TWO-TIER NEWBORN SCREENING FOR CYSTIC FIBROSIS

A Practical Perspective

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In this presentation, we cover:

- Rationale for newborn screening for cystic fibrosis (CF-NBS)
- New IRT/DNA protocol, and reasons for replacing the IRT/IRT protocol in Virginia
- Interpretation of results, and actions required
- Sweat testing, indeterminate values, and "CRMS"
- Informational resources about CF newborn screening

Objectives

Newborn Screening in Virginia

Mandatory since 1965

- Centralized lab for dried blood spot testing
- Division of Consolidated Laboratory Services

Expanded NBS (28 conditions) since March 1, 2006

- Introduction of screening for CF (IRT/IRT)

Revised CF-NBS methodology since December 1, 2011

- Change to IRT/DNA protocol

~ 1 in 3,300	**Cystic fibrosis ⇨ most common!**
~ 1 in 4,000	Hypothyroidism
~ 1 in 10,000	MCAD deficiency
~ 1 in 15,000	PKU
~ 1 in 60,000	Galactosemia (classical)
~ 1 in 100,000	Maple syrup urine disease
~ 1 in 100,000	Homocystinuria

Relative Incidence of Inherited Metabolic Disorders

Diagnosis of CF Prior to the Introduction of NBS

- Earliest clinical presentation is meconium ileus at birth: ~20% of patients
- Excluding those patients with meconium ileus, the average age to diagnosis of CF-based on symptoms was **14 months**

Side note:

Newborns with meconium ileus occasionally have false negative NBS by IRT (low IRT).

Always test for CF in babies with meconium ileus!

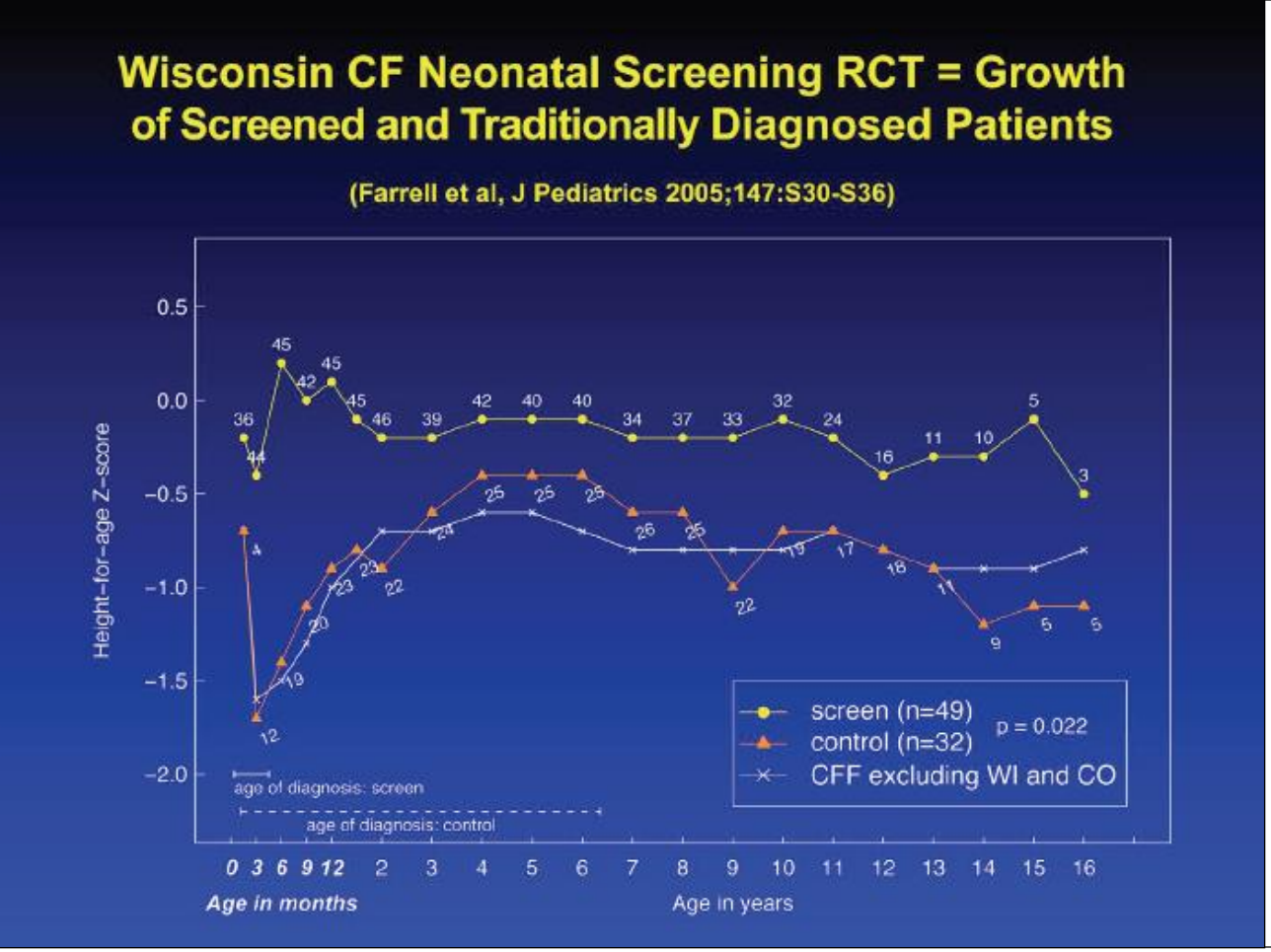
Why Should We Screen?

- Prevent early malnutrition and vitamin deficiency
  (often present by 2 months of age)
- Reduce early pulmonary complications
  (atelectasis, pneumonia, establishment of airway infection)
- Improve long-term outcomes
  including growth, cognitive function, survival
- Prevent prolonged "diagnostic odyssey"
- Ability to offer genetic counseling to families
  regarding future risk

RCT and Epidemiologic Data (US, Europe, Australia, others)

- Nutritional benefit of CF-NBS strongly verified: not only at diagnosis, but persistent advantage
- Cognitive benefit seen in Wisconsin study
- Variable strength of evidence for long-term pulmonary function benefit of CF-NBS but trend for positive effect
- Survival benefit emerging

