Overview of CF and CFTR genotyping

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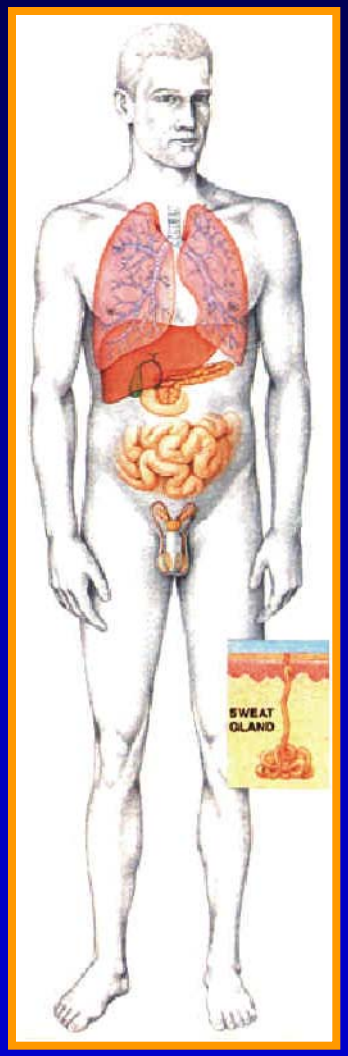
Colorado School of
PUBLIC HEALTH

Organ Dysfunction in CF

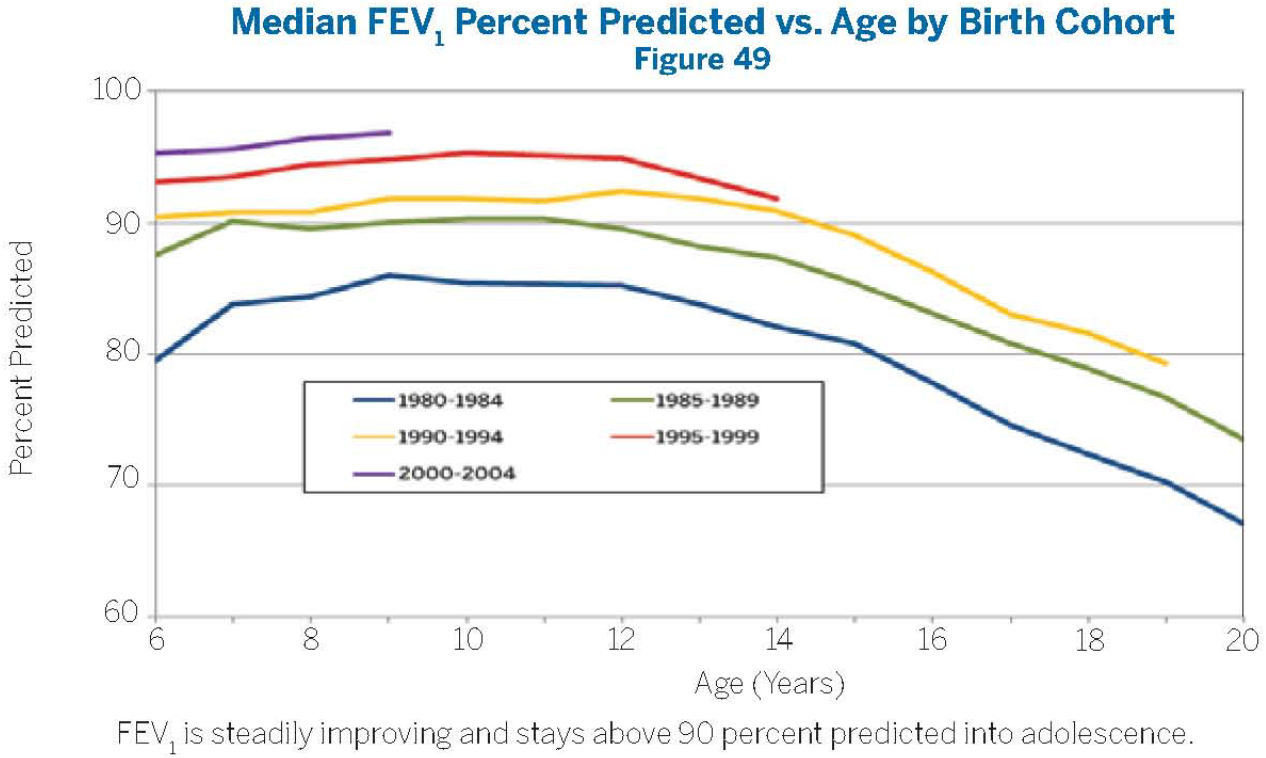


- *Sinuses* – Sinusitis, nasal polyps
- *Lung* – Endobronchitis, bronchiectasis
- *Pancreas* – Exocrine Insufficiency
  CF Related Diabetes
- *Intestine* – Meconium ileus
  Constipation/DIOS
- *Liver* – Focal sclerosis
- *Vas Deferens* – failure to develop
- *Sweat gland* – salt-losing dehydration

Adapted from Welsh and Smith, Sci Am, 1995

Cystic Fibrosis

- **Genetic condition – 1/3,500 births; 35,000 individuals in US**
- **Progressive lung disease**



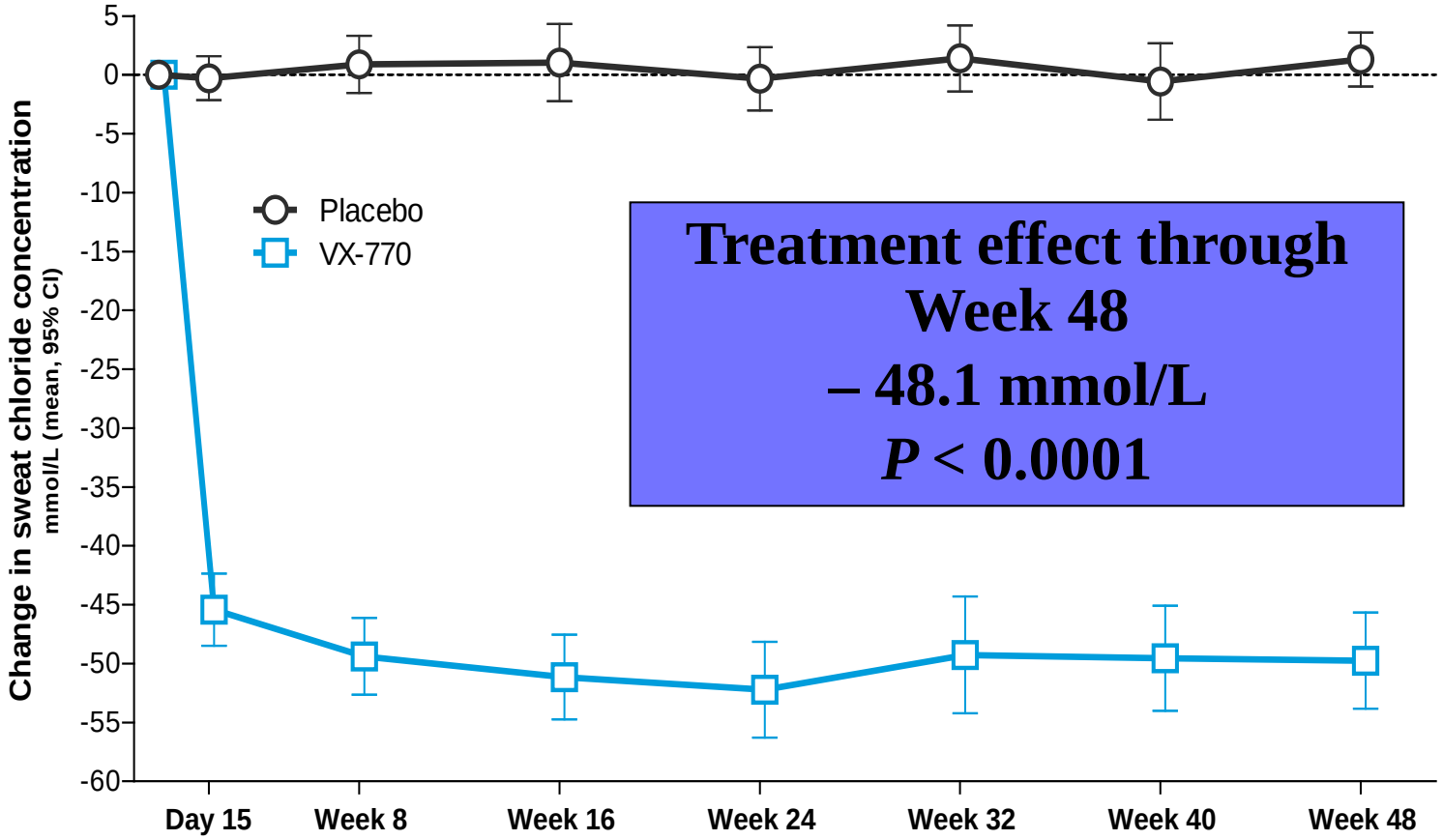
- **Median Predicted Survival:**
  - **37 years**
- **Median Age at Death:**
  - **26 years**

Patient Registry, Cystic Fibrosis Foundation, 2008, Bethesda MD, USA (N=c.25,000)

Hypothesis: Improving CFTR function will result in clinical benefit in patients with G551D

