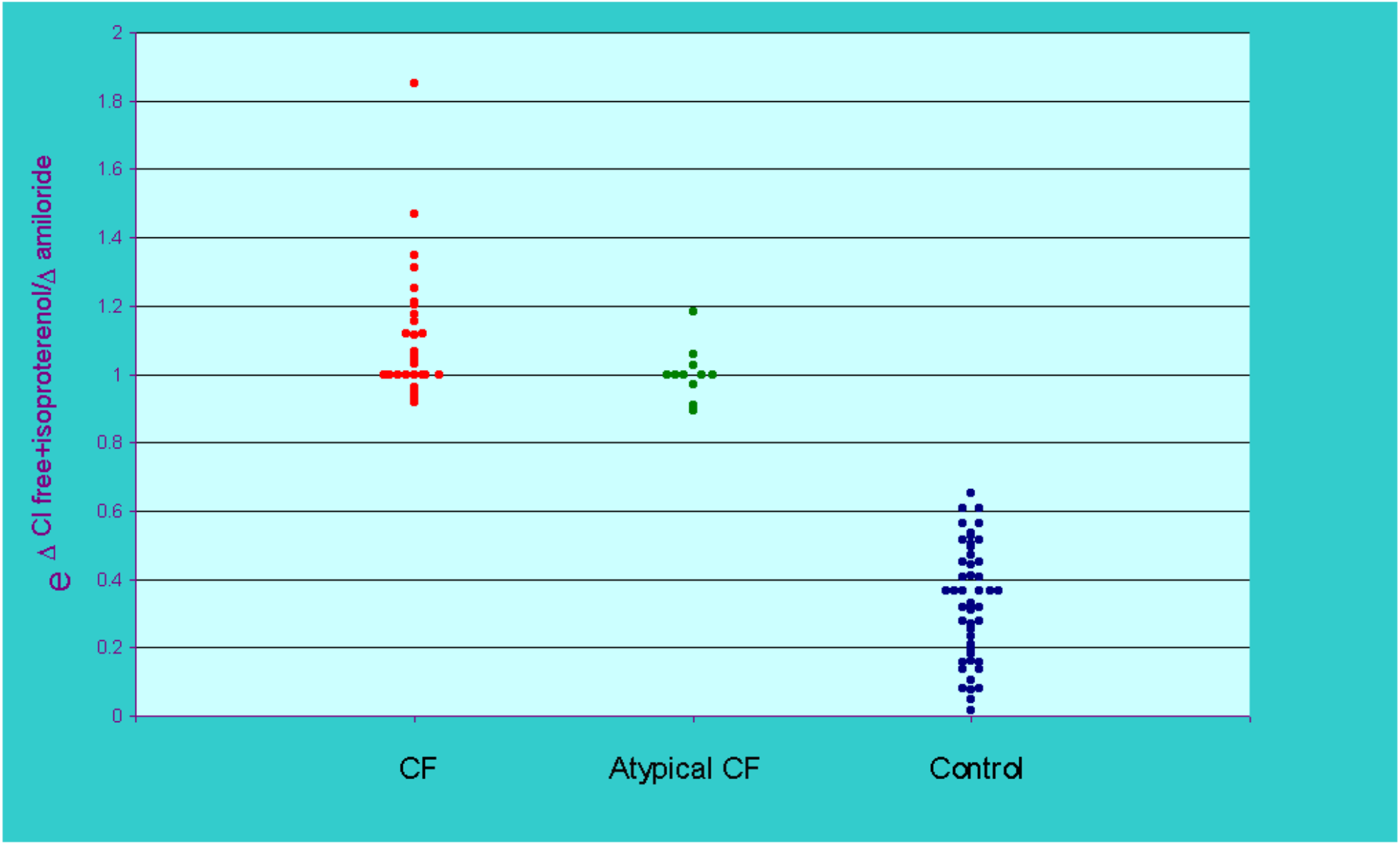
Amiloride

Cl^- free

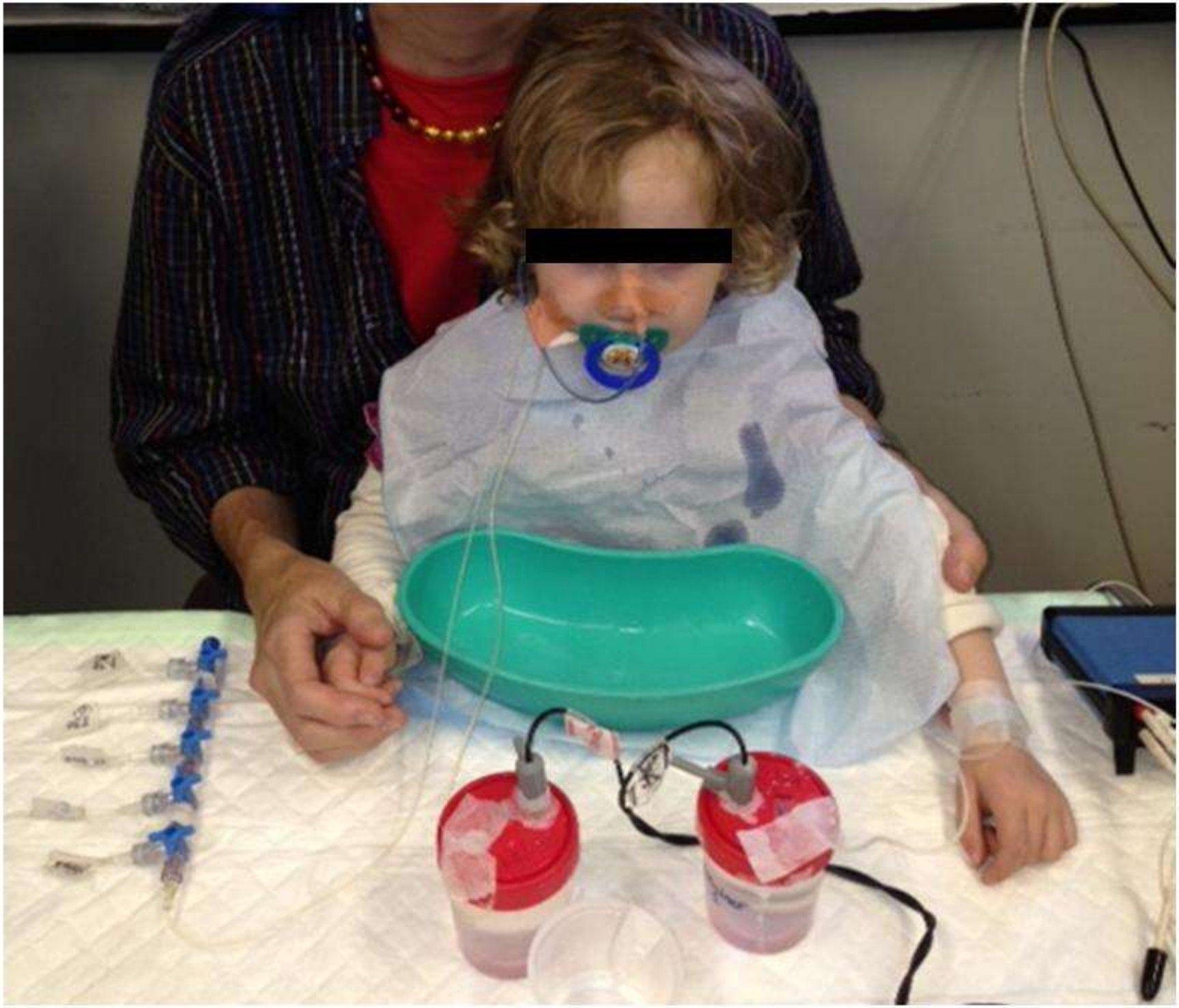
Isoproteranol



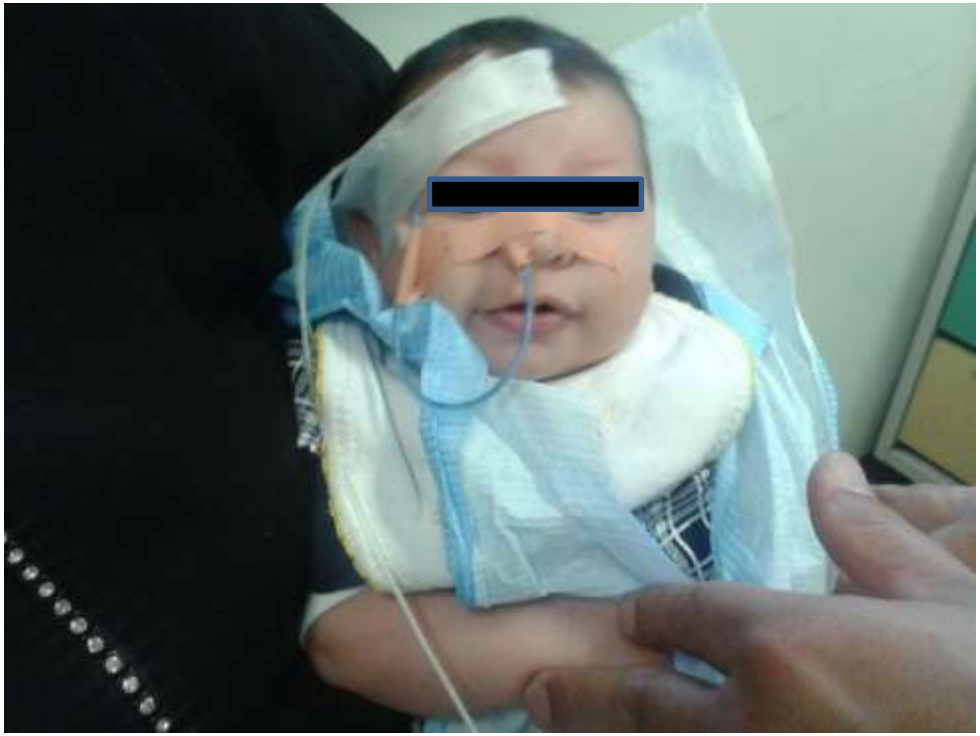
NPD measurements in CF, Atypical CF and Controls



Wilschanski Eur Resp J 2001;17:1208-15



NPD – BABY



Dr Michael Cohen ECFS 2017

METHODS:

Patients with suspected CF were referred from Israeli CF centers between 2003 and 2014 who performed sweat test (Gibson Cooke or Macroduct) and NPD (Knowles) in one tertiary center.

(Dr Hanna Goldstein MD Thesis
ECFS Seville 2017)

• Sweat test was defined as:
Normal <40mmol/L
Borderline 40-60 mmol/L
Positive >60mmol/L.

• The measurement of $e^{\Delta chlor/\Delta Amil} > 0.7$ was defined as an abnormal NPD while $e^{\Delta chlor/\Delta Amil} < 0.7$ was defined as a normal NPD*.