Benefits of CF-NBS



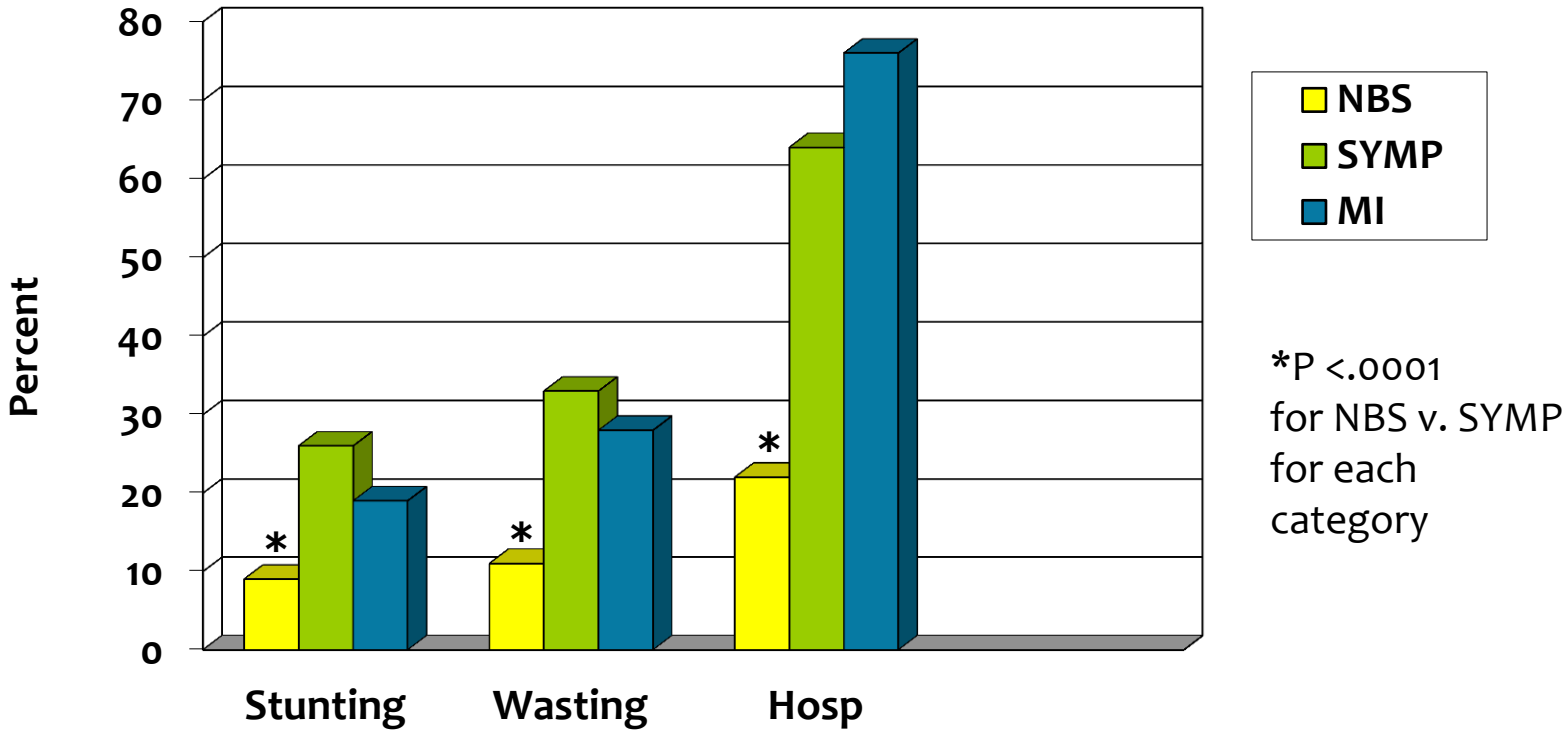
Patients with CF who were diagnosed by newborn screening have better linear growth than patients diagnosed after they developed clinical symptoms

(Pancreatic insufficient patients only; meconium ileus patients excluded)

Linear Growth

Complication rates in the year of diagnosis for CF infants <12 months old

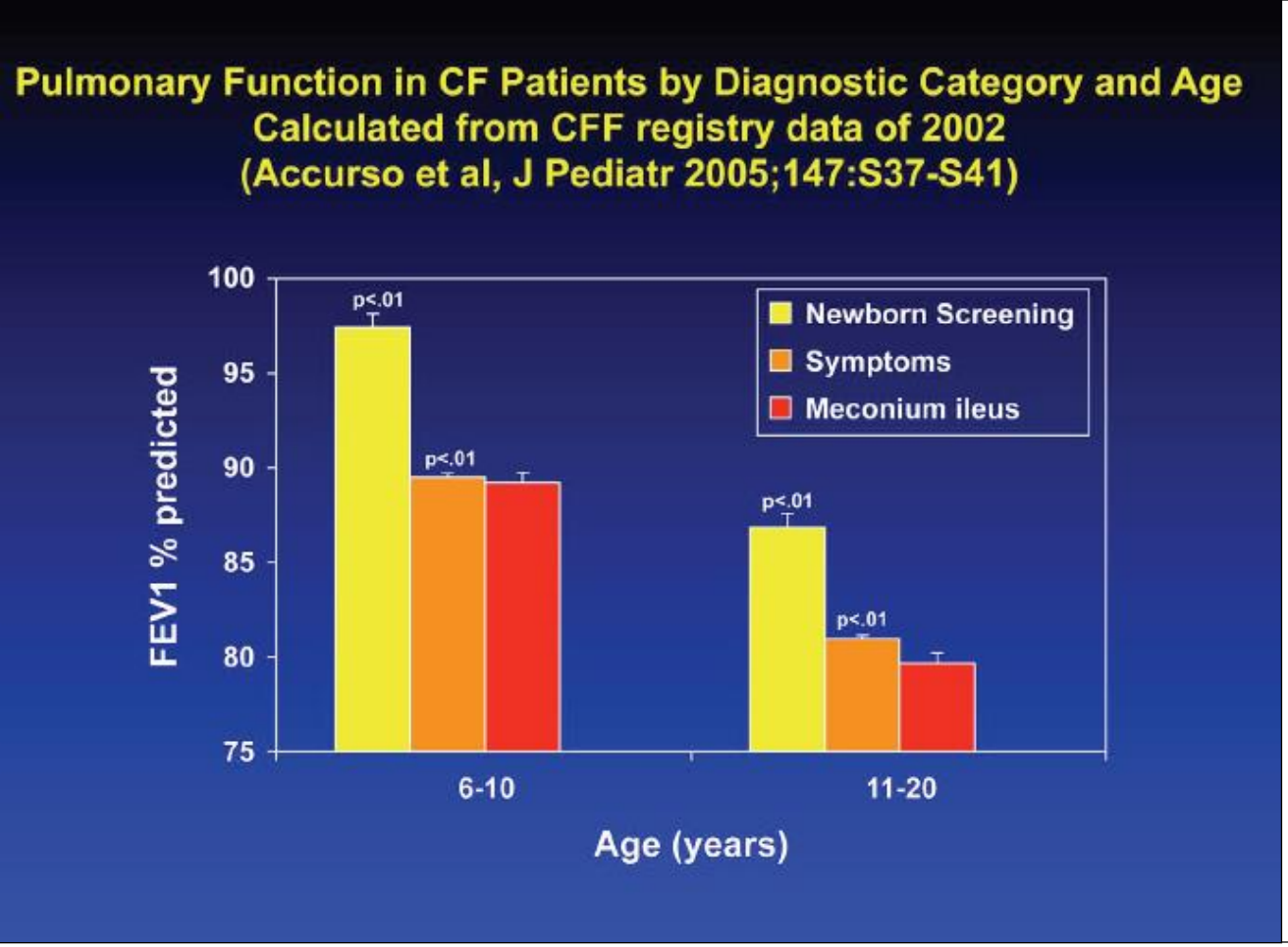
(Diagnosis by NBS vs. symptoms vs. meconium ileus)