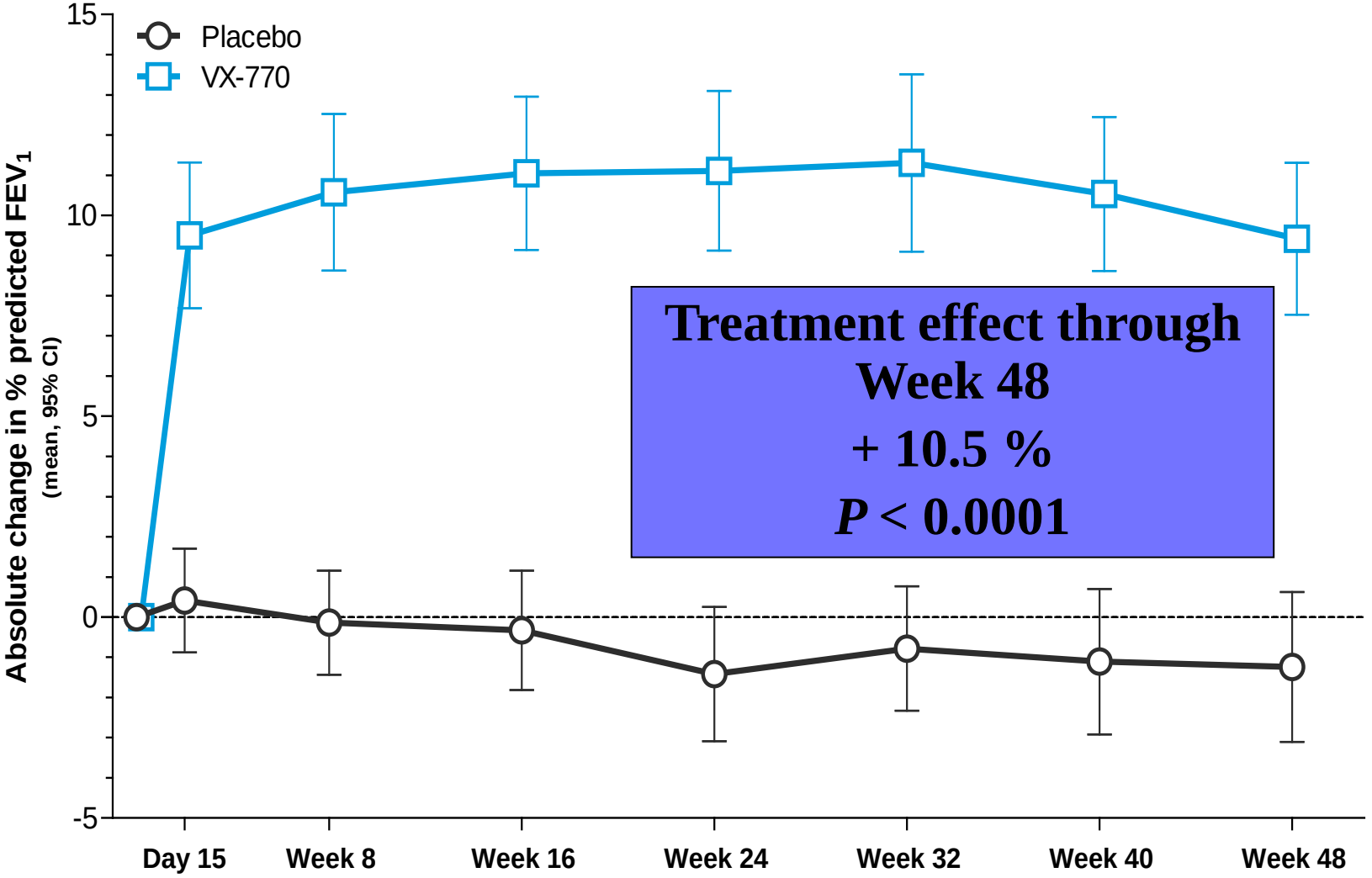
First suggested: (Accurso et al, NEJM, 2010, N=39)

Sweat Chloride



Phase 3 Trial (Ramsey et al, NEJM, 2011)

Lung Function Improves with VX-770



Phase 3 Trial (Ramsey et al, NEJM, 2011)

CDC

MMWR

Morbidity and Mortality Weekly Report

Recommendations and Reports | October 15, 2004 / Vol. 53 / No. RR-13

Newborn Screening for Cystic Fibrosis

Evaluation of Benefits and Risks and Recommendations for State Newborn Screening Programs

Image courtesy of Natus Medical Incorporated

INSIDE: Continuing Education Examination

DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION

"On the basis of a preponderance of evidence, the health benefits to children with CF outweigh the risk of harm and justify screening for CF."

"Newborn screening systems should ensure parental and provider education…"

Supplement to

THE JOURNAL OF PEDIATRICS

CYSTIC FIBROSIS NEWBORN SCREENING:
EVIDENCE FOR BENEFIT AND CURRENT EXPERIENCE

PROCEEDINGS FROM A WORKSHOP
CO-SPONSORED BY THE
CENTERS FOR DISEASE CONTROL AND PREVENTION
AND THE CYSTIC FIBROSIS FOUNDATION, ATLANTA, GEORGIA,
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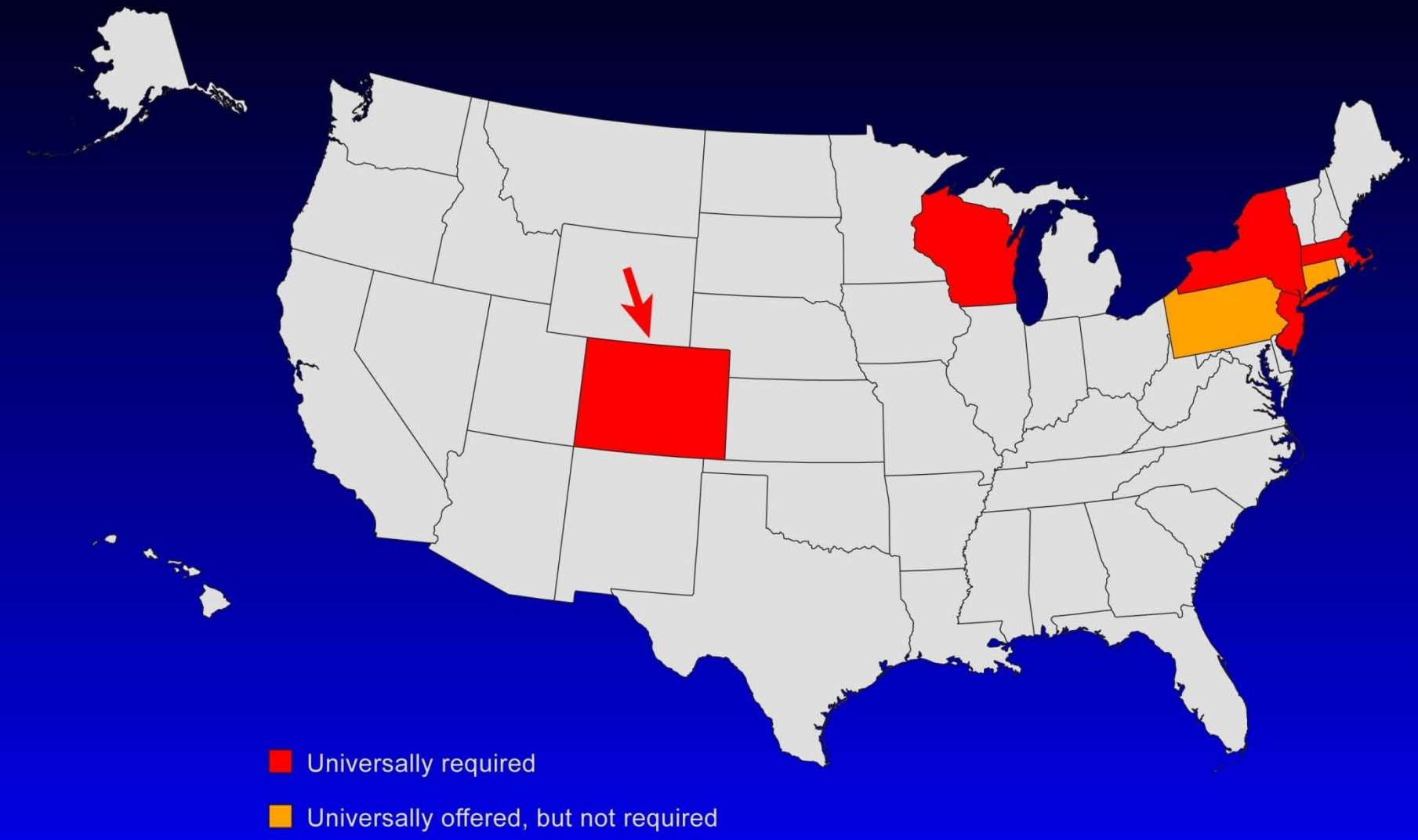
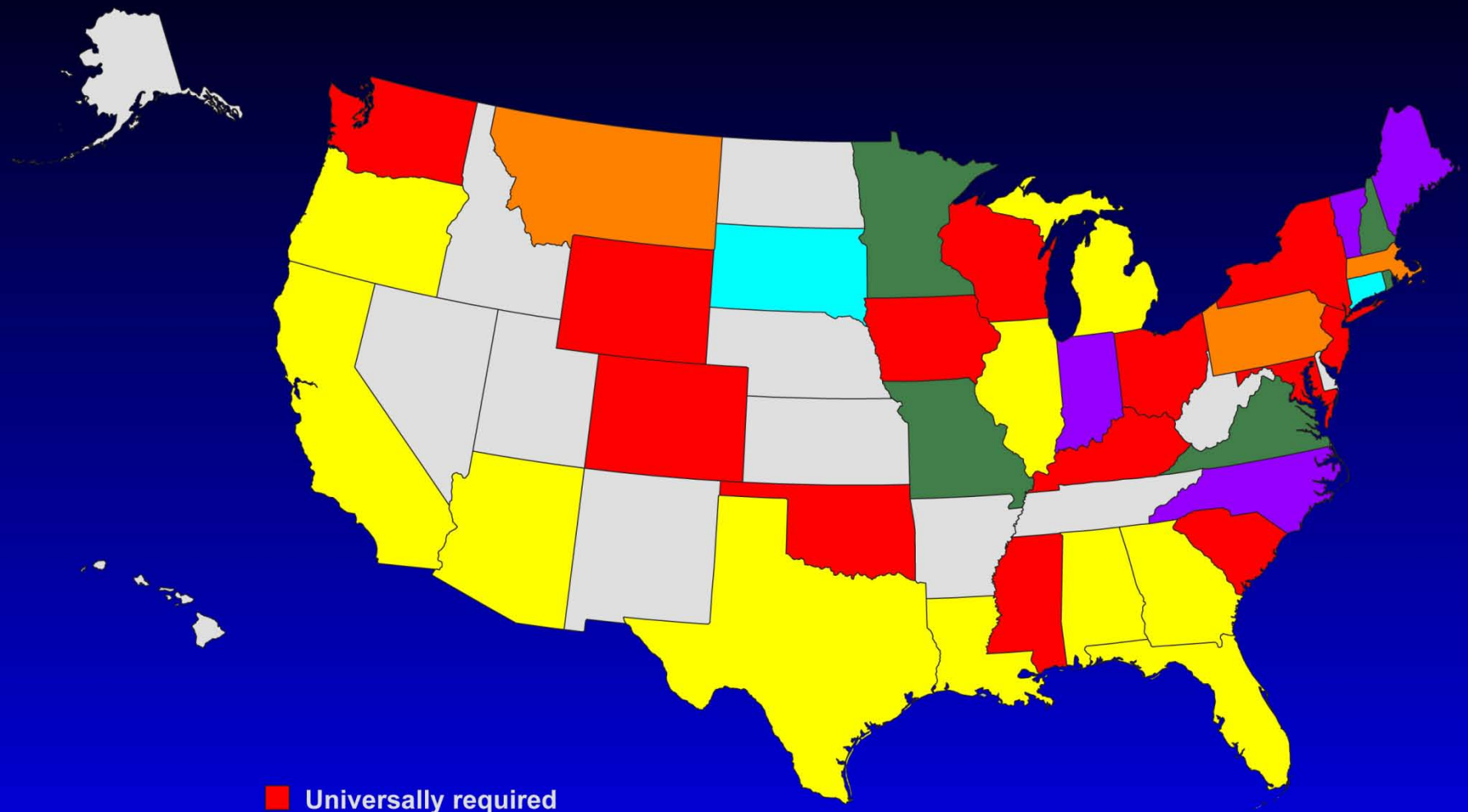
Status of CF NBS in 2004