• Final diagnosis was defined as registration in the Israeli CF Registry.

*Eur Respir J. 2001 Jun;17(6):1208-15.

- Of the 170 patients with borderline sweat test
- 5 out of 140 (3.6%) with normal NPD are in the registry while of the 30 patients with abnormal NPD, 7 (23%) are in the registry.

Conclusions:

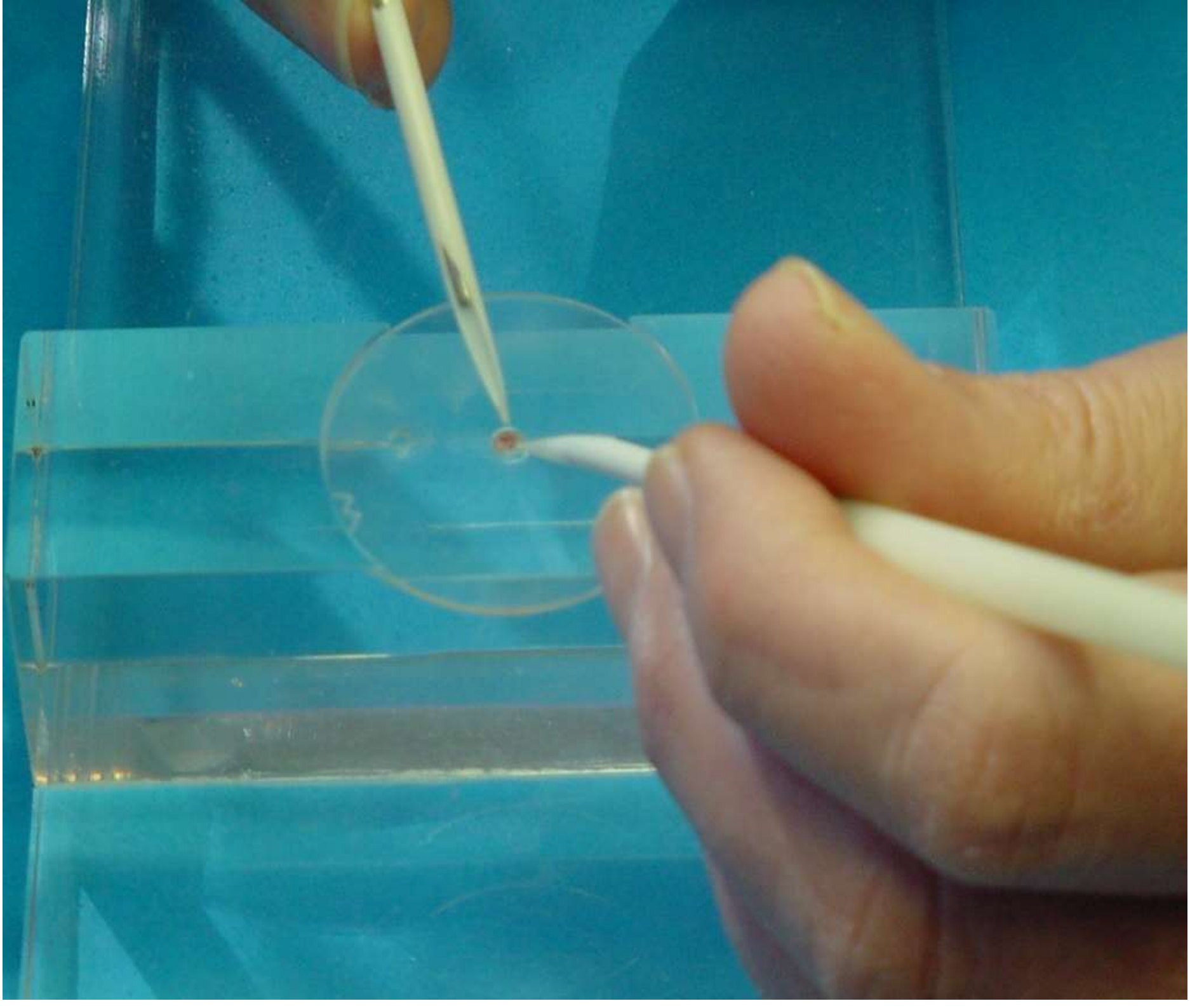
- In patients with a questionable diagnosis of CF, the NPD is very useful and is best at ruling out the diagnosis in the presence of a false positive sweat test.

- Nasal PD should be recommended for patients in whose diagnosis of CF is questionable.

Intestinal Current Measurement (ICM)