*P <.0001 for NBS v. SYMP for each category

Data from CF Registry, presented in Accurso FJ, Sontag MK, Wagener JS: Complications associated with symptomatic diagnosis in infants with cystic fibrosis. J. Pediatr. 2005, 147: S37-41

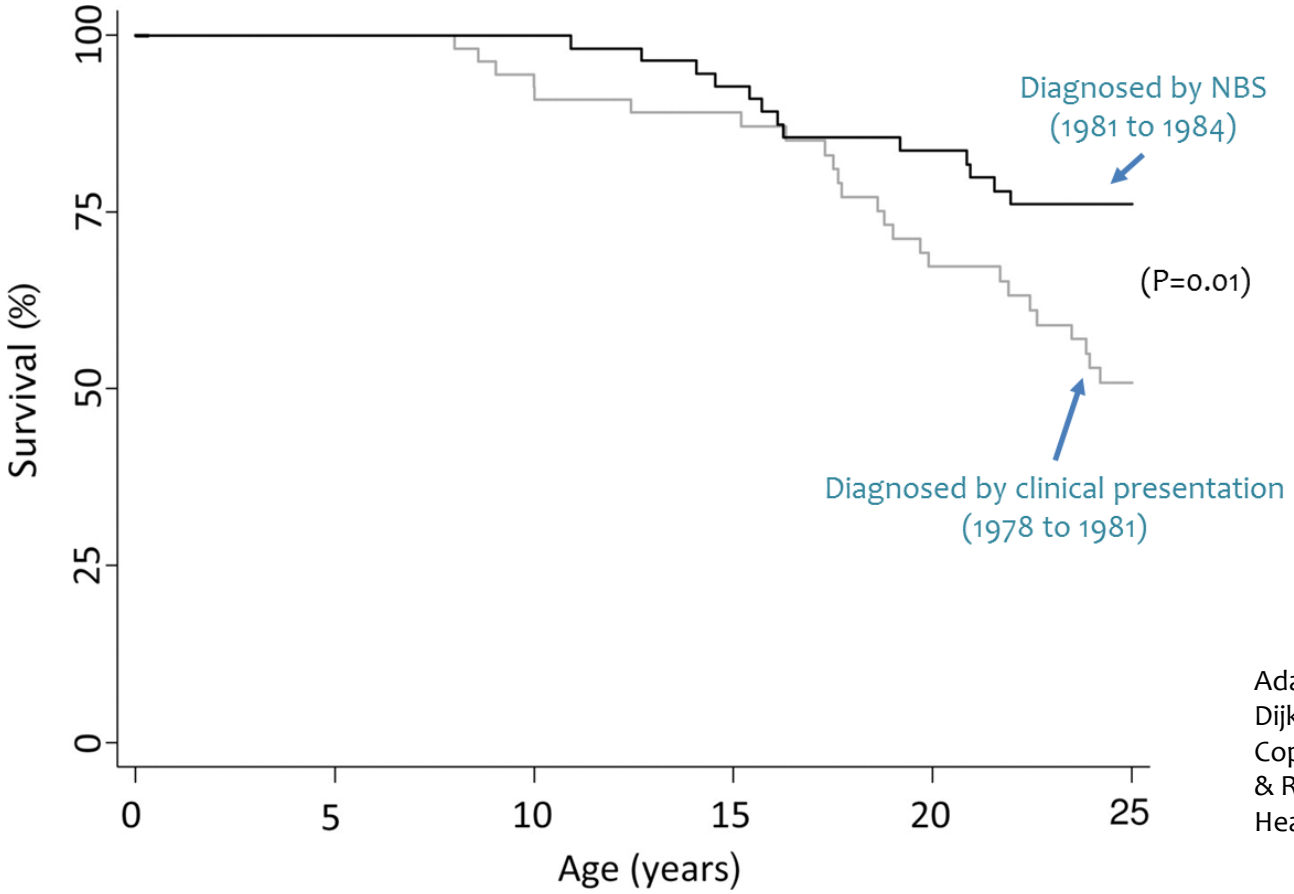
Complications



CF patients diagnosed by NBS have better pulmonary function in later childhood than those diagnosed after clinical symptoms or CF patients who had meconium ileus.

Pulmonary Function

CF Diagnosis by NBS confers survival benefit (Data from New South Wales)



Adapted from:
Dijk FN et al, Arch Dis Child 2011.

Survival Benefit

IRT: immunoreactive trypsinogen in dried blood spots

- Trypsinogen is an enzyme precursor made by pancreas
- Detectable in blood of normal and CF newborns
- IRT is elevated in CF newborns, even those with "pancreatic sufficiency" (approx. 10-15% of CF patients)
- Damaged pancreatic acinar cells "leak" this enzyme precursor into bloodstream
- Nonspecific elevation can occur with perinatal stress

DNA: CFTR mutations

- Over 1,500 known mutations
- ΔF 508 mutation = 69% of alleles
- Defined additional panel of 25-40 mutations can allow identification of 88-96% of CF alleles

Screening Methods

Prior to December 2011, CF Newborn Screening in Virginia relied on IRT only, with repeat IRT on a new sample if the initial values were elevated.

