CLSI Guidelines for Cystic Fibrosis Newborn Screening and Challenges with Using IRT as a Biomarker

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[on behalf of the CLSI Subcommittee & the IRT Workshop (23-24 May 11)organizers/participants]

Newborn Screening for Cystic Fibrosis

Historical Perspective: The Beginning in Auckland, NZ

Jeannette Crossley

1979: IRT* discovery in NZ-- "the shot heard around the world" for CF NBS

(Crossley JR, Elliott RB, Smith PA, Lancet 1:472,1979)

***Immunoreactive trypsinogen**

Result: Potential of IRT recognized (retrospectively)

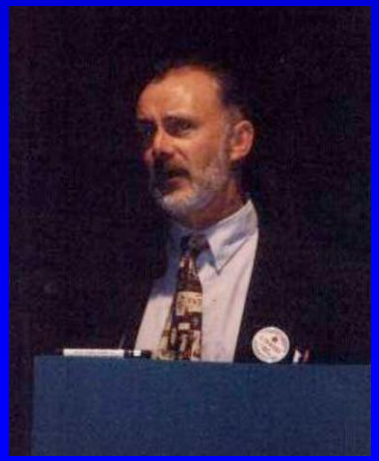
Historical Perspective: International Extensions

1979: Australia proceeds "full speed ahead" thanks to NSW/Wilcken (J Pediatr 102:383,1983)

Bridget Wilcken

1980: France (Normandy) initiates IRT/IRT for screening linked to more organized care (Arch Fr Pediatr 40:295, 1983)

Georges Travert



Result: Validity of IRT demonstrated prospectively

Historical Perspective: International Extensions with QI

1980's: In England, efforts were devoted to define more clearly those intrinsic (analytical) and extrinsic (pathophysiological) variables which were likely to be important in screening outcomes.

- Heeley AF, Heeley ME, et al. Screening for cystic fibrosis by dried blood spot trypsin assay. Arch Dis Child 1982; 57:18-21

Mary and Anthony Heeley

Result: IRT analysis clarified and improved

Historical Perspective: Increased Research

1982: Colorado initiates IRT/IRT as a clinical tool linked to research (NEJM 325:769, 1991)

1983: CFF Task Force paper published recommending more research– 7 issues (Pediatrics 72:741, 1983)