1-a new diagnostic procedure for Cystic Fibrosis in infants and young children

2- Possible use as another electrophysiological endpoint for treatments for CF

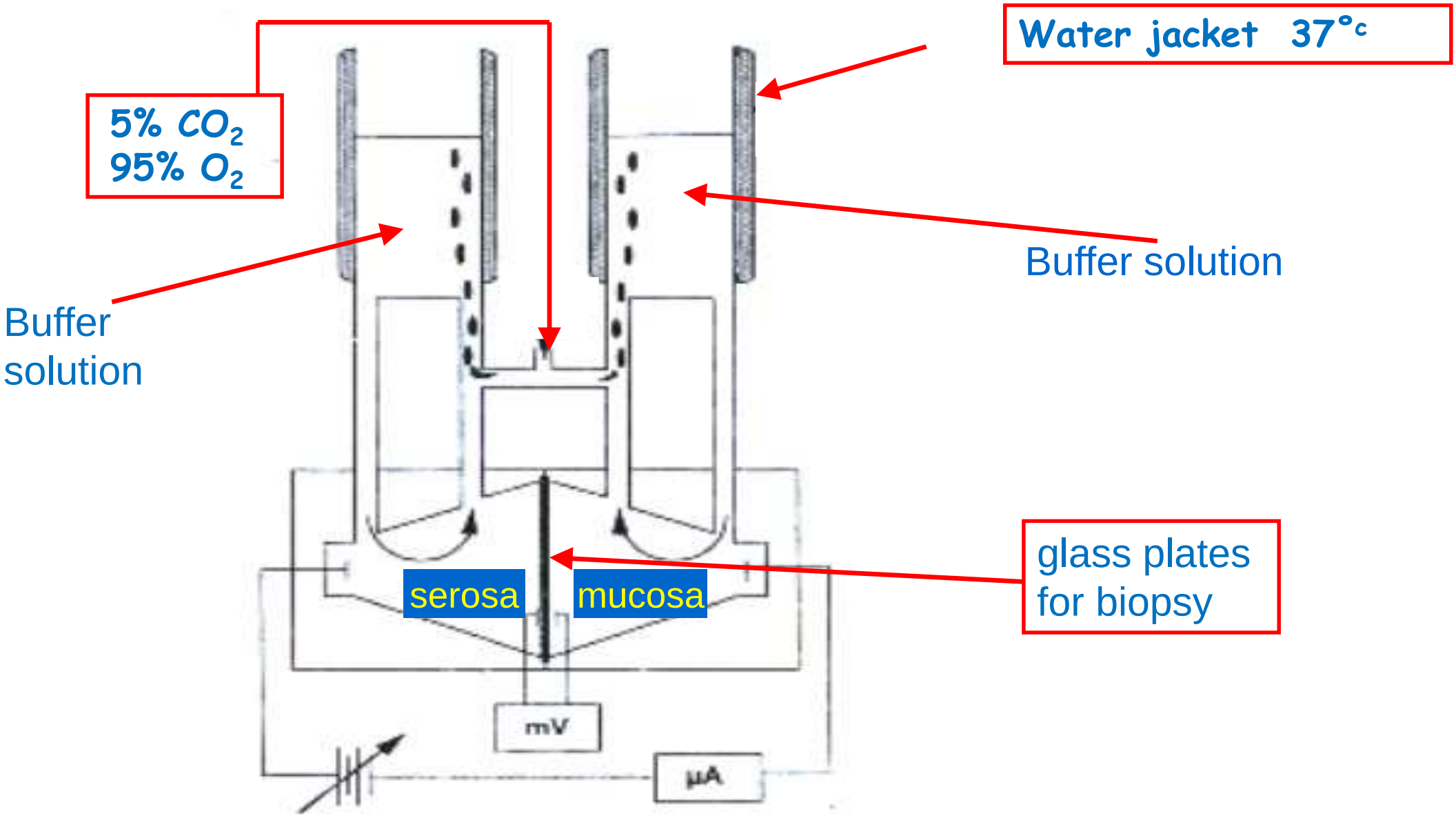


Protocol:

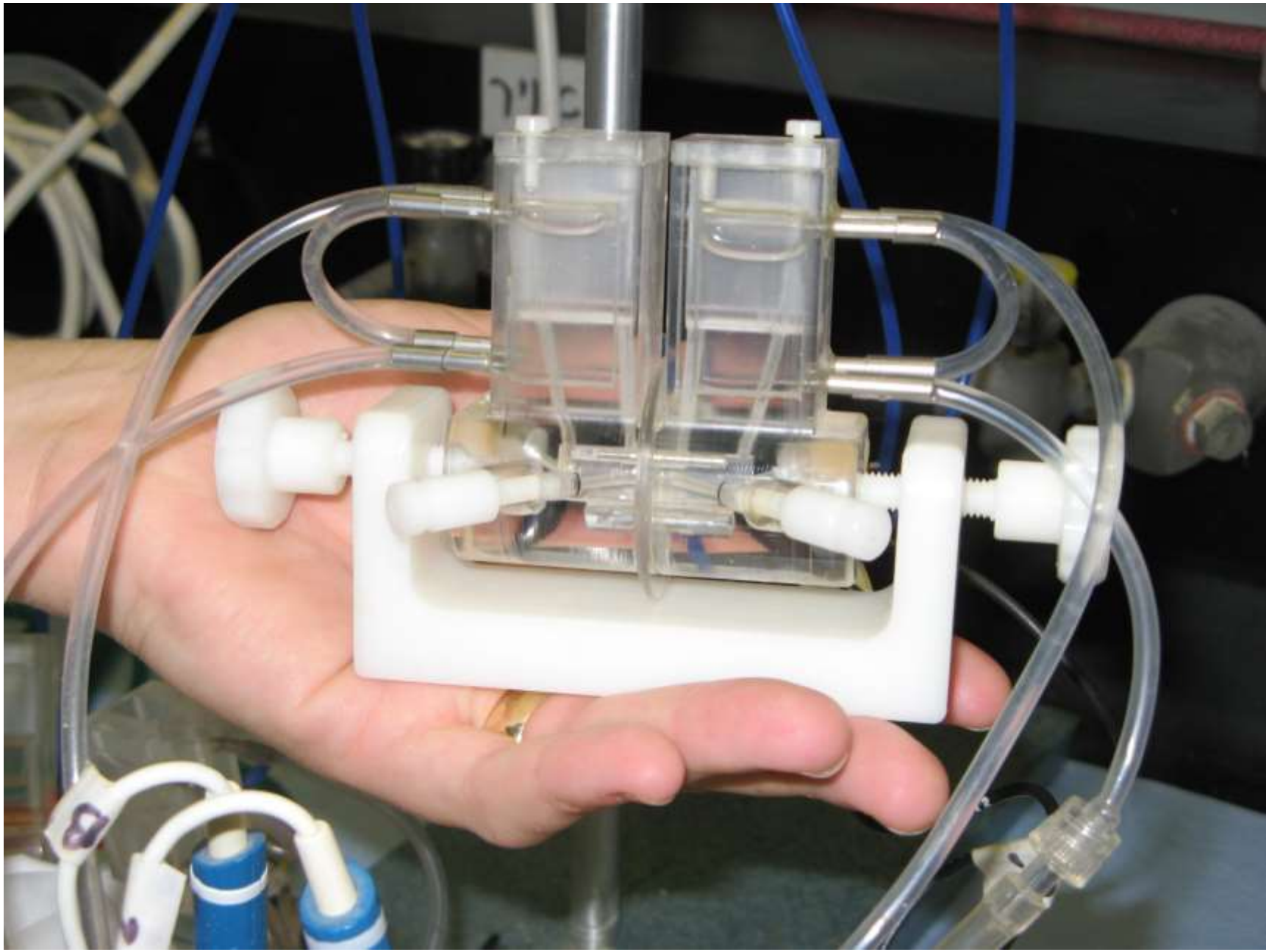
1. Suction rectal biopsy (4mm)

2. Placing the biopsy between 2 plastic plates and positioning in a modified Ussing chamber

Ussing chamber



Ussing Chamber



Protocol continued

3. Solutions are added on one or/and two sides of the biopsy

4. Current measurement at voltage clamp (0mV)

Glucose : energy source to prove viability of the tissue

Indomethacin: endogenic prostaglandin inhibitor

↓

↓ formation cAMP

↓

↓ apical chloride secretion

Amiloride : Inhibition of epithelial sodium channel (ENaC)

Carbachol : induction of Ca^{+2}

↓

Ca^{+2} sensitive K^{+} channels

↓

hyperpolarization

↓

apical chloride secretion in presence of cAMP

DIDS: Inhibition of non-CFTR Chloride Channels,
Ca^{+2} Activated Chloride Channels (CaCC)