Prior Screening

Low sensitivity

- Sensitivity of 80% with standard IRT cutoffs

Need second sample after a "1st abnormal"

- Up to 20% of infants lost to follow-up in states without a mandatory second sample
- Timing of second sample is important as IRT declines naturally with age

IRT/IRT misses or delays diagnosis of CF in 50% of infants with CF

Issues with IRT/IRT Protocol for Newborn Screening

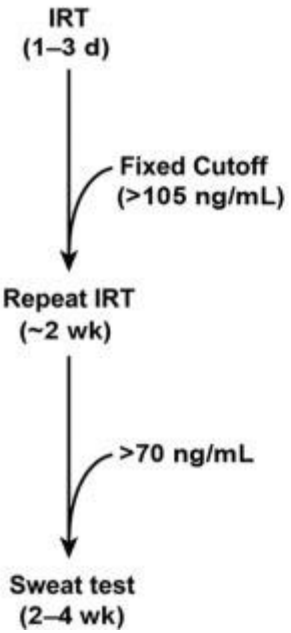
- Use IRT in dried blood spots as a “first tier” newborn screening test
- Those samples with an abnormal IRT are then studied for common CFTR mutations
- Samples with an elevated IRT and one or two identified CFTR mutations are “screen positive”
- Those “screen positive” patients are referred for sweat chloride testing to confirm or rule out CF

A Solution

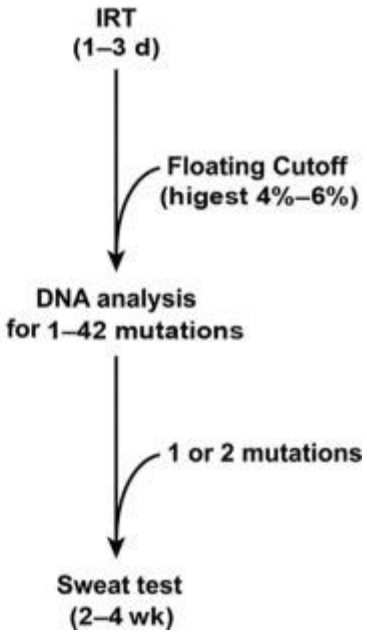
Advantages
- No detection of carriers
- No need to have genetic counseling services

Disadvantages
- Need for 2nd specimen
- Potential for incomplete f/u
- Burden on PCPs and NICUs, and NBS program
- Delay in collection of repeat sample blurs value of measurement due to naturally declining IRT

IRT/IRT



IRT/DNA



Advantages
- All on one specimen
- Potentially faster turnaround, earlier dx
- Better sensitivity

Disadvantages
- Need for genetic counseling services
- Higher cost

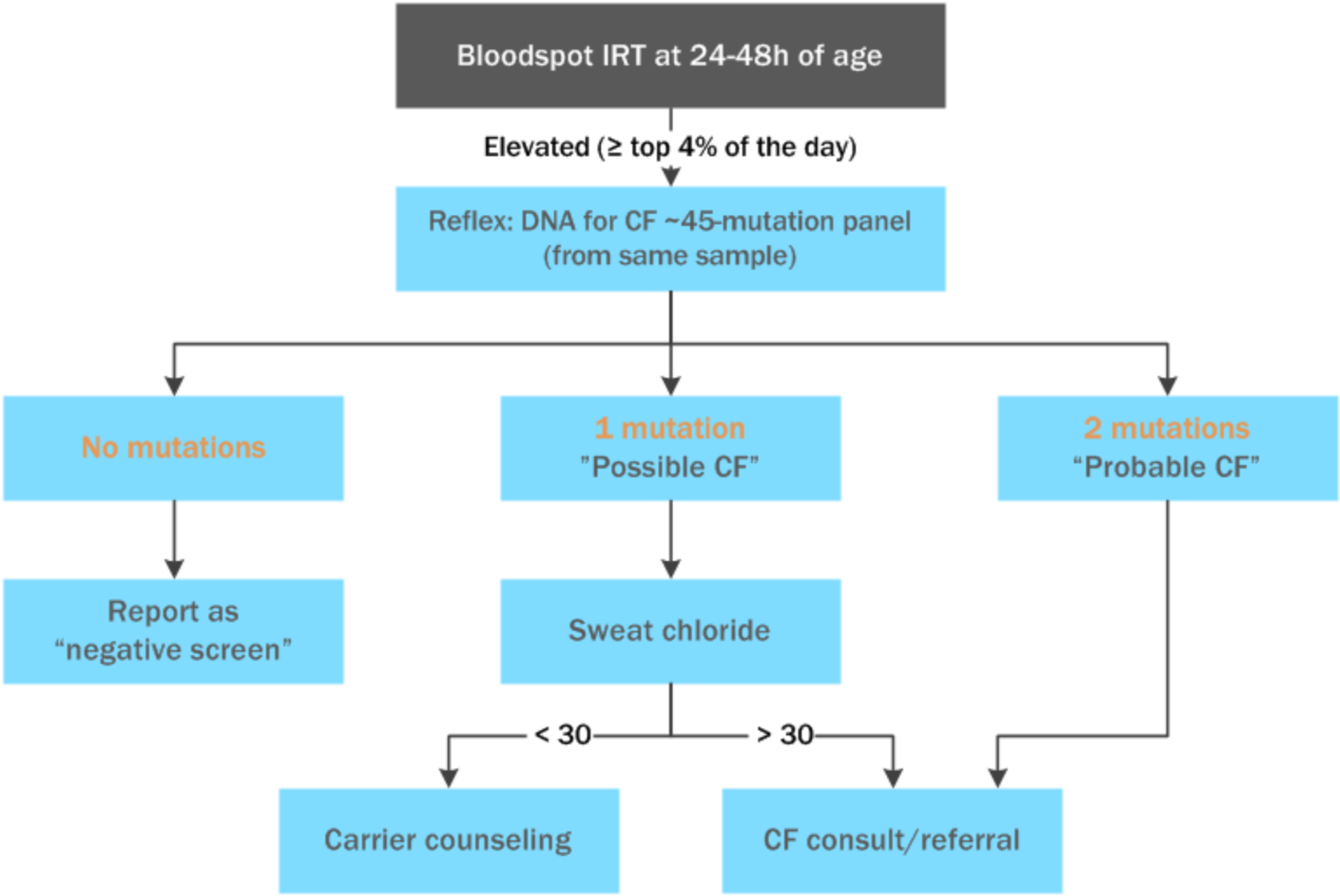
Adapted from Kloosterboer et al, 2009

Comparison of Common CF-NBS Protocols

Comparative Sensitivities of Various Algorithms

Screening Algorithm	% Sensitivity (excluding infants with meconium ileus)
IRT>105 ng/ml; Post-2 week IRT>70 ng/ml	80.2%
IRT>daily top 4%; DNA: DF508 only	93.1%
IRT>daily top 4%; DNA: 25 mutation panel	96.2%

Adapted from Kloosterboer et al, 2009:
Data from 660,443 Wisconsin newborns 1994-2004



IRT/DNA Newborn Screening Algorithm

- How many NBS samples will have "elevated" IRT?
  By definition, about 4% (for cutoff at 96th percentile)
- Of those samples with elevated IRT, how many will be normal on "reflex" DNA testing (i.e., No Mutations)? *About 94%*
- Of those patients with elevated IRT and …
  One Mutation, how many will actually have CF?
  About 3% … thus the need for the sweat test
  Two Mutations, how many will have CF?
  Almost all

Frequently Asked Questions
about IRT/DNA Screening Algorithm

- Sample mix-up at birth hospital or state lab
  wrong baby, wrong label, or misidentified sample
- 2 different CF mutations are present,
  but are in *cis* (both mutations are in one CFTR gene), and are balanced by a normal CFTR gene on the other chromosome
- Presence of at least one identified CF mutation of potentially subclinical severity: R117H
  R117H is 4th most common CF-associated allele (1.3%)
  "PolyT variant" in Intron 8 affects pathogenicity (5T > 7T/9T)
  Variable clinical manifestations

How could a "2 mutation result" *not* represent CF?