Slide courtesy P. Farrell

Current Status of CF NBS (2006)



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Slide courtesy P. Farrell

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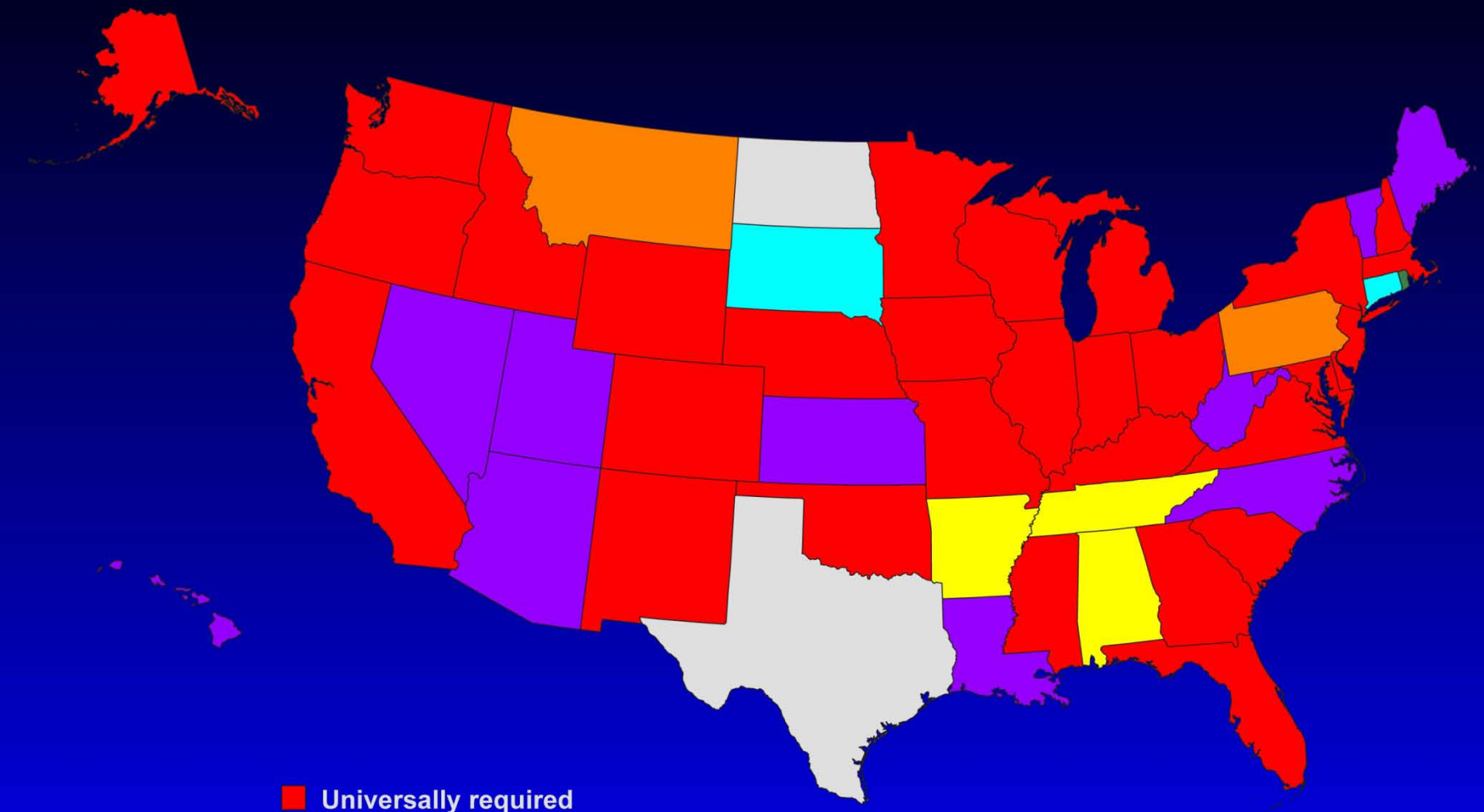

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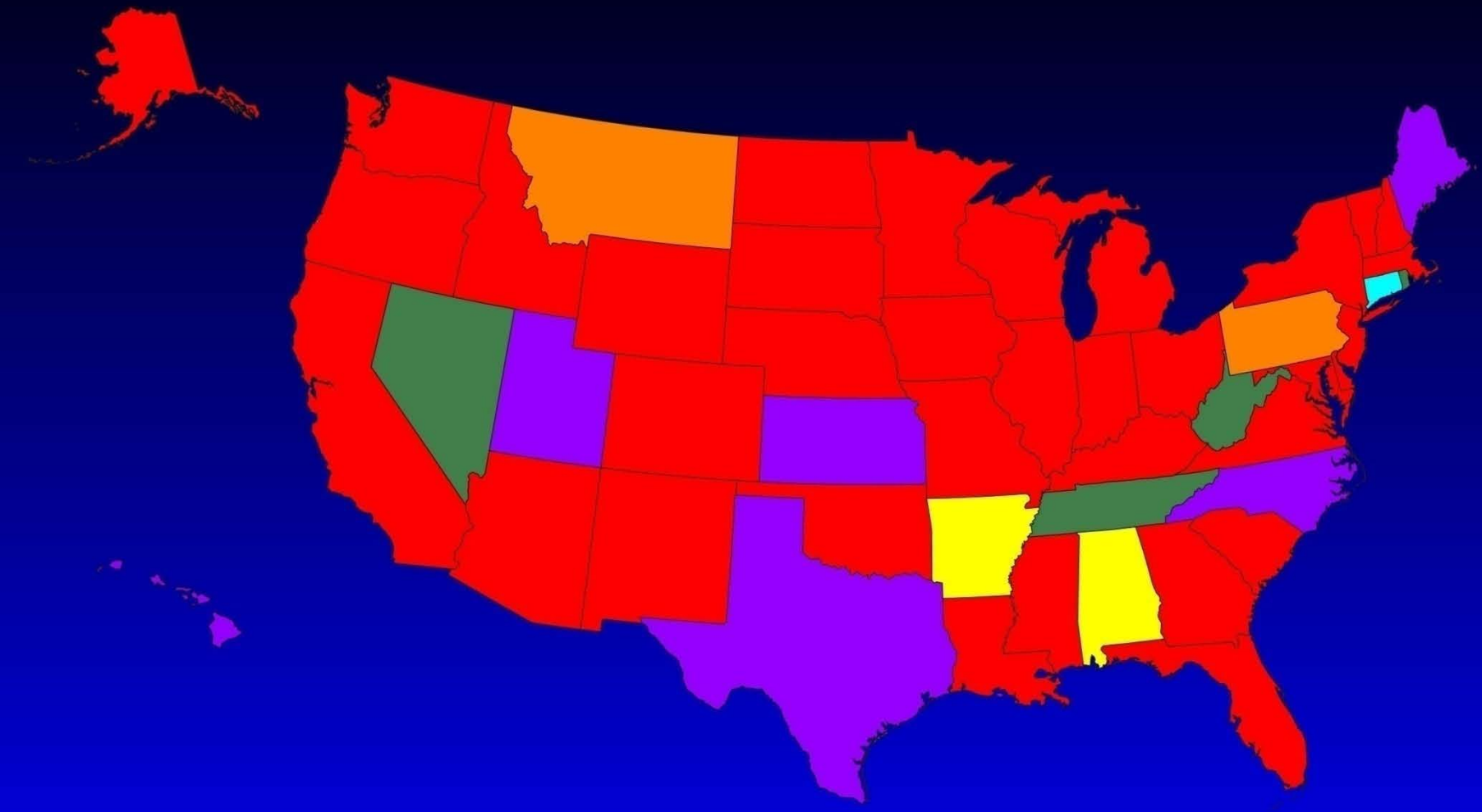
Current Status of CF NBS (2008)