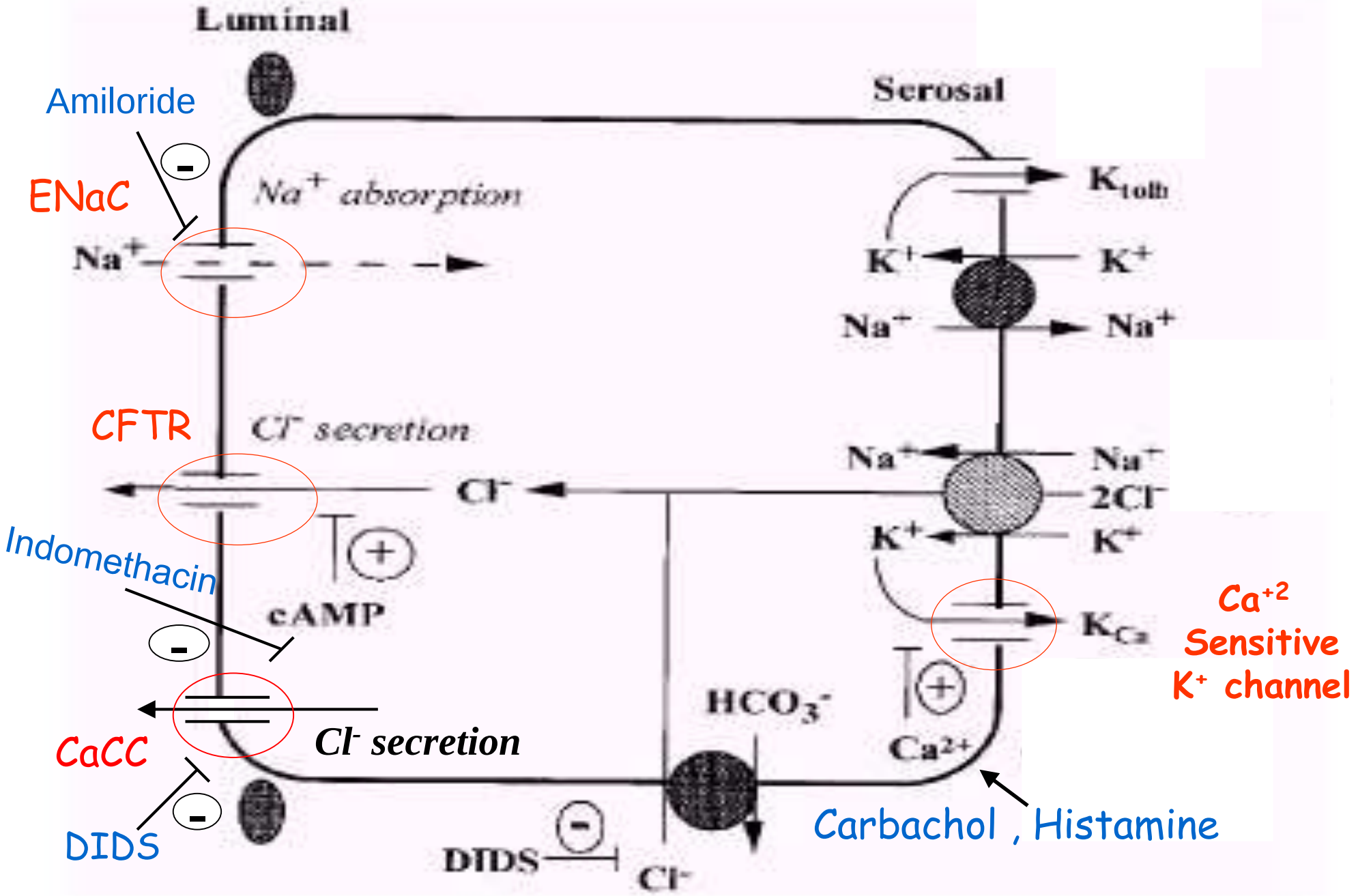
Histamine: similar effect to carbachol

cAMP & forskolin : Activation of CFTR

↓

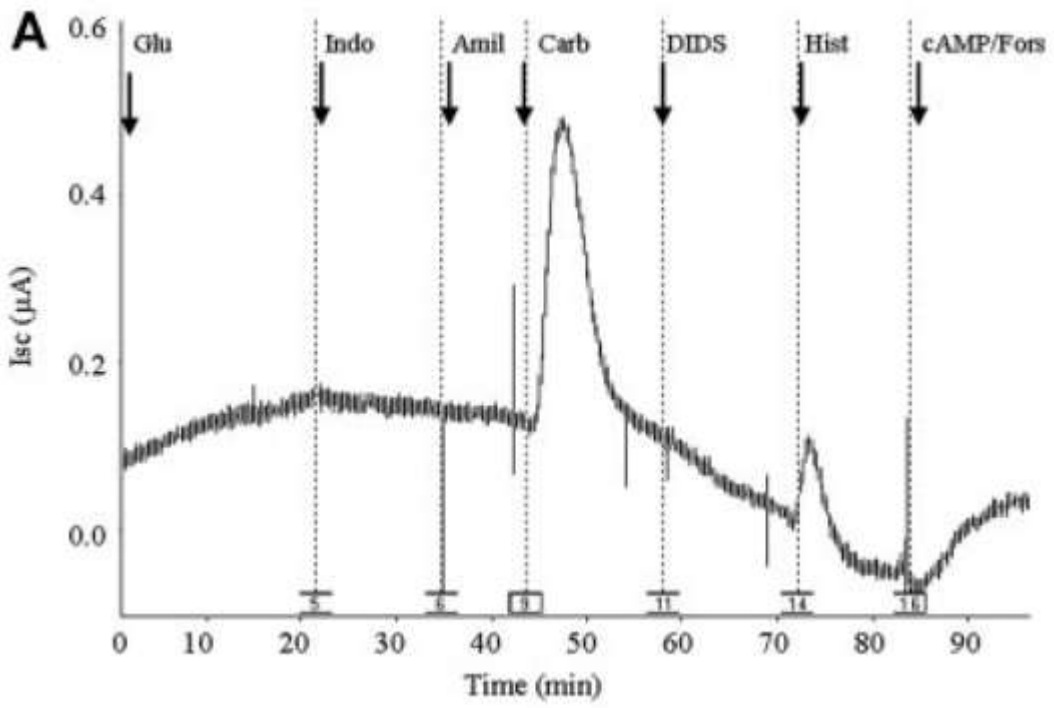
Cl^{-} secretion ↑

Cell model of the membrane transport processes in the human distal colon

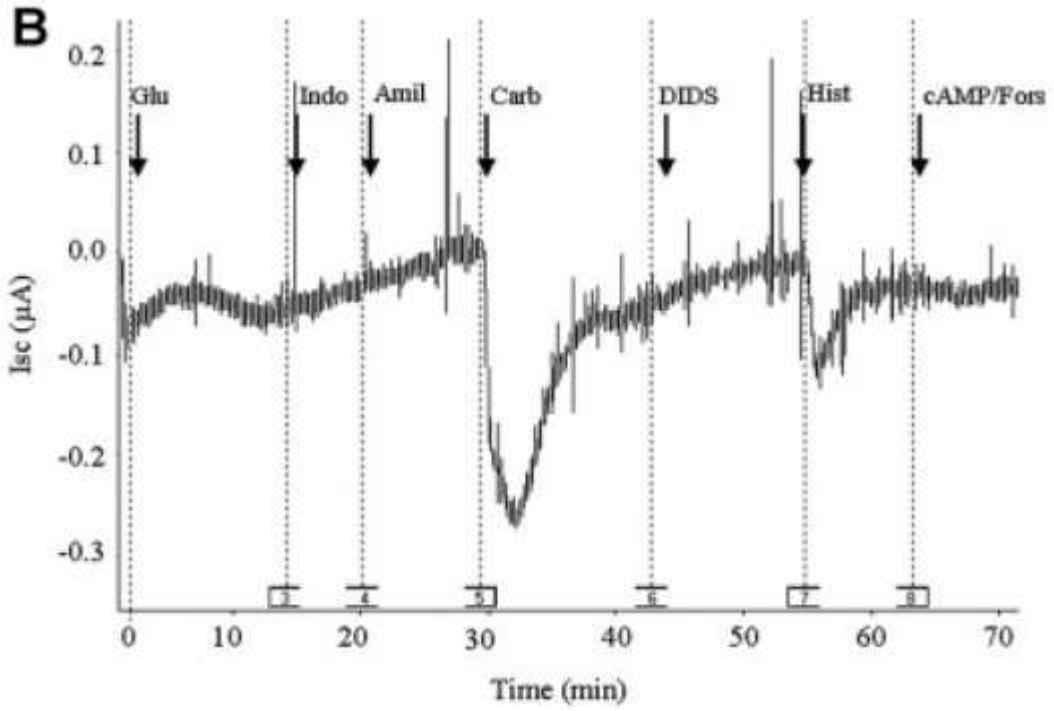


Journal of Physiology(1999) 519.1,pp.251-260

Healthy control



Known CF



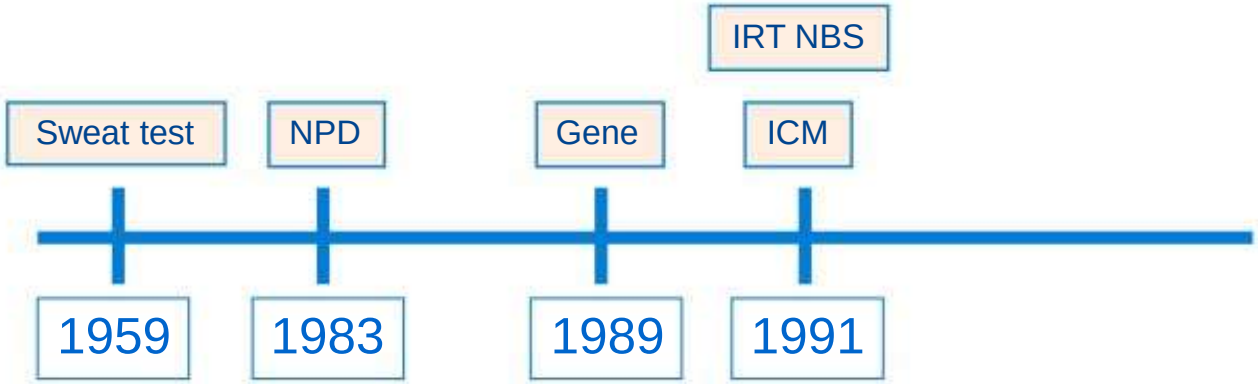
CF diagnostic consensus timeline

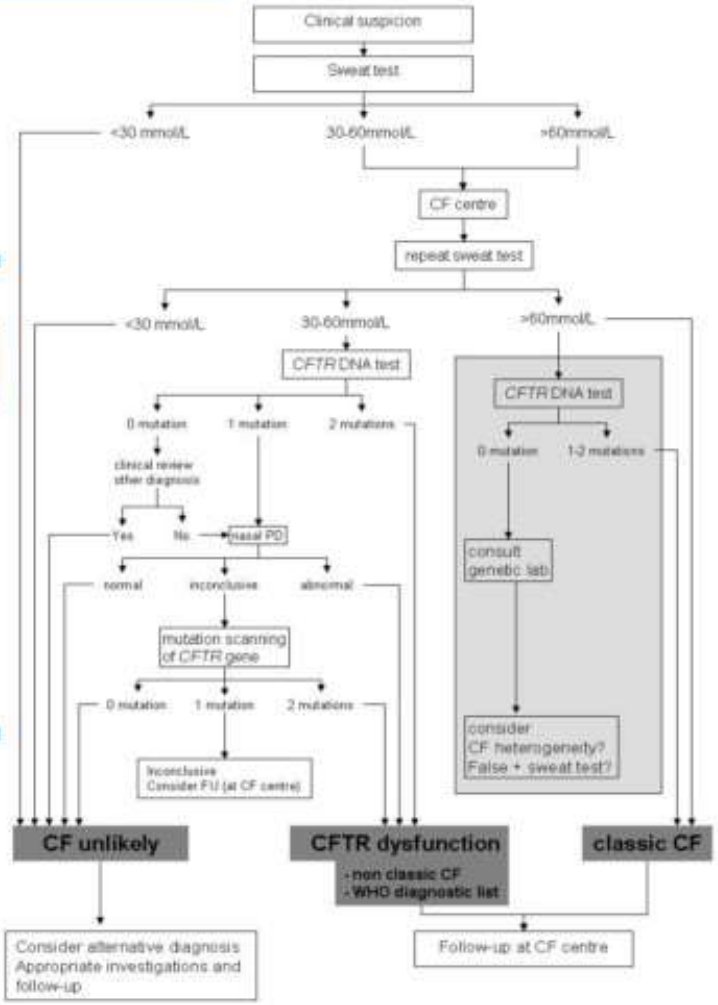
CFF diagnosis consensus
Rosenstein et al.

Cystic fibrosis: terminology and diagnostic algorithms

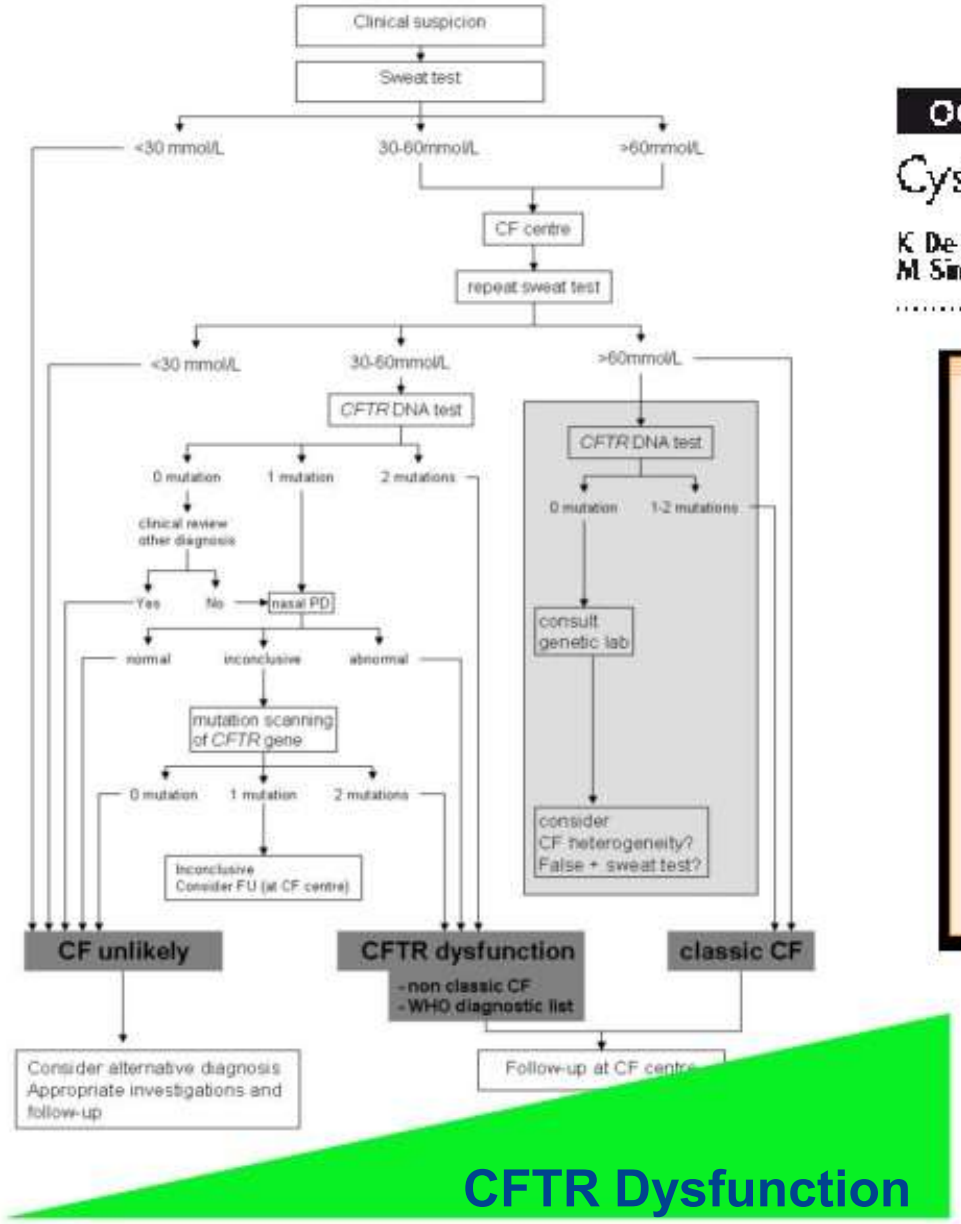