Factors accounting for a missed/delayed diagnosis of CF after newborn screening:

In the newborn nursery (or out-of-hospital birth)

1. NBS specimen is *not obtained*
2. NBS specimen *quality is unacceptable*
3. NBS specimen *labeling error* in the neonatal nursery

Adapted from Rock MJ et al, 2011

Missed/Delayed Diagnosis: *Nursery*

Factors accounting for a missed/delayed diagnosis of CF after newborn screening:

In the centralized testing laboratory

4. NBS specimen mix-up in the laboratory
5. Initial immunoreactive trypsinogen (IRT) cutoff level is inappropriate
6. Infant's IRT level is below the cutoff (biologic false negative)
7. In IRT/IRT method, a second specimen is not obtained and there is no follow-up
8. In IRT/IRT method, the second IRT result is not above the cutoff value
9. In IRT/DNA method, uncommon mutation(s) is/are present and not identified
10. Lab errors (e.g., errors measuring IRT, or DNA mutation analysis)
11. Clerical/central error in recording and reporting the newborn screen result to the primary care provider

Adapted from Rock MJ et al, 2011

Missed/Delayed Diagnosis: *Laboratory*

Factors accounting for a missed/delayed diagnosis of CF after newborn screening:

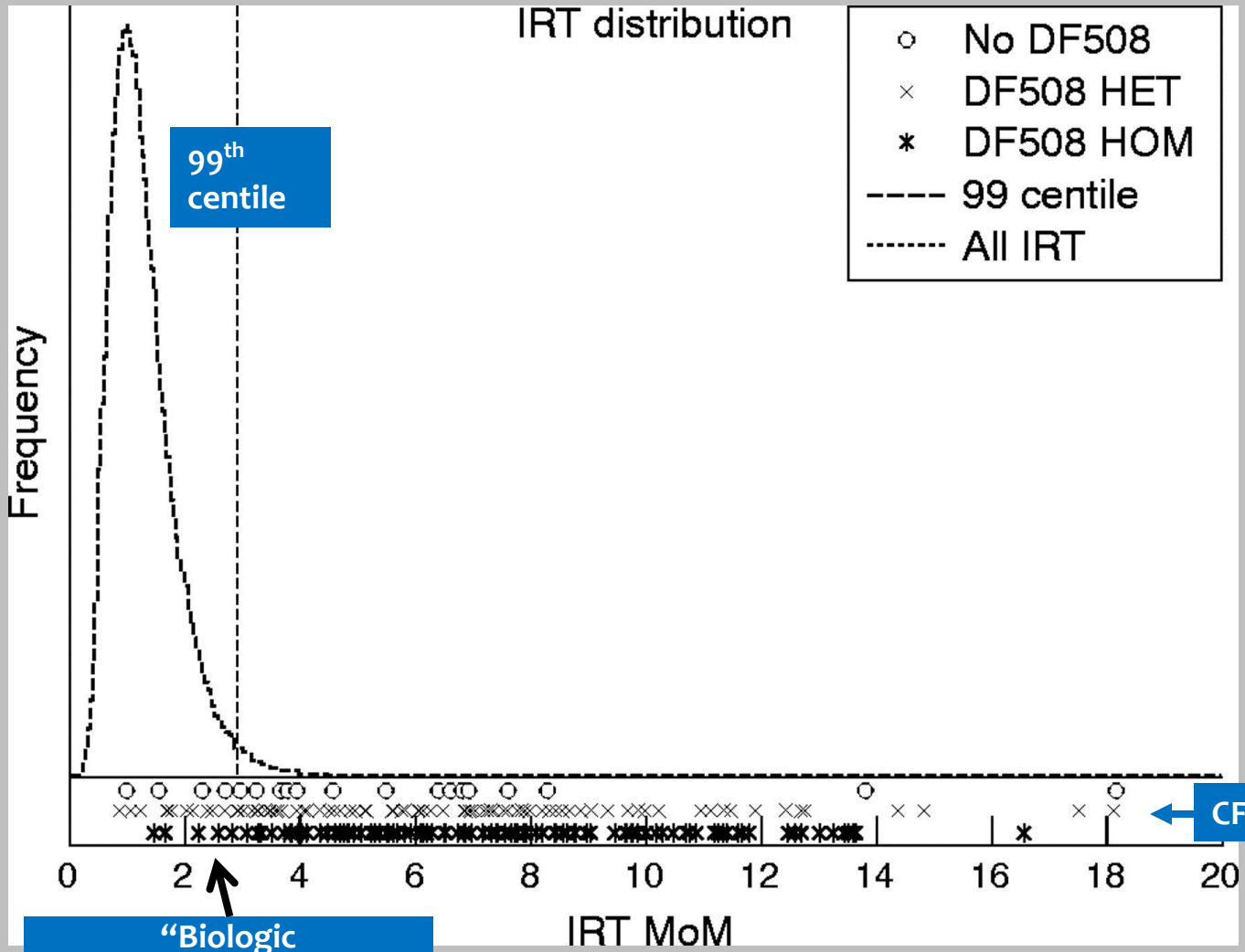
Follow-up

12. *Miscommunication* of newborn screen result between primary care provider and family (e.g., sweat test not performed)
13. *Error in measurement* of sweat chloride
14. *Inappropriate cutoff value* of sweat chloride