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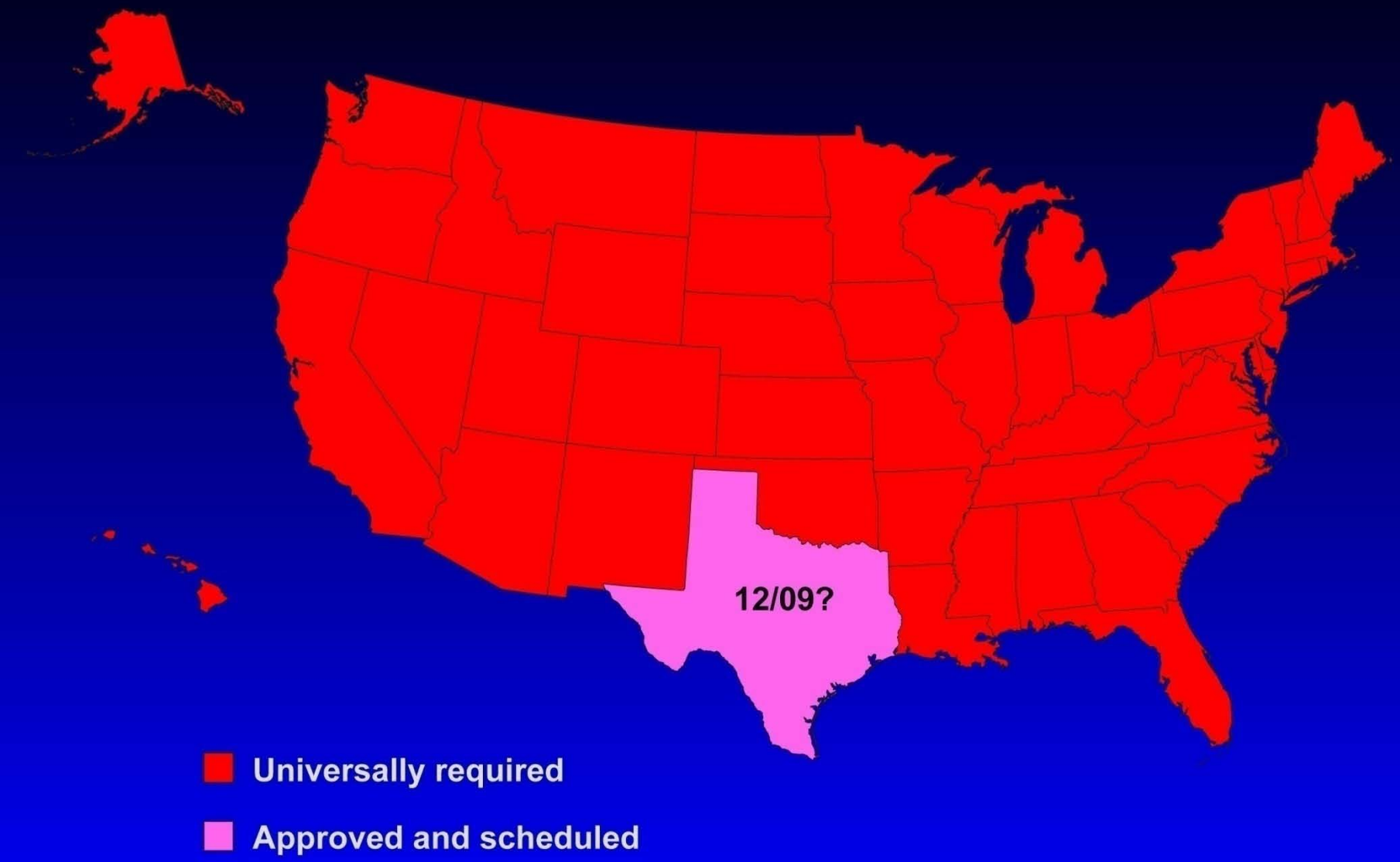
Current Status of CF NBS (2009)



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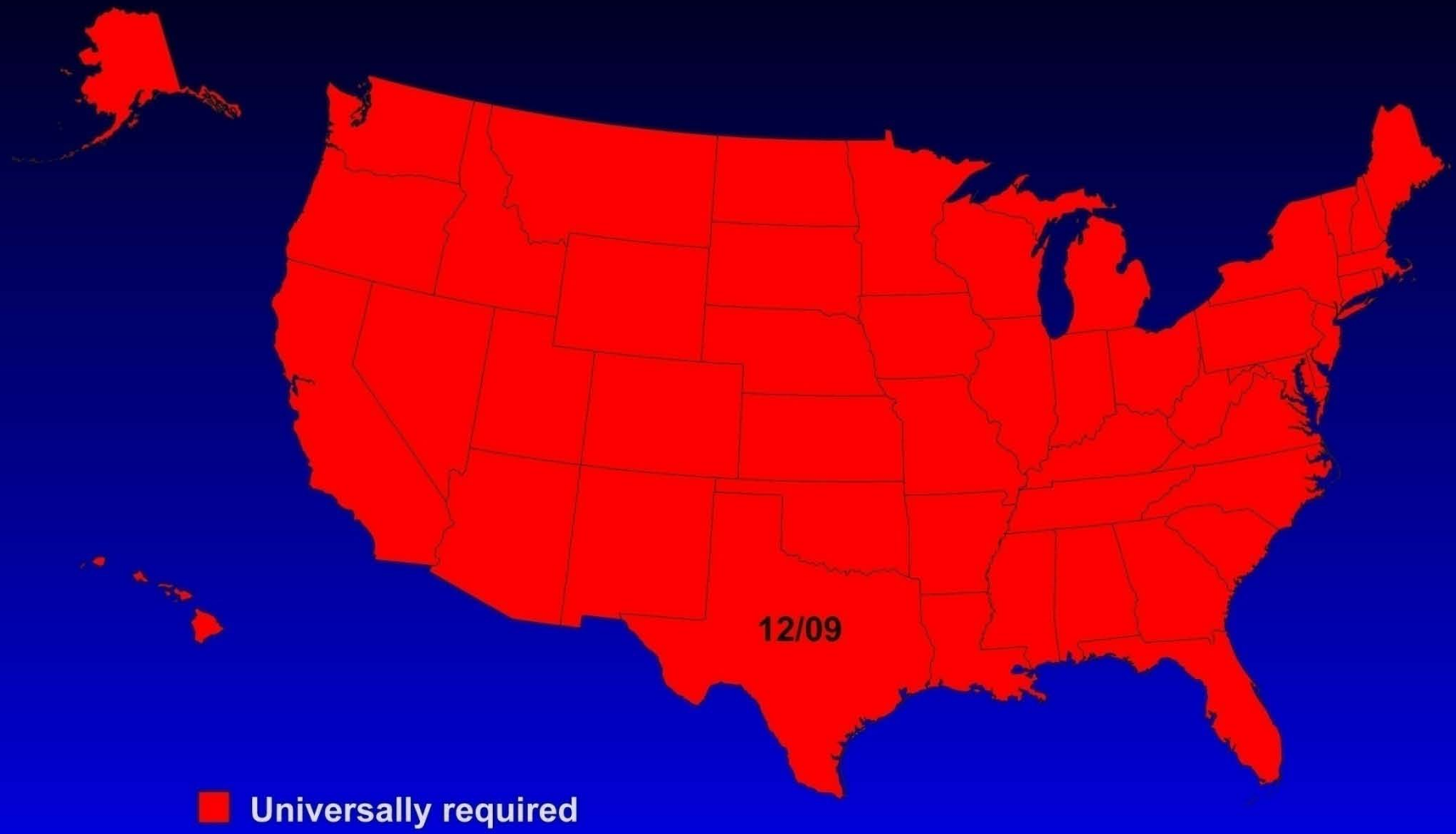
Current Status of CF NBS (2010)