De Boeck K[1], Wilschanski M[2], Castellani C[3], Taylor C[4], Cuppens H[5], Dodge J[6], Sinaasappel M[7]

ECFS diagnostic algorithm
De Boeck et al.





ECFS CF Diagnosis guideline (2006)



OCCASIONAL REVIEW

Cystic fibrosis: terminology and diagnostic algorithms

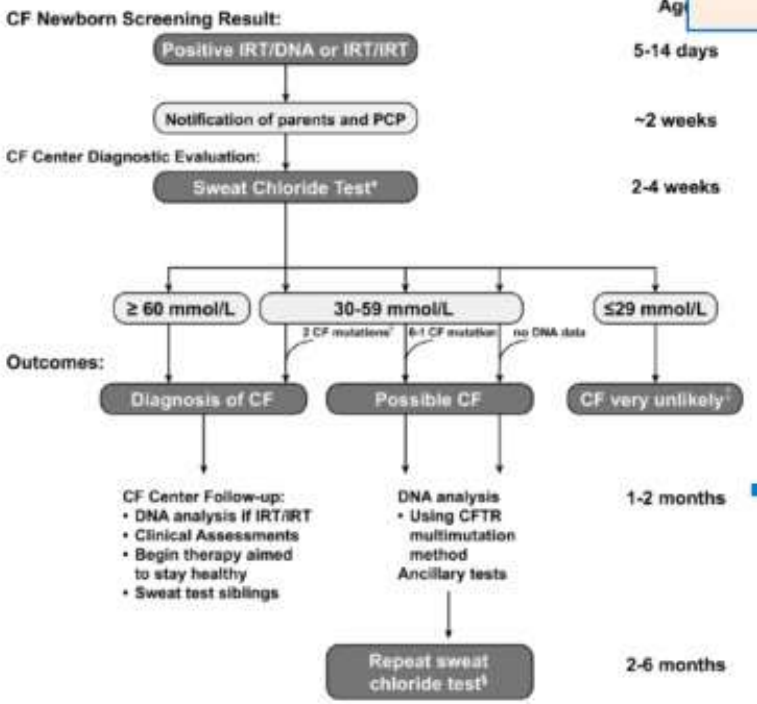
K De Boeck, M Wilschanski, C Castellani, C Taylor, H Cuppens, J Dodge, M Sinaasappel, on behalf of the Diagnostic Working Group

Main points:

- Sweat Cl- intermediate 30-60 mmol/l
- Nasal PD recommended; ICM mentioned
- Intermediate category:
  CFTR dysfunction/non-classic CF

De Boeck et al. Thorax 2006

CF diagnostic consensus timeline



CFF diagnosis consensus
Rosenstein et al.