Adapted from Rock MJ et al, 2011

Missed/Delayed Diagnosis: *Follow-up*

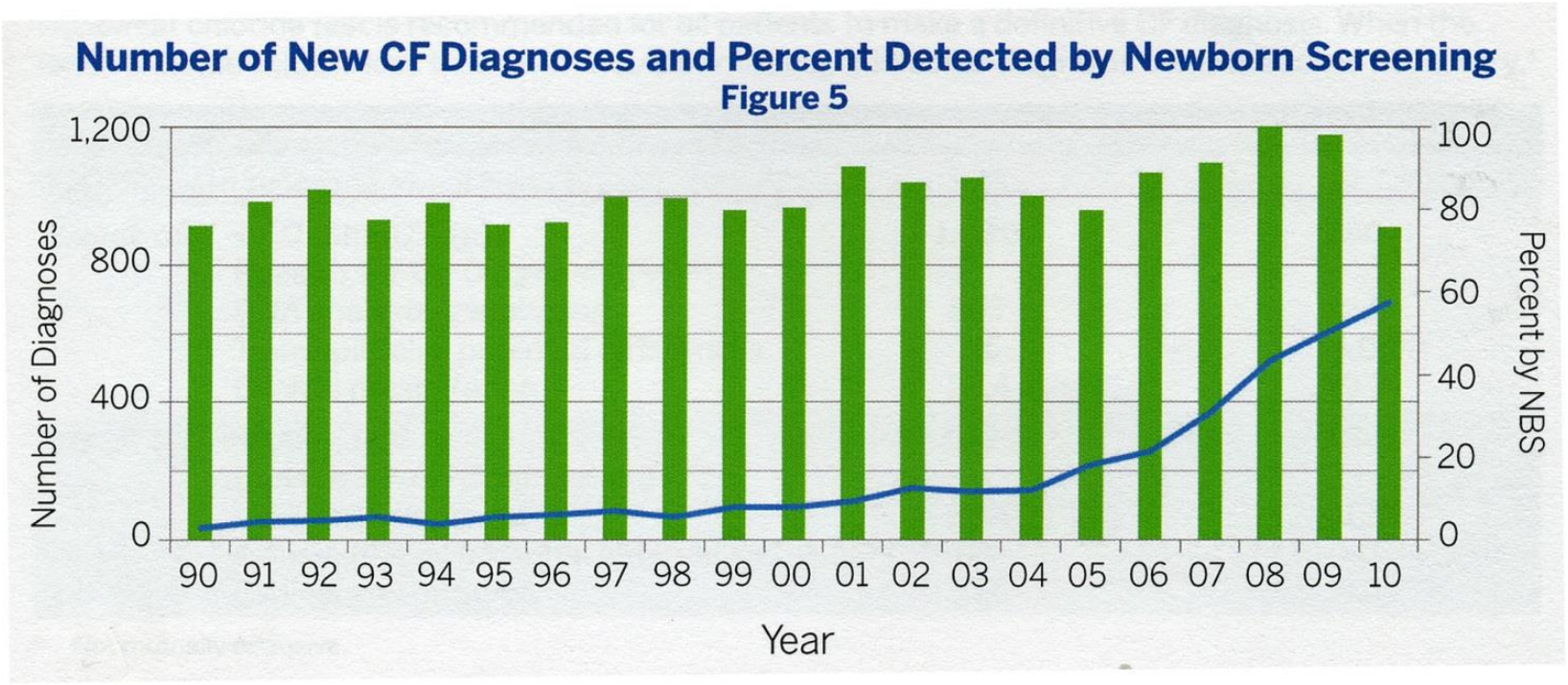
Distribution of IRT values: CF patients in relation to normal newborn population (MoM= multiple of the median for IRT value)



Massie, J et al. Arch Dis Child 2006; 91:222-225

Newborn Screening Is Now the Main Pathway to CF Diagnosis

(2010 National CF Registry Data)



Pathway to Diagnosis

Benefits of IRT/DNA Screening

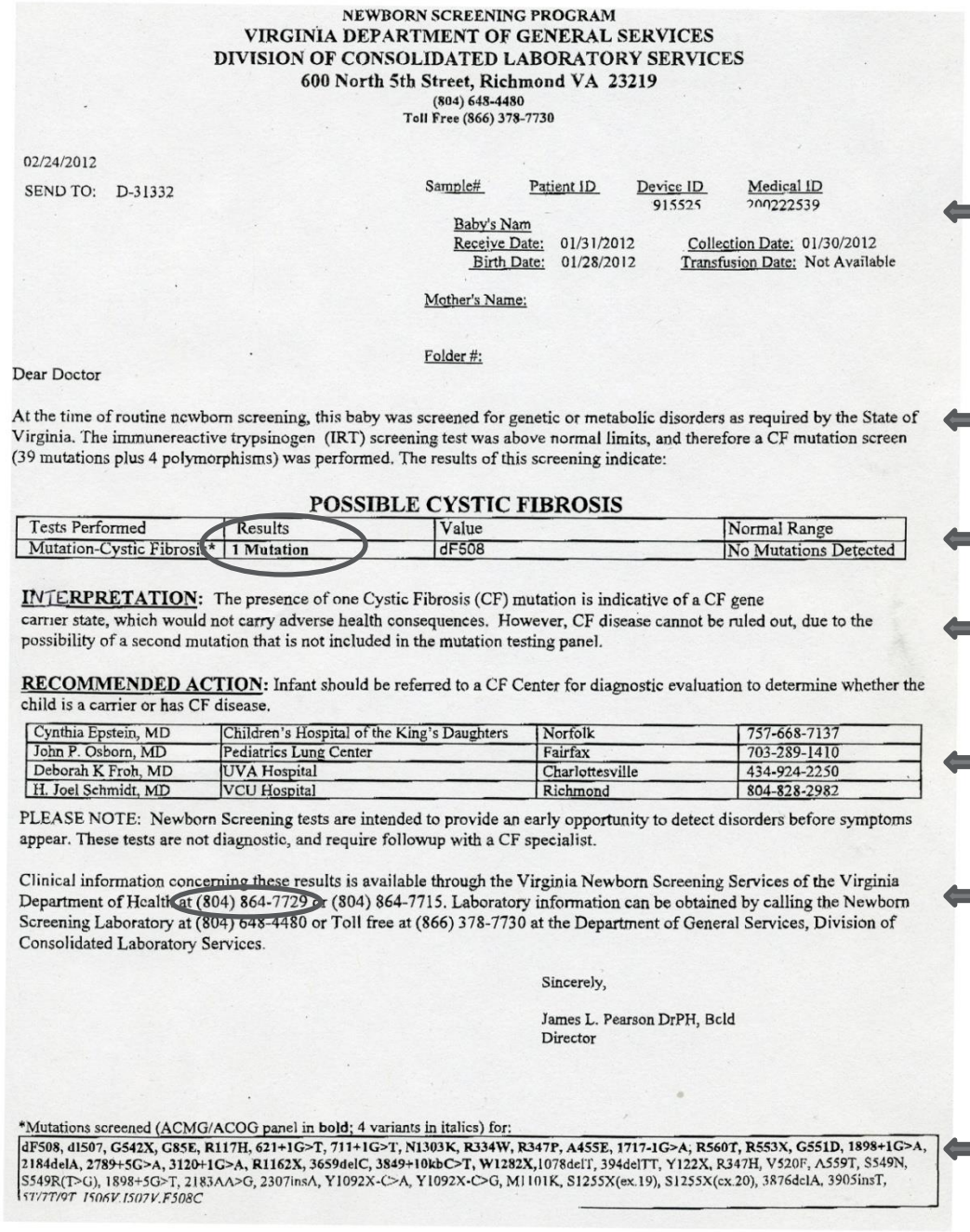
- Higher sensitivity due to lower initial IRT cut-off
- No need for second NBS sample
  DNA testing done on the initial sample if the IRT is elevated
- Shorter time to diagnosis and treatment
  2.3 weeks (IRT/DNA) to diagnosis vs. 4 weeks (IRT/IRT)
  5.9 weeks (IRT/DNA) to initial CF center visit vs. 7.7 weeks (IRT/IRT)
- Clarifies borderline sweat test results
  Up to 10% of infants with CF will have borderline sweat chloride results
  17% of those infants will have 2 mutations on more extensive mutation testing