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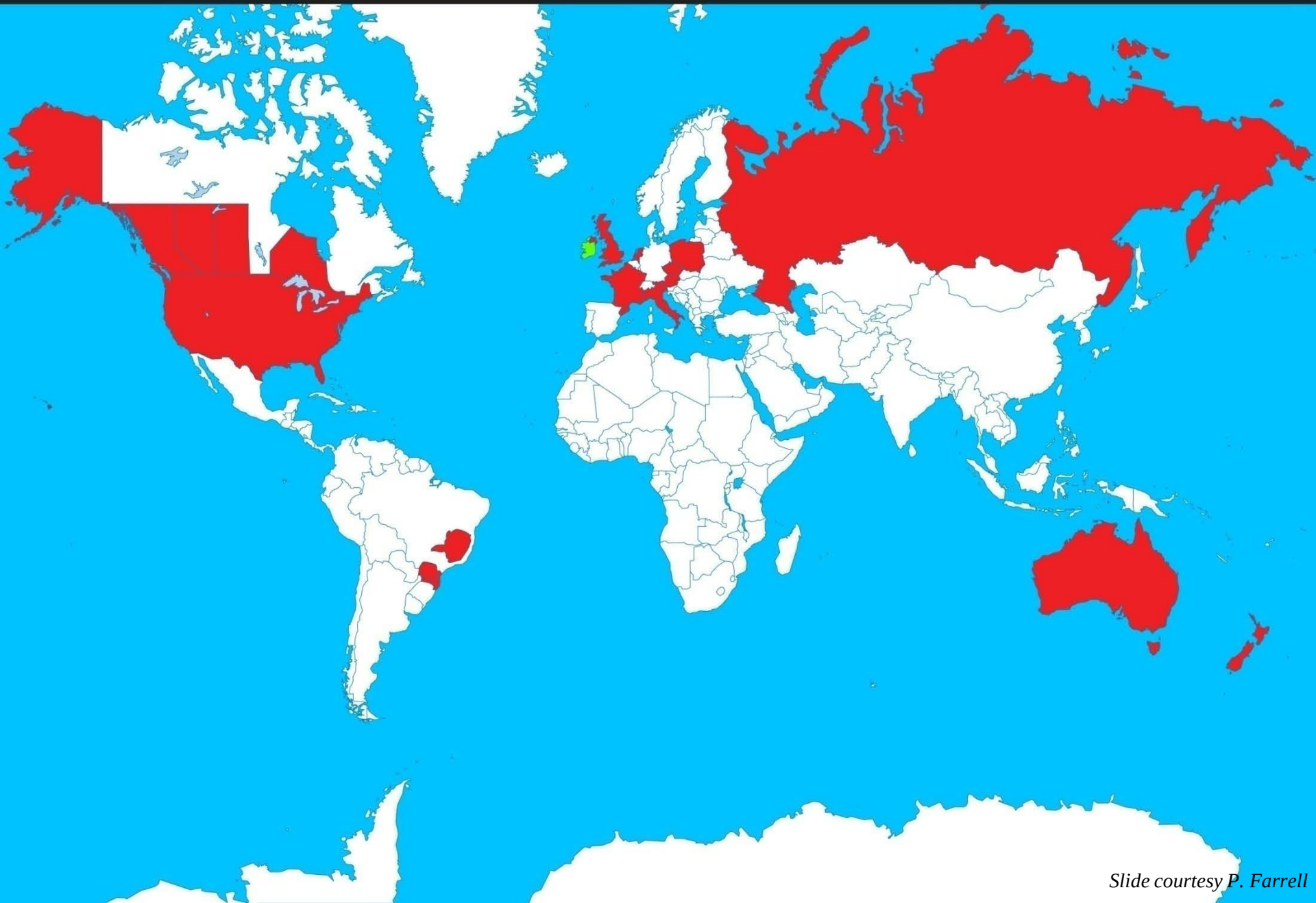
Global Distribution of CF Newborn Screening in 2010