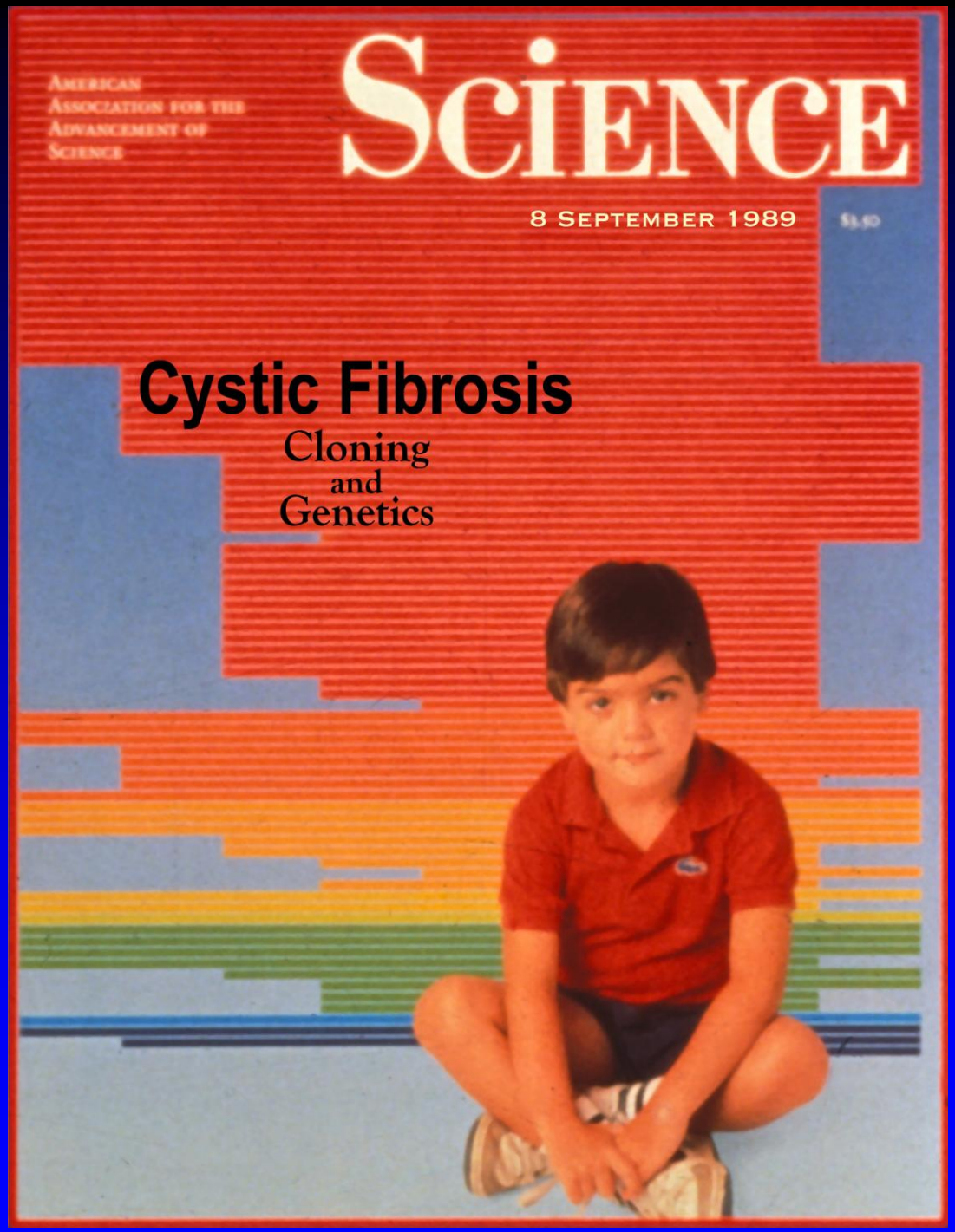
Frank Accurso

Keith Hammond

Identification of the Problems and Challenges with the IRT/IRT Test

1989: Wisconsin's first four years of screening with IRT reveal age-related declines and false negative problems (*Pediatrics* 85:1001-1007, 1990)

1990: France suspends their national IRT/IRT program!!!



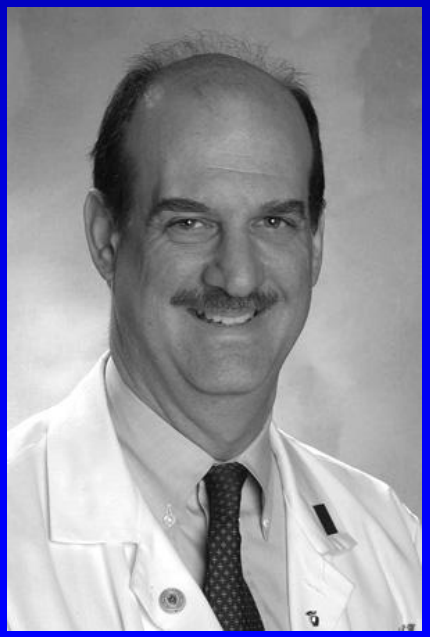
Discovery of the ΔF508 CFTR Mutation

Research teams led by Lap-Chee Tsui, Jack Riordan, and Francis Collins

Four Key Developments in CF NBS During 1989-2004

1. IRT/DNA [F508del] method (AJHG 52:616, 1993; BMJ 308:1469, 1994)
2. Improved nutritional outcomes clearly demonstrated (NEJM 337:963,1997)
3. IRT/DNA [CFTR multi-mutation method] (Pediatr 113:1573, 2004)
4. Improved cognitive outcomes with better nutrition (Pediatr 113:1549, 2004)

Anne Comeau



Richard Parad

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

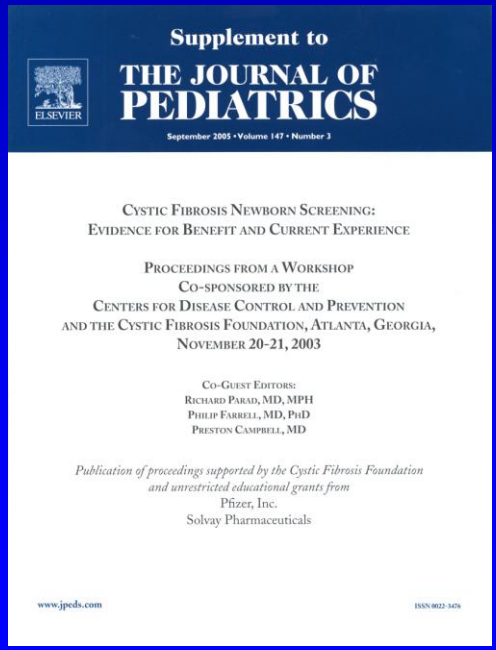
Image courtesy of Natus Medical Incorporated

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

September 2005 • Volume 147 • Number 3

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,
NOVEMBER 20-21, 2003

CO-GUEST EDITORS:
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PHILIP FARRELL, MD, PhD
PRESTON CAMPBELL, MD

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