4 Possible Results

"Normal Screen"	IRT value in the top 4% but <170 ng/ml, and No Mutations identified on screening using the mutation panel *"Screen normal"; no further action required*
"Low Risk of CF"	IRT >170 ng/ml, but No Mutations identified *No sweat test required: not a failed screen* *Likely to include infants with severe neonatal problems* ****Discuss with CF consultant if no obvious reason for high IRT*
"Possible CF"	IRT top 4%, and One Mutation identified *Needs sweat test (accredited CF Center) to confirm CF diagnosis (vs carrier)* *Needs genetic counseling*
"Probable CF"	IRT top 4%, and Two Mutations identified *Contact CF center without delay* *CF center will arrange sweat test plus clinical visit within a few days*

Anatomy of a CF-NBS Report: Tier 2 (DNA)

ANATOMY OF A VIRGINIA CF-NBS REPORT



NEWBORN SCREENING PROGRAM
VIRGINIA DEPARTMENT OF GENERAL SERVICES
DIVISION OF CONSOLIDATED LABORATORY SERVICES
600 North 5th Street, Richmond VA 23219
(804) 648-4480
Toll Free (866) 378-7730

02/24/2012

SEND TO: D-31332

Sample# Patient ID Device ID Medical ID
915525 200222539

Baby's Nam
Receive Date: 01/31/2012 Collection Date: 01/30/2012
Birth Date: 01/28/2012 Transfusion Date: Not Available

Mother's Name:

Folder #:

Dear Doctor

At the time of routine newborn screening, this baby was screened for genetic or metabolic disorders as required by the State of Virginia. The immunereactive trypsinogen (IRT) screening test was above normal limits, and therefore a CF mutation screen (39 mutations plus 4 polymorphisms) was performed. The results of this screening indicate:

POSSIBLE CYSTIC FIBROSIS

Tests Performed	Results	Value	Normal Range
Mutation-Cystic Fibrosis*	**1 Mutation**	dF508	No Mutations Detected

INTERPRETATION: The presence of one Cystic Fibrosis (CF) mutation is indicative of a CF gene carrier state, which would not carry adverse health consequences. However, CF disease cannot be ruled out, due to the possibility of a second mutation that is not included in the mutation testing panel.

RECOMMENDED ACTION: Infant should be referred to a CF Center for diagnostic evaluation to determine whether the child is a carrier or has CF disease.

Cynthia Epstein, MD	Children's Hospital of the King's Daughters	Norfolk	757-668-7137
John P. Osborn, MD	Pediatrics Lung Center	Fairfax	703-289-1410
Deborah K Froh, MD	UVA Hospital	Charlottesville	434-924-2250
H. Joel Schmidt, MD	VCU Hospital	Richmond	804-828-2982

PLEASE NOTE: Newborn Screening tests are intended to provide an early opportunity to detect disorders before symptoms appear. These tests are not diagnostic, and require followup with a CF specialist.

Clinical information concerning these results is available through the Virginia Newborn Screening Services of the Virginia Department of Health at (804) 864-7729 or (804) 864-7715. Laboratory information can be obtained by calling the Newborn Screening Laboratory at (804) 648-4480 or Toll free at (866) 378-7730 at the Department of General Services, Division of Consolidated Laboratory Services.

Sincerely,

James L. Pearson DrPH, Bcld
Director

*Mutations screened (ACMG/ACOG panel in **bold**; 4 variants in italics) for:

dF508, d1507, G542X, G85E, R117H, 621+1G>T, 711+1G>T, N1303K, R334W, R347P, A455E, 1717-1G>A, R560T, R553X, G551D, 1898+1G>A, 2184delA, 2789+5G>A, 3120+1G>A, R1162X, 3659delC, 3849+10kbC>T, W1282X,1078delT, 394delTT, Y122X, R347H, V520F, A559T, S549N, S549R(T>G), 1898+5G>T, 2183AA>G, 2307insA, Y1092X-C>A, Y1092X-C>G, M1101K, S1255X(ex.19), S1255X(cx.20), 3876delA, 3905insT, *I507/7T/9T I506V.I507V.F508C*

Sample identifiers

Reminder that IRT was elevated and thus CF mutation screen was done

Key results

Interpretation and recommendation

CF Center contact info

State NBS program contact information

List of mutations in test panel

Virginia: IRT/DNA Protocol Statistics

December 1, 2011 – March 9, 2012

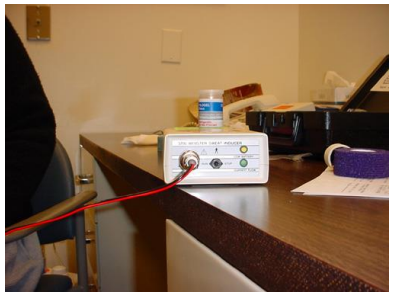
n=1,103 samples analyzed for CF mutations following IRT above cutoff

DNA Results	Virginia number/percent	Wisconsin percent*
0 Mutations	1,033 = 93.7%	93.9%
1 Mutation	66 = 6.0%	4.6%
2 Mutations	4 = 0.36%	0.35%

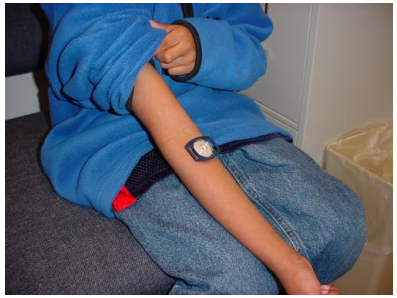
*Wells J, Rosenberg M, Hoffman G, Anstead M, Farrell PM. A decision-tree approach to cost comparison of newborn screening strategies for cystic fibrosis. Pediatr 2012; 129: e339-e347.