CFF diagnosis consensus, including NBS
Farrell et al.

ECFS diagnostic algorithm
De Boeck et al.

1998 2006 2008

IRT NBS

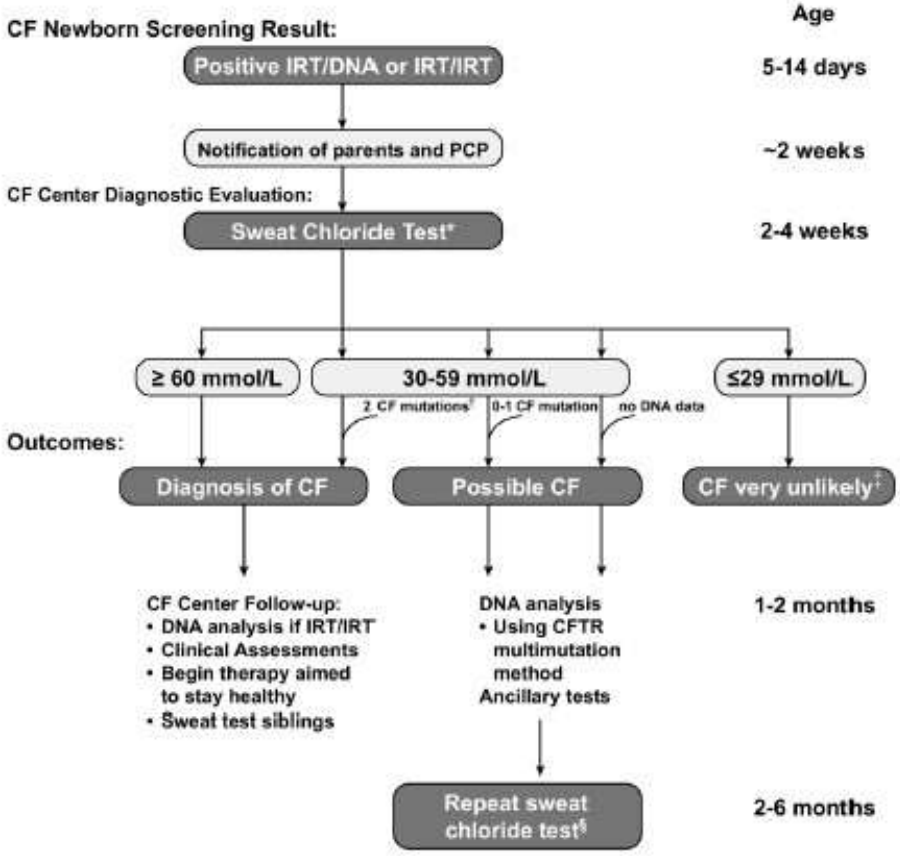
Sweat test NPD Gene ICM

1959 1983 1989 1991

CFF Diagnosis Guideline (2008)

Guidelines for Diagnosis of Cystic Fibrosis in Newborns through Older Adults: Cystic Fibrosis Foundation Consensus Report

PHILIP M. FARRELL, MD, PHD, BERYL J. ROSENSTEIN, MD, TERRY B. WHITE, PHD, FRANK J. ACCURSO, MD, CARLO CASTELLANI, MD, GARRY R. CUTTING, MD, PETER R. DURIE, MD, FRCP, VICKY A. LEGRYS, DRA, CLS, JOHN MASSIE, MBBS, FRACP, PHD, RICHARD B. PARAD, MD, MPH, MICHAEL J. ROCK, MD, AND PRESTON W. CAMPBELL, III, MD



Main points:

- Sweat Cl^- intermediate 40-60 mmol/l
- NPD mentioned/ no ICM
- Intermediate category: Possible CF

Farrell et al., J Pediatr 2008

ECFS Diagnosis Guideline after NBS

ELSEVIER

Journal of Cystic Fibrosis xx (2015) xxx – xxx

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID): A new designation and management recommendations for infants with an inconclusive diagnosis following newborn screening

A. Munck [a,b], S.J. Mayell [c], V. Winters [d], A. Shawcross [c], N. Derichs [e], R. Parad [f,g], J. Barben [h], K.W. Southern [c,d,*]

J Cyst Fibros 2015

NORTH AMERICA: CF METABOLIC SYNDROME!!!!!

CFTR-related disorder

ELSEVIER

Journal of Cystic Fibrosis Volume 10 Suppl 2 (2011) S86–S102

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Recommendations for the classification of diseases as CFTR-related disorders

C. Bombieri[a], M. Claustres[b], K. De Boeck[c], N. Derichs[d] J. Dodge[e], E. Girodon[f], I. Sermet[g], M. Schwarz[h], M. Tzetis[i], M. Wilschanski[j], C. Bareil[b], D. Bilton[k], C. Castellani[l], H. Cuppens[m], G.R. Cutting[n], P. Dřevínek[o], P. Farrell[p], J.S. Elborn[q], K. Jarvi[r], B. Kerem[s], E. Kerem[t], M. Knowles[u], M. Macek Jr[v], A. Munck[w], D. Radojkovic[x], M. Seia[y], D.N. Sheppard[z], K.W. Southern[aa], M. Stuhrmann[ab], E. Tullis[ac], J. Zielenski[ad], P.F. Pignatti[a], C. Ferec[ae,*]

„A clinical entity associated with CFTR dysfunction that does not fulfil diagnostic criteria of CF “

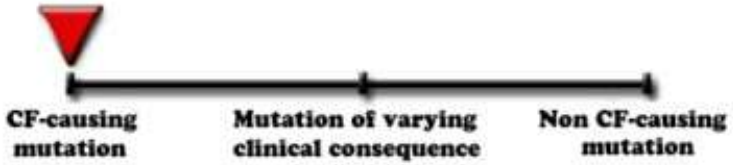
Defining CF-causing mutations

CFTR1: >2000 known CFTR mutations

Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice

C. Castellani [a,*], H. Cuppens [b], M. Macek Jr. [c], J.J. Cassiman [b], E. Kerem [d], P. Durie [e], E. Tullis [f], B.M. Assael [a], C. Bombieri [g], A. Brown [h], T. Casals [i], M. Claustres [j], G.R. Cutting [k], E. Dequeker [b], J. Dodge [l], I. Doull [m], P. Farrell [n], C. Ferec [o], E. Girodon [p], M. Johannesson [q], B. Kerem [r], M. Knowles [s], A. Munck [t], P.F. Pignatti [g], D. Radojkovic [u], P. Rizzotti [v], M. Schwarz [w], M. Stuhrmann [x], M. Tzetis [y], J. Zielenski [e], J.S. Elborn [z]

CFTR2:



CFTR2 Clinical and Functional Translation of CFTR

CFTR3: Personalized characterisation of rare CFTR genotypes

CFF Diagnosis Consensus Process 2015

CFF initiative

3 chairs

Preparation and literature review

32 experts, 9 countries

Premeeting online survey

Fullday Consensus Conference as premeeting to NACFC

Review Talks to all relevant subtopics of CF diagnosis

Plan to vote at the meeting (failed)

Refining by writing group

Voting rounds after the meeting

CFF Diagnosis Consensus Conference
6 Oct 2015, Phoenix

CYSTIC FIBROSIS FOUNDATION
THERAPEUTICS

Conference Participants from ECFS:

-Kris De Boeck
-Michael Wilschanski
-Kevin Southern
-Jane Davies
-Silvia Gartner
-Isabelle Sermet
-Carlo Castellani
-Olaf Sommerburg
-Hannah Blau
-Anne Munck
-Nico Derichs

Working/Writing Group:
-Phil Farrell
-Patrick Sosnay
-Sarah Hempstead
-M. Howenstine
-Bruce Marshall
-Susanna McColley
-Clement Ren
-Michael Rock
-Margaret Rosenfeld
-Isabelle Sermet
-Kevin Southern
-Nico Derichs
-Terry White
-Preston Campbell

SUPPLEMENT TO

The JOURNAL of PEDIATRICS

www.jpeds.com

February 2017 · Volume 181S

Cystic Fibrosis Foundation Consensus Guidelines for Diagnosis of Cystic Fibrosis

Just published !