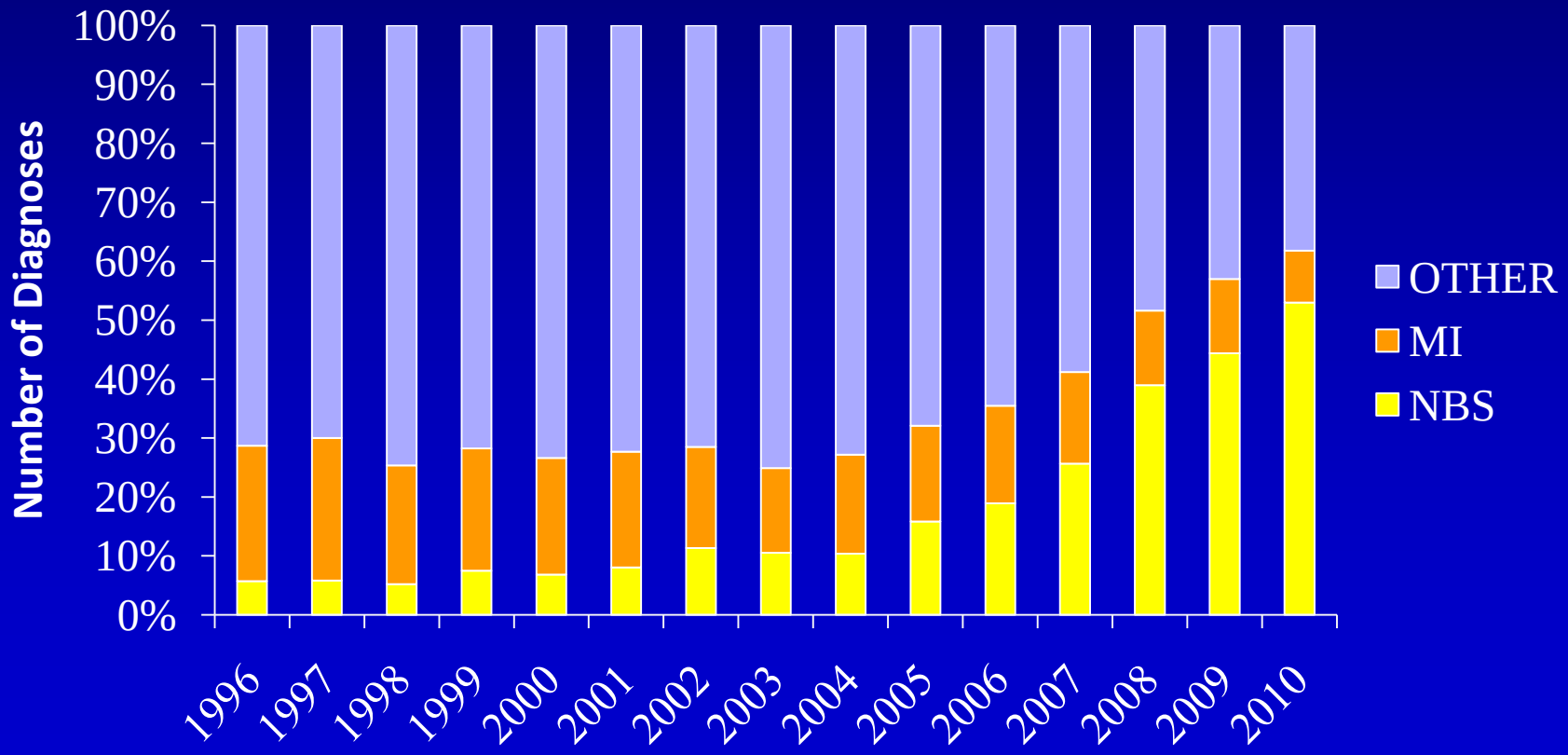


Slide courtesy P. Farrell

By 2010, newborn screening was the most common diagnostic indication

U.S. CF Foundation Registry

All new diagnoses reported to CFF in each year

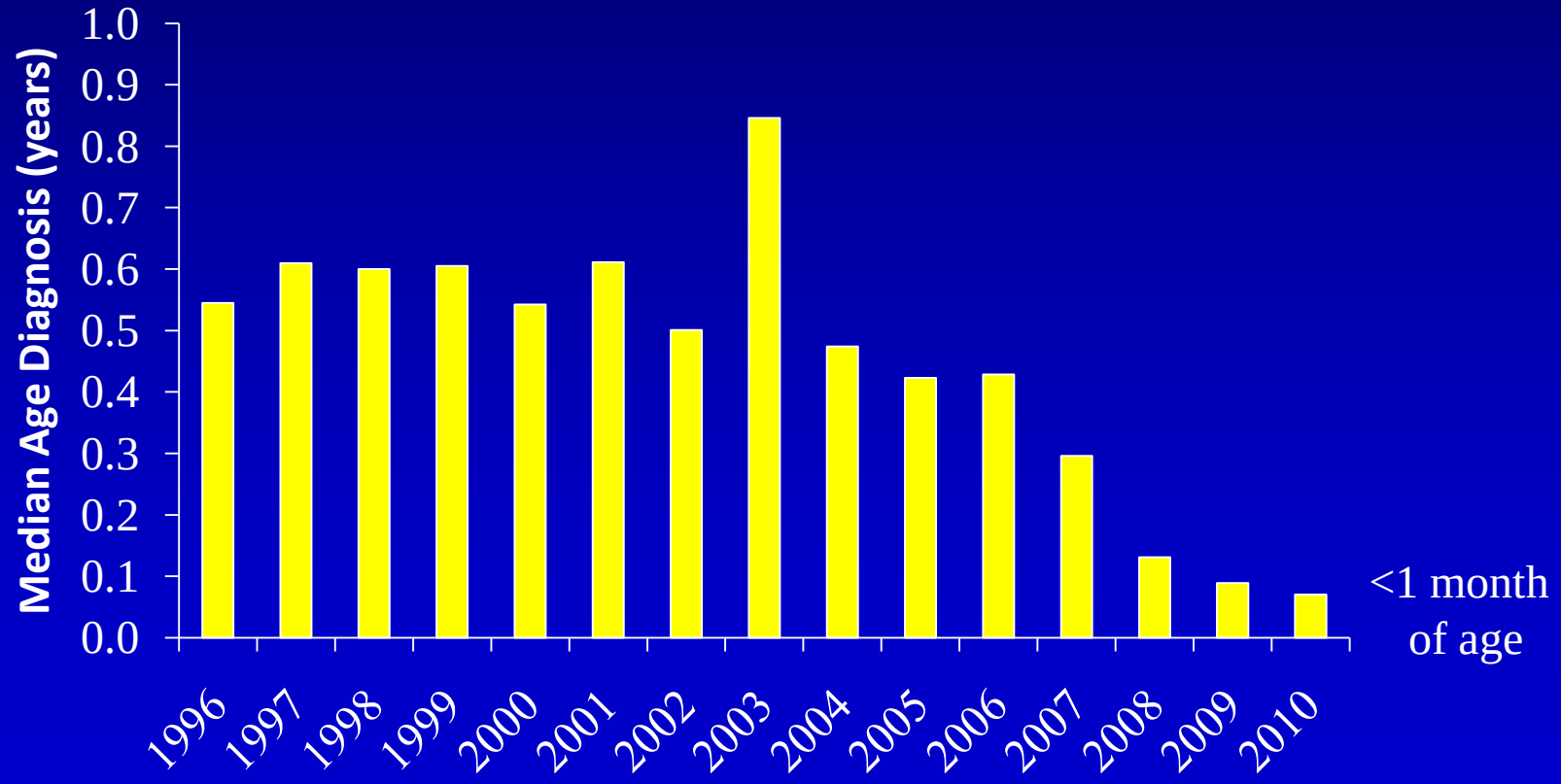


Presented at NACFC, November 2011, Anaheim

Age of diagnosis has decreased with newborn screening

U.S. CF Foundation Registry

All new diagnoses reported to CFF in each year



Presented at NACFC, November 2011, Anaheim

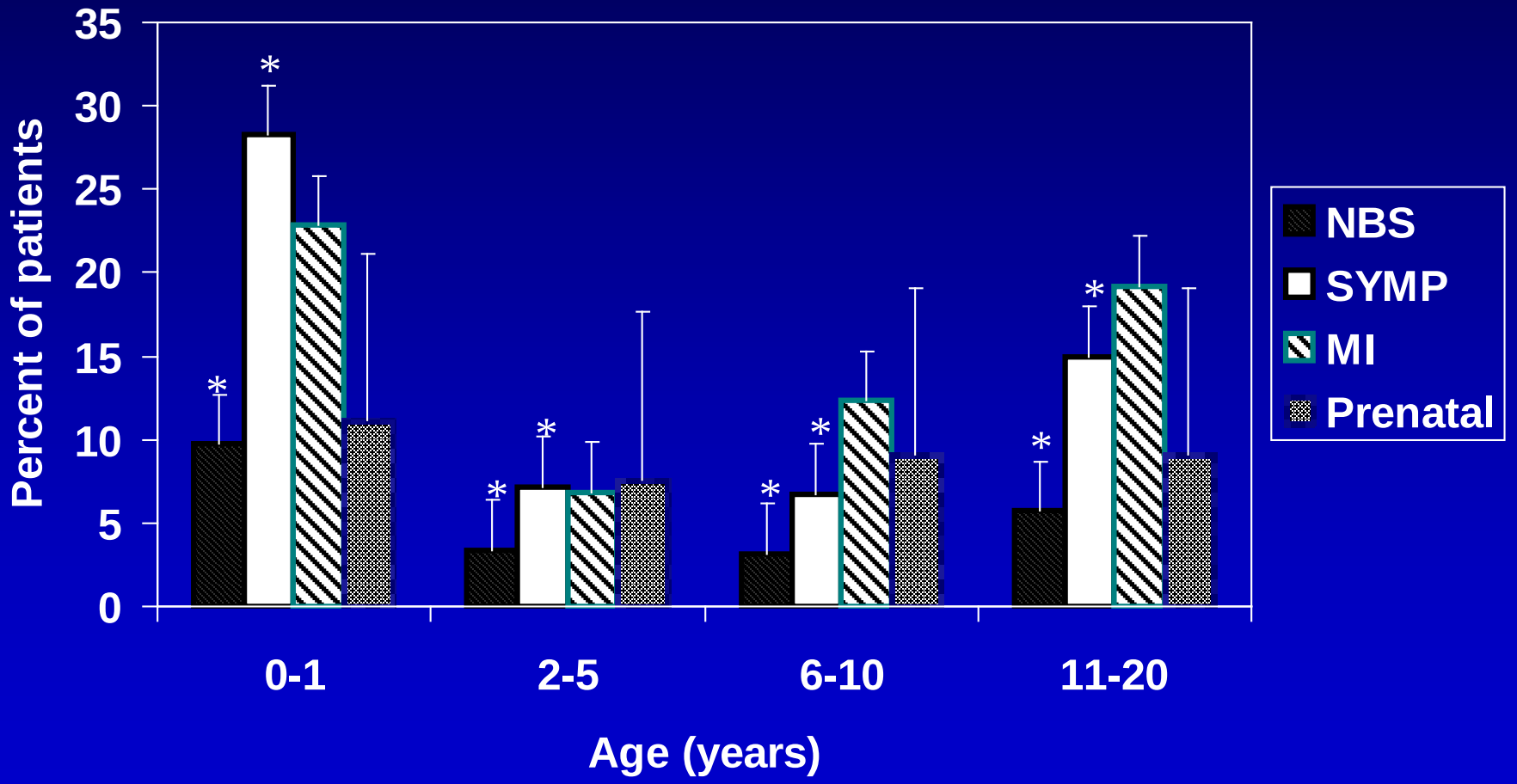
Complications in US

- U.S. CF Foundation Patient Registry, 2000-2002
- Comparison of
  - Newborn Screening (NBS)
  - Symptomatic Diagnosis (SYMP)
  - Meconium Ileus (MI)
  - Prenatal
- Weight for age
- Height for age
- Hospitalizations
- Pseudomonas aeruginosa infections

Accurso, Sontag, Wagener, J Pediatr 2005;147:S37-S41)

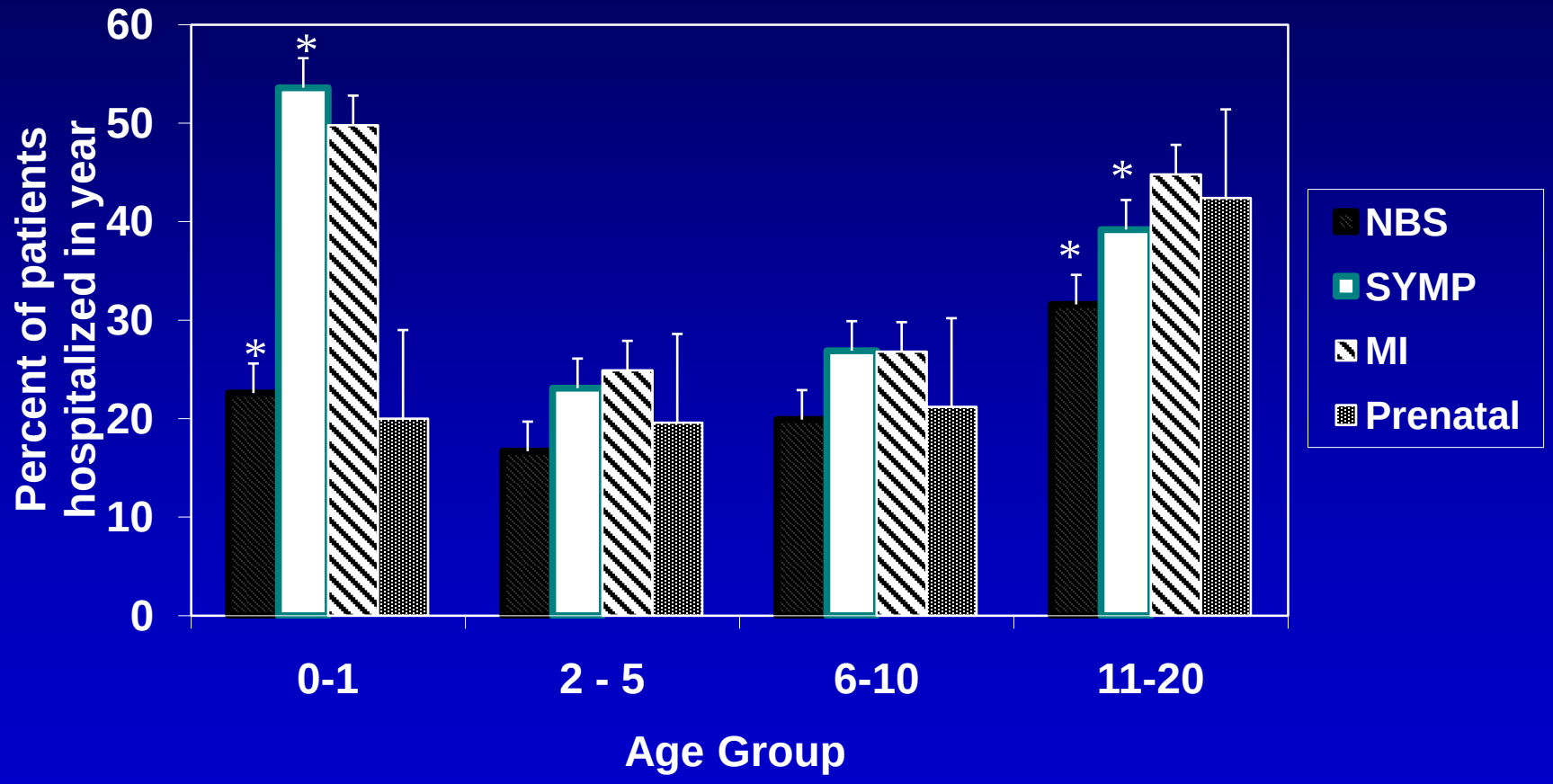
Newborn screened infants were less likely to be malnourished