1. Stimulation of sweat production





2. Collection of sweat




3. Analysis of chloride concentration of sweat


Sweat Testing

Sweat Chloride Results for Infants

Interpretation	Age <6 months	Age > 6 months
Normal	< 30	< 40
Intermediate	30-59	40-59
Abnormal	≥ 60	≥ 60

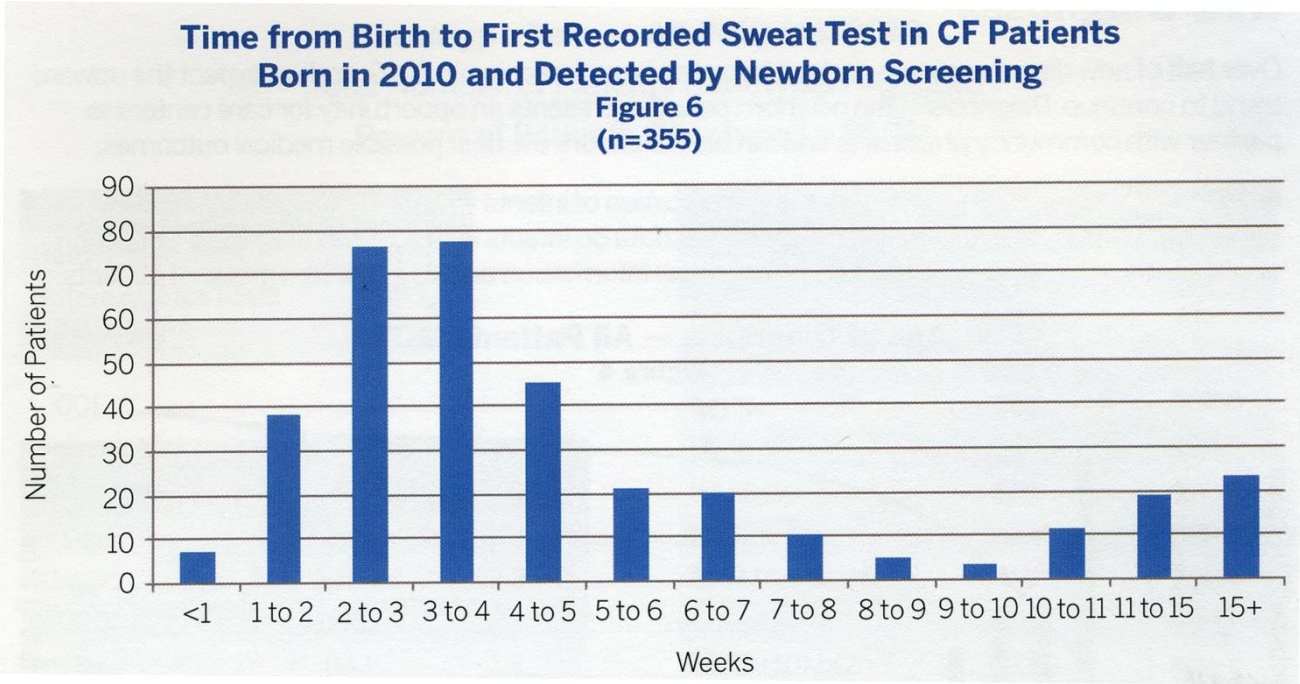
Babies with a positive NBS and intermediate sweat test value should be followed at a CF Center.

Tentative diagnosis for those infants with a positive NBS result and an intermediate sweat chloride result is "CRMS" or "CFTR-related metabolic syndrome". With serial sweat testing and clinical follow-up, these infants may ultimately be reclassified as either "normal" or as having CF.

Genetic Counseling

- If one or two CF mutations are found:
  - Explanation of "carrier" vs CF
  - Explanation of need for additional mutation testing (if indicated)
  - Genetic risk for CF within the family
  - Options regarding testing of family members
- Who should provide this information?
  Genetic counselor, CF provider, primary care physician
- Availability and accessibility may be problematic

Improvement Needed Nationally



An important goal of newborn screening is early diagnosis of CF and initiation of specialized CF care (pre-symptomatic, if possible)

Prompt Processing and Follow-up

Cystic Fibrosis Foundation
www.cff.org

National Human Genome Research Institute
www.genome.gov/10001213

The "Gene Reviews"
www.ncbi.nlm.nih.gov/books/NBK1250/

Virginia Newborn Screening Program website
www.vahealth.org/VNSP

Selected Websites

PEARLS of the Day

- IRT/DNA protocols offer better CF detection than IRT/IRT, **but newborn screening will still miss a small number of cases**. *Order sweat testing if there are symptoms concerning for CF.*
- Be sure to follow up NBS results promptly, even if they suggest that a carrier situation is most likely. Avoid delays in follow-up.
- Genetic counseling should be offered if **any** CF mutations are found.
- Sweat testing should preferably occur at a CF center.
- Call your state NBS program or CF center for advice when needed, and consult the cff.org website for additional info or parent-oriented materials.

References

Cavanagh et al, 2010. Long-term Evaluation of Genetic Counseling Following False-Positive Newborn Screen for Cystic Fibrosis. J Genet Couns, 19: 199-210. PubMed

Collins et al, 2012. Factors that influence parents' experiences with results disclosure after newborn screening identifies genetic carrier status for cystic fibrosis or sickle cell hemoglobinopathy. Patient Educ Couns, 90: 378-385. PubMed

Comeau et al, 2007. Guidelines for Implementation of Cystic Fibrosis Newborn Screening Programs: Cystic Fibrosis Foundation Workshop Report. Pediatrics, 119: e495-518. PubMed

Kloosterboer et al, 2009. Clarification of Laboratory and Clinical Variables That Influence Cystic Fibrosis Newborn Screening with Initial Analysis of Immunoreactive Trypsinogen. Pediatrics, 123: e338-346. PubMed

Rock et al, 2005. Newborn screening for cystic fibrosis in Wisconsin: Nine-year experience with routine trypsinogen/DNA testing. J Pediatr, 147: S73-S77. PubMed

Sanders et al, 2011. Comparing age of cystic fibrosis diagnosis and treatment initiation after newborn screening with two common strategies. Journal of Cystic Fibrosis, 11: 150-153. PubMed

Tluczek et al, 2011. Factors Associated with Parental Perception of Child Vulnerability 12 Months After Abnormal Newborn Screening Results. Research in Nursing & Health, 34: 389-400. PubMed

Tluczek et al, 2011. Psychosocial Consequences of False-Positive Newborn Screens for Cystic Fibrosis. Qual Health Res, 21: 174-186. PubMed

Wagener et al, 2012. Newborn screening for cystic fibrosis. Curr Opin Pediatr, 24: 329-335. PubMed

Wells et al, 2012. A Decision-Tree Approach to Cost Comparison of Newborn Screening Strategies for Cystic Fibrosis. Pediatrics, 129: e339-347. PubMed