SUPPLEMENT TO

The JOURNAL of PEDIATRICS

www.jpeds.com

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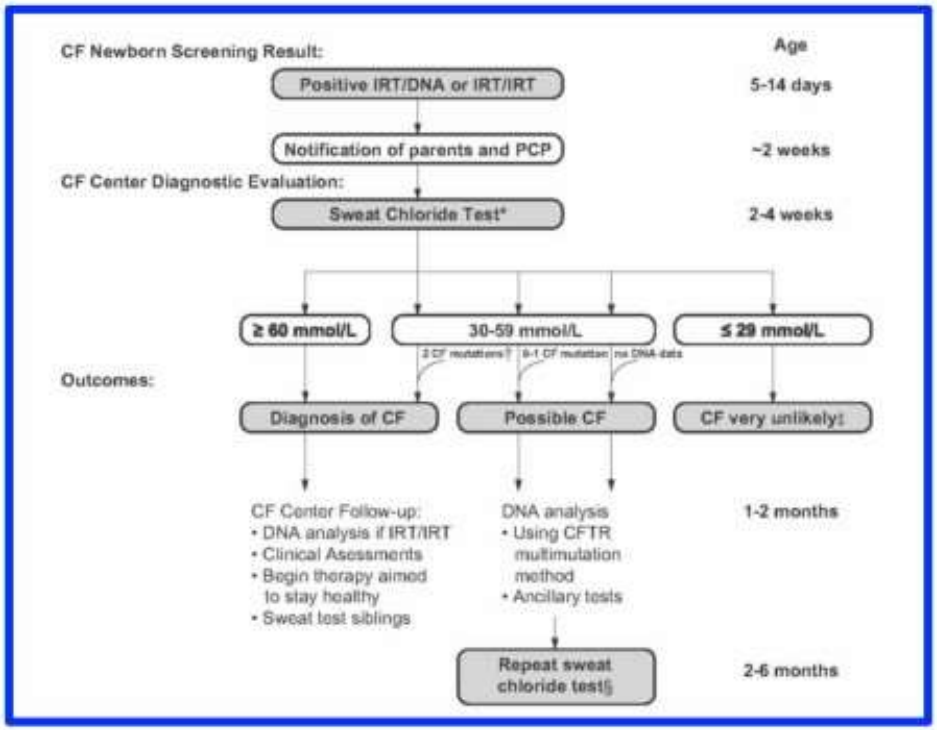
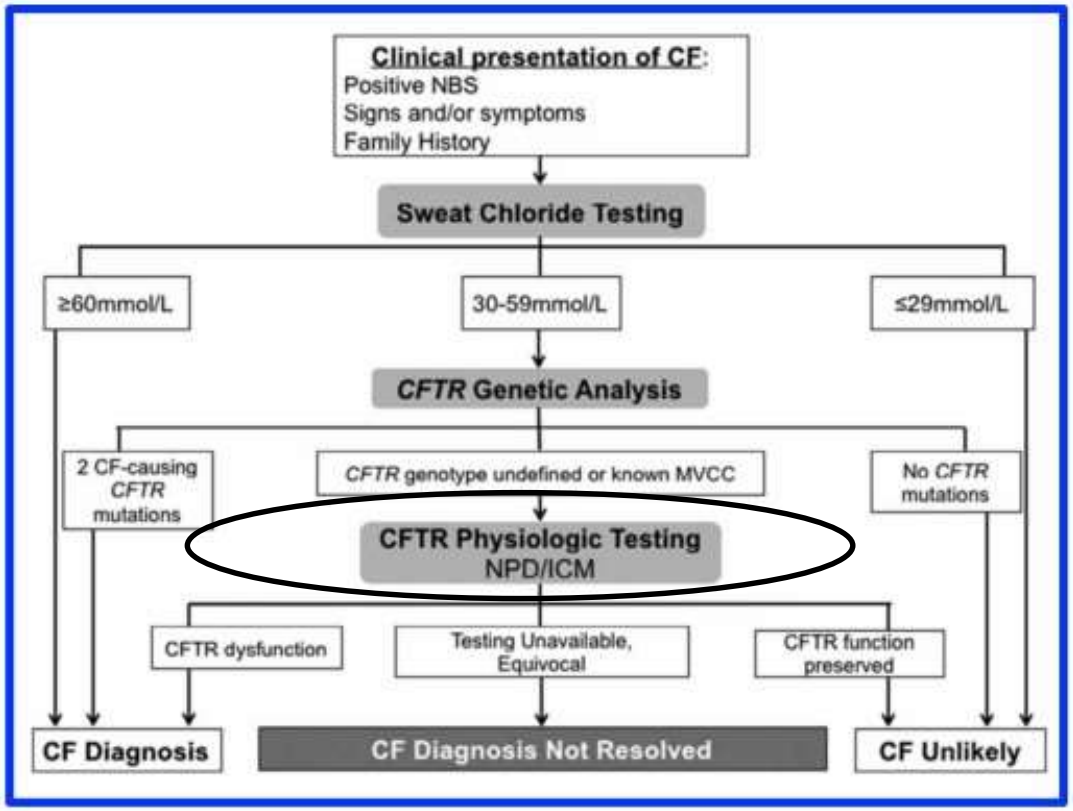
CFF Diagnosis Consensus Guidelines 2008 vs 2015





CFF Diagnosis Consensus Guidelines 2008 vs 2015

Table III. Summary of revisions to the 2008 CF Foundation guidelines for diagnosis of CF

Revisions to guidelines for screened populations

2015 Consensus	2008[24] Comparison
• Sweat testing: same recommendation in 2008, but is not being followed and is, therefore, re-emphasized here	• Sweat testing: should be done in everyone
• Sweat Cl^-: < 30 mmol/L is normal threshold for all ages (exceptions occur)	• Sweat Cl^-: < 40 mmol/L was normal threshold for ages ≥6 mo (exceptions occur)
• NPD/ICM: useful; testing should be conducted in a validated lab	• NPD: limited to contributory evidence; ICM: used only in research
• CFTR mutations: use CFTR2 mutation list, with guidelines given for mutations not included in CFTR2	• *CFTR* mutations: Used ACMG/ACOG panel of 23 mutations[51]
• Presumptive diagnosis of CF: can be made (NBS+ and 2 CF mutations or signs and symptoms of CF; or meconium ileus) and treatment started; diagnosis must be confirmed with a sweat test	• Not discussed
• Genetic analysis: recommended in addition to that done during NBS	• Genetic analysis: recommended if not part of NBS

Revisions to guidelines for CRMS/CFSPID

2015 Consensus	2008[24] Comparison
• CRMS = CFSPID: now a harmonized definition	• (Neither term in use)
• Repeat sweat testing recommended; NPD/ICM testing may be considered	• Repeat sweat testing: every 6-12 mo, but recommendation considered to be "evolving"
• Clinical assessment: by age 2 mo; duration and frequency of follow-up remains to be determined	• Clinical assessment: by age 2 mo; repeat every 6-12 mo

Revisions to guidelines for nonscreened population with inconclusive sweat chloride values

2015 Consensus	2008[24] Comparison
• Sweat Cl^-: < 30 mmol/L is normal threshold for all ages (exceptions occur)	• Sweat Cl^-: < 40 mmol/L was normal threshold for ages ≥ 6 mo (exceptions occur)
• Ancillary testing: NPD/ICM	• Ancillary testing: NPD only

Revisions to general definitions

2015 Consensus	2008[24] Comparison
• CFTR-related disorder: a symptomatic entity that does not meet diagnostic criteria for CF	• CFTR-related disorder: Individuals with 0-1 CF-causing mutations and clinical signs (possibly multiple-organ) suggestive of CF
• Avoid terms like "atypical" or "nonclassical" CF because there is no consensus definition of these terms	• Recommendation unchanged but greater emphasis now given to the importance of avoiding these imprecise, potentially confusing terms in the US.