(weight for age < 3rd percentile)



Accurso, Sontag, Wagener, J Pediatr 2005;147:S37-S41)

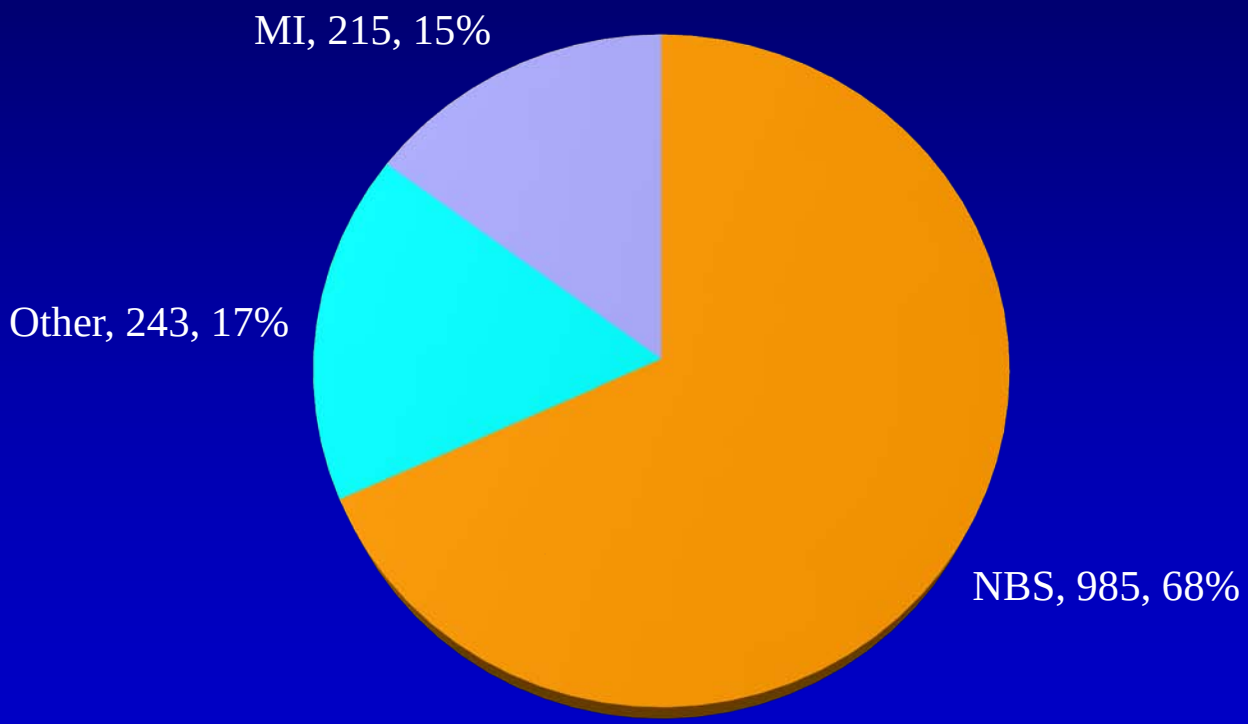
Children with CF who were newborn screened as infants fewer hospitalizations



Accurso, Sontag, Wagener, J Pediatr 2005;147:S37-S41)

Most infants under 2 years in 2010 were diagnosed early

U.S. CF Foundation Registry

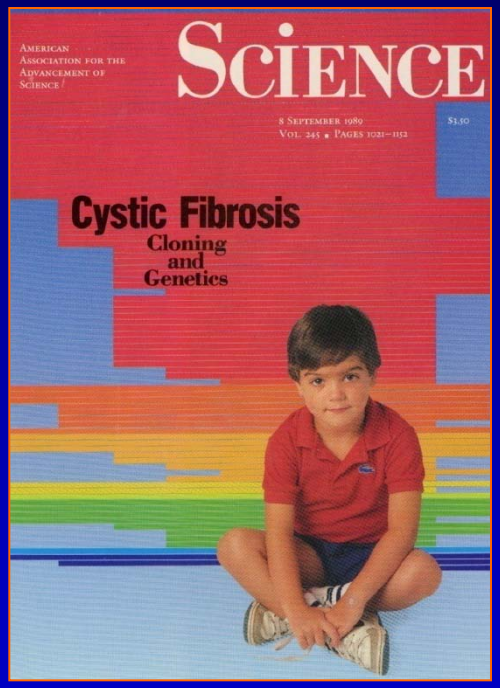


- 83% of children < 2 years by the end of 2010 were identified by NBS or MI
- The oldest baby in Texas identified under newborn screening was <1 in 2010 (~400,000 births/year, 60 babies with CF/year)

IRT/IRT

- Newborns receive 2 newborn screen tests
  - 1st before hospital discharge
  - 2nd at 2 week well baby check (mandated or extra sample collected)
- IRT is tested on both newborn screen blood spots
- If both IRTs are elevated, child is recalled for a sweat test (e.g. cutoffs at 100ng/ml and 70ng/ml)
- No genetic testing is performed – no carriers are identified

Introduction of mutation analysis to CF NBS



(Riordan et al, Science, 1989)

- CF Mutation identified in 1989
- Wisconsin NBS program: 1991-92 introduced F508 (Gregg at al Am J Hum Genet 1993)
- Massachusetts: Multiplex CFTR Mutation Testing – 1999-2003 (Comeau at al Pediatrics)

IRT/DNA

- Newborns receive 1 newborn screen tests
- IRT is tested on dried blood spot
- If IRTs is elevated, same sample is tested for CFTR mutations.
- If 1 or more CFTR mutations are identified child is recalled for a sweat test
  - 2 mutations – presumptive positive (sweat test)
  - 1 mutation – possible CF (sweat test)

Comparisons of Different Screens

IRT/IRT		IRT/DNA
Tend to be >99th %	IRT Cutoffs	Tend to be 96-98 %
Must wait for 2nd test	Timing	Earlier Diagnosis
No genetic info	Genetic Results	Genetic Counseling Required

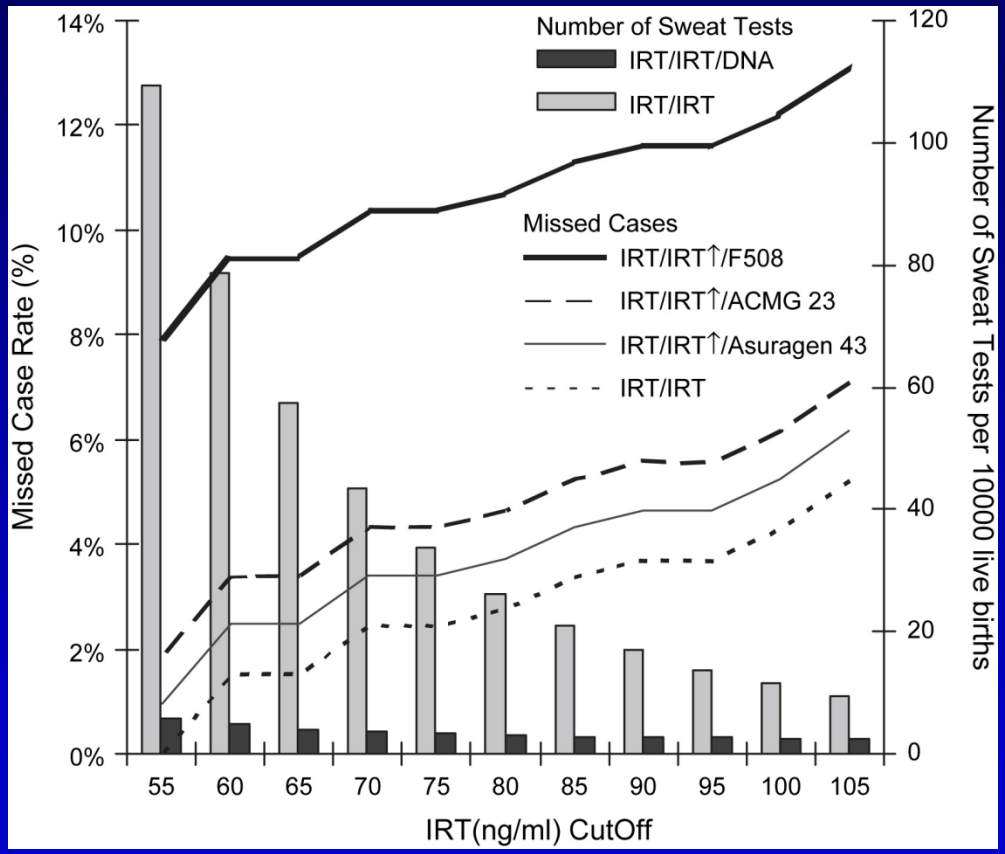
IRT/IRT$_{1\uparrow}$/DNA

- Decrease 1st screen cutoff
  - 105ng/ml (99.7 %ile) to 97th %ile (~55ng/ml)
- **Link** 1st and 2nd screen specimens for each baby
- Test 2nd screen ONLY if first screen > 97%ile
- Mutation analysis if BOTH first and second screen results > 97%

IRT/DNA-EGA

- Newborns receive 1 newborn screen tests
- IRT is tested on dried blood spots
- If IRTs is elevated, same sample is tested for CFTR mutations.
  - If 2 CFTR mutations are identified child is recalled for a sweat test, presumptive positive
  - If 1 CFTR mutation is identified same blood spot tested by expanded genetic analysis methods
    - If additional mutation(s) identified – sweat test
    - If no additional mutation identified – genetic counseling
- Fewer babies recalled for sweat tests

IRT/IRT has the highest sensitivity for the same cutoffs



However the positive predictive value is poor (many more sweat tests)

Sontag et al, J Peds 2009

Goals for NBS Tests in CF

- Minimize false negatives (Sensitivity)
- Balance the number of false positives (PPV)
- Provide a more specific diagnosis, i.e. DNA
- *Minimize the need for genetic counseling for detection of carriers*
- Reduce parental stress
  - Reduce the time to a diagnosis
  - Reduce the number of children/parents recalled for testing
- Reduce costs of screening and follow-up

Advantages to adding DNA testing to CF NBS

- Offers a more specific result in many cases
  - >60% of CF cases had 2 mutations.
- Can provide additional genetic information
  - Allow genetic counseling of parents of carriers

Challenges to adding DNA testing to CF NBS

- Clinicians ‘trust’ DNA
  - Need to educate clinicians that mistakes can happen in all tests
- Identification of carriers requires counseling
- May miss individuals with rare mutations (especially challenging in Hispanic populations in CF)

Selection of CFTR mutations

- Only mutations known to cause CF should be included in a panel
- 23-mutation ACMG
  - High degree of sensitivity
  - All mutations known to cause disease (special case R117H*)
- Additional mutations added when needed for population coverage for regional differences

CLSI. *Newborn Screening for Cystic Fibrosis; Approved Guidelin*. CLSI document I/LA35-A. Wayne PA: Clinical and Laboratory Standards Institute, 2011

Allele Frequencies of CFTR Mutations From the ACMG-23 Panel Reported in Cohorts Detected Through CF NBS

	CA* (23)	MA* (24)	NY* (24)	CO* (25)	WI* (26)
	N=70	N=112	N=108	N=317	N=21
F508	75.3	67.9	57.4	71.3	66.7
G542X	6.2	1.3	3.2	3.8	
G551D	3.7	3.1	1.4	1.4	
W1282X	3.7	1.8	0.9	1.1	2.4
621+1G>T	2.5	0.4	0.5	1.6	
R553X	2.5	0.4	0.9	1.8	
3120+1G>A	1.2		0.5		2.4
I507del	1.2		0.5	0.7	
G85E	1.2	1.8	0.9		
R1162X	1.2		0.5		
N1303K	1.2	2.2	0.5	1.1	
2789+5G-A		0.4	3.2		2.4
3849+10kbC>T	3.7	0.9	0.5		2.4
R334W	2.5		0.5		
R117H	†	4.0	0.9		‡
R347P			0.5		2.4

* CA = California; MA = Massachusetts; NY = New York; CO = Colorado; WI = Wisconsin.

† Detection of this allele trans to a disease-causing mutation was excluded from percentages reported by these authors, but would have been > 1%.

‡ Not tested in this mutation panel.

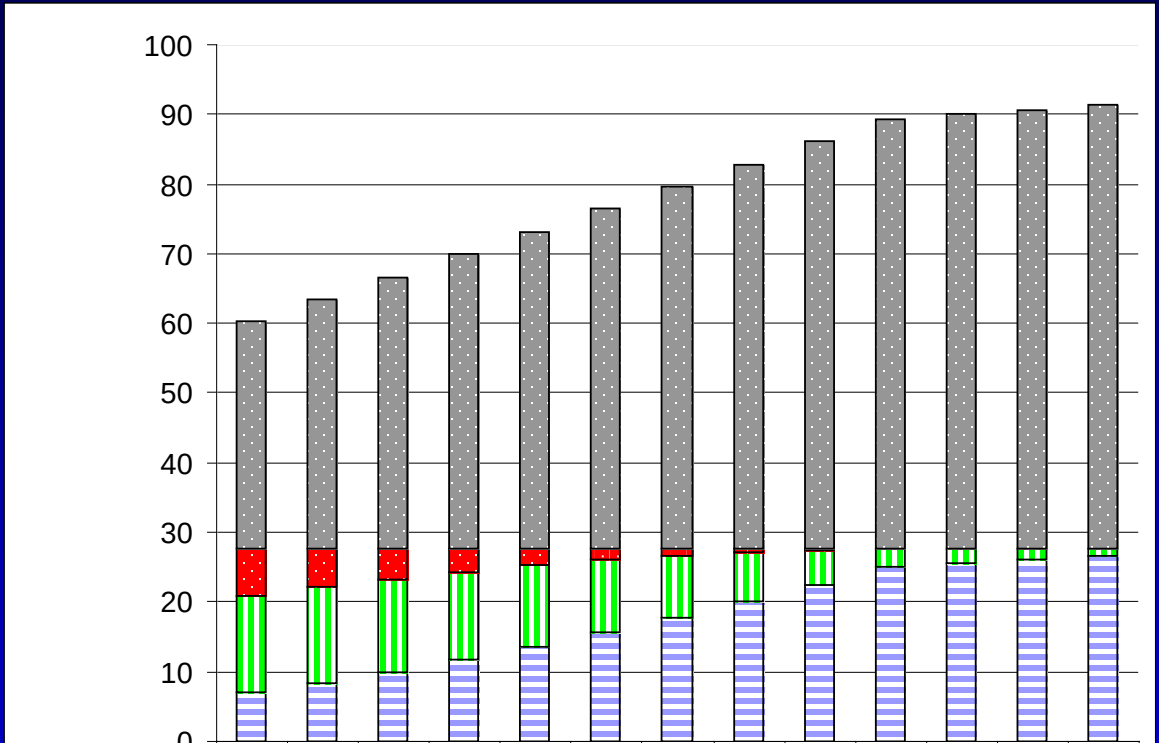
CLSI. *Newborn Screening for Cystic Fibrosis; Approved Guidelin*. CLSI document I/LA35-A. Wayne PA: Clinical and Laboratory Standards Institute, 2011

Balance of sensitivity/PPV

- Sensitivity: as long as one mutation from an affected patient is on panel, infant will be referred for sweat testing
- PPV: With the inclusion of too many mutations, more carriers will be called back for sweat testing

Detection of CF Cases and Carriers at Different Levels of Mutation Panel Sensitivity

Theoretical Population of 1000 Newborns With High IRT Referred for DNA Testing



	50	55	60	65	70	75	80	85	90	95	96	97	98
Carriers	32	36	39	42	45	49	52	55	58	62	62	63	64
0 Mutations	7	6	4.4	3.4	2.5	1.7	1.1	0.6	0.3	0.1	0.0	0.0	0.0
1 Mutation	14	14	13	13	12	10	9	7	5	3	2	2	1
2 Mutations	7	8	10	12	14	16	18	20	23	25	26	26	27

CLSI. *Newborn Screening for Cystic Fibrosis; Approved Guidelin.* CLSI document I/LA35-A. Wayne PA: Clinical and Laboratory Standards Institute, 2011

Methods used

- Most state labs that are doing multiple CF mutation detection are using:
  - Luminex based assay (all FDA approved)
  - Hologic Inplex assay (ACMG23 FDA approved)

  - ACMG 23
  - ACMG 23 plus additional mutations.

Reporting of results IRT/DNA

Test	Result	Value to Report	Action Required
IRT – no second tier	Normal	IRT level	CF screen normal
Mutation analysis	No mutations	IRT level No mutations detected	CF screen normal
Mutation analysis	One mutation	IRT level and mutation	Sweat chloride testing
Mutation analysis	Two mutations	IRT level and mutations	Call PCP Sweat chloride testing

Reporting of results IRT/IRT/DNA

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