CFTR genotypes

Table I. Categories of mutations, as defined by CFTR2

	Clinical criteria	Functional analysis	Population/penetrance
CF-causing	Mean sweat chloride ≥60 mmol/L in patients with the mutation present in *trans* with a known CF-causing mutation*	Mutation of the type expected to produce no protein (nonsense, frameshift, canonical splice)† *OR* Functional evidence of: • <10% wild-type CFTR mRNA present • <10% wild-type CFTR protein folding/processing • <10% wild-type CFTR-specific chloride conductance	Evidence suggests completely penetrant for CF: • Not seen in the nontransmitted allele in fathers of offspring with CF • Allele frequency in the population with CF higher than in the general population
MVCC	Variant may or may not meet CF-causing criteria	Variant may or may not meet CF-causing criteria	Lack of CF phenotype in some individuals with mutation in *trans* with CF-causing mutation
	Variant does not satisfy *both* clinical and functional criteria above (but may satisfy one or the other, or may satisfy neither)		
Non-CF-causing	Clinical evidence not considered	Variant must not meet CF-causing criteria	Evidence that the variant is nonpenetrant: • Allele frequency in the population with CF lower than allele frequency in the general population • Observed as the nontransmitted allele in *trans* with a CF-causing mutation in the father of offspring with CF
Unknown	Analysis incomplete *OR* Unable to assign a disease liability characterization		

mRNA, messenger RNA.
*If too few patients with sweat chloride data, pancreatic function data are used.[20]
†Not all mutations that cause premature terminations will result in nonsense-mediated decay and no protein (ie, those with terminations in the last exon); in which case, laboratory-based functional analysis is required.

MVCC—Mutation of Varying Clinical Consequence

CFTR genotypes

Table II. 2015 CF Foundation diagnosis consensus conference recommendations for diagnosis of CF using CFTR2

Statement numbers	Consensus statements
11	The latest classifications identified in the CFTR2 project[25] should be used to aid with CF diagnosis: • CF-causing mutation: individuals with 2 copies on separate alleles will likely have CF (clinical sweat confirmation needed) • Mutation of varying clinical consequence (MVCC): a mutation that in combination with a CF-causing mutation or another MVCC mutation may result in CF • Uncharacterized mutation/mutation of UNK: mutation that has not been evaluated by CFTR2 and may be disease causing or of variable clinical consequence or benign • Non-CF-causing mutation: individuals with 1 or more are unlikely to have CF (as a result of that allele)
12	In individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, the identification of 2 CF-causing mutations (defined by CFTR2) is consistent with a diagnosis of CF. Sweat chloride testing is necessary, though, to confirm the diagnosis.
13	The absence of detection of 2 CF-causing *CFTR* mutations does not exclude a diagnosis of CF.

Table III. Effects on diagnosis recommendations of different categories of *CFTR* mutations in the presence of a CF-causing mutation (in *trans*)

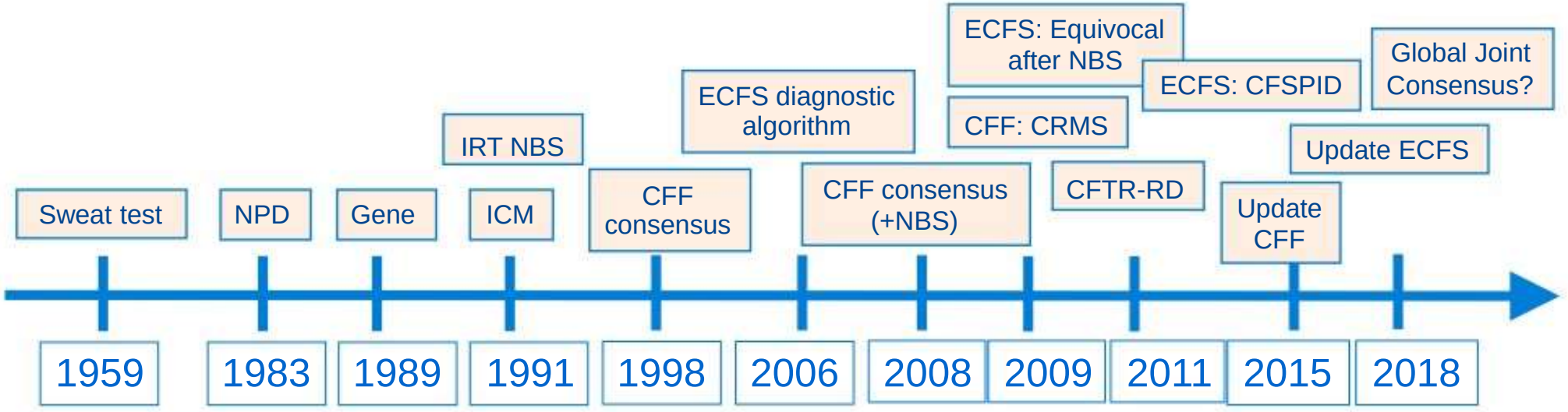
CFTR genotype		Recommendations for interpretation
Allele 1	Allele 2	
CF-causing mutation	Variant not characterized by CFTR2 (or categorized as "unknown")	This category includes mutations not annotated by CFTR2. Therefore, this genotype may or may not result in CF, depending on the disease liability of the uncharacterized variant. In some cases, there may be existing literature on the variant. If so, the same criteria used by CFTR2 can be used to define the pathologic potential for that variant. The literature on the variant should include clinical evidence (described in well-phenotyped patients with CF), functional evidence (either predicted to result in no protein, tested for RNA or protein levels, or tested for chloride conductance), and finally, population evidence. The population evidence can be investigated by looking for the variant in public databases such as 1000 genomes[33] or the Exome Aggregator Consortium.[34] A high allele frequency of the variant in these public databases would suggest that it is not fully penetrant.
	Mutation of varying clinical consequence	The likelihood that this genotype will result in CF will depend on the penetrance of the mutation with varying clinical consequence. In most cases, that is not well known. The clinical scenario becomes the key determinate of the diagnostic label. This can become challenging, especially if this genotype is detected in a newborn (eg, Extended Genetic Testing as Part of NBS in California). Because lung and pancreatic phenotypes progress over time, a clinical scenario that meets CF criteria may not occur until later in life.
	No variant identified	The key to interpreting a result in which only one variant identified is evaluating the extent of genetic testing. If only a panel containing common mutations was used, there may be an unidentified mutation. If there is suspicion from either clinical criteria or from CFTR physiologic testing, extended CFTR analysis should be performed. Sequencing and deletion duplication testing have a very high negative predictive value, but cannot completely exclude the CF.
	Non-CF-causing	The non-CF-causing mutations in CFTR2 were all identified in patients believed to have CF (enough to be entered in a registry). This occurred because the individual: (1) does not actually have CF, and the diagnosis is incorrect; (2) has mild organ system manifestations that do not typically meet diagnostic criteria for CF, but may be an example of a CFTR-related disorder; or (3) has CF, but 1 of the causative variants has not been identified (and the non-CF-causing variant is inappropriately assumed the culprit). This (2 mutations on the same chromosome) would be an example of a complex allele.

Screened populations: CRMS/CFSPID

Table I. Consensus recommendations related to CRMS/CFSPID

Statement numbers*	Consensus statements
16	The term CRMS is used in the US for healthcare delivery purposes and CFSPID is used in other countries, but these both describe an inconclusive diagnosis following NBS.
17	The term CRMS/CFSPID is reserved for individuals who screen positive without clinical features consistent with a diagnosis of CF.
18	The definition of CRMS/CFSPID is an infant with a positive NBS test for CF and either: • A sweat chloride <30 mmol/L and 2 *CFTR* mutations, at least 1 of which has unclear phenotypic consequences *OR* • An intermediate sweat chloride value (30-59 mmol/L) and 1 or 0 CF-causing mutations
19	Children designated as CRMS/CFSPID should undergo at least one repeat sweat chloride test at CF centers with suitable expertise, such as an accredited CF center.
20	Children designated as CRMS/CFSPID should have clinical evaluation performed by CF providers to identify the minority that may develop clinical symptoms.
21	Children designated as CRMS/CFSPID can be considered for extended *CFTR* gene analysis (sequencing and/or deletion duplication testing), as well as CFTR functional analysis (NPD/ICM) testing to further define their likelihood of developing CF.
22	The decision to reclassify children designated as CRMS/CFSPID as CF is an integrated decision that should take into account functional assessment of CFTR (sweat chloride, and possibly NPD/ICM), *CFTR* genetic analysis, and clinical assessment by the CF clinicians caring for the patient.
23	Genetic counseling should be offered to families of individuals followed for CRMS/CFSPID, including a discussion of the risk in future pregnancies.
24	Research recommendation: Infants with a designation of CRMS/CFSPID (by definition) do not have clinical features consistent with a diagnosis of CF and further research is needed to determine the prognosis and best practices for frequency and duration of follow-up.

CF diagnosis: global consensus plans



Continue to work towards global CF diagnosis consensus (ECFS, CFF, Australia, Latin America, Africa...)

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THANK YOU!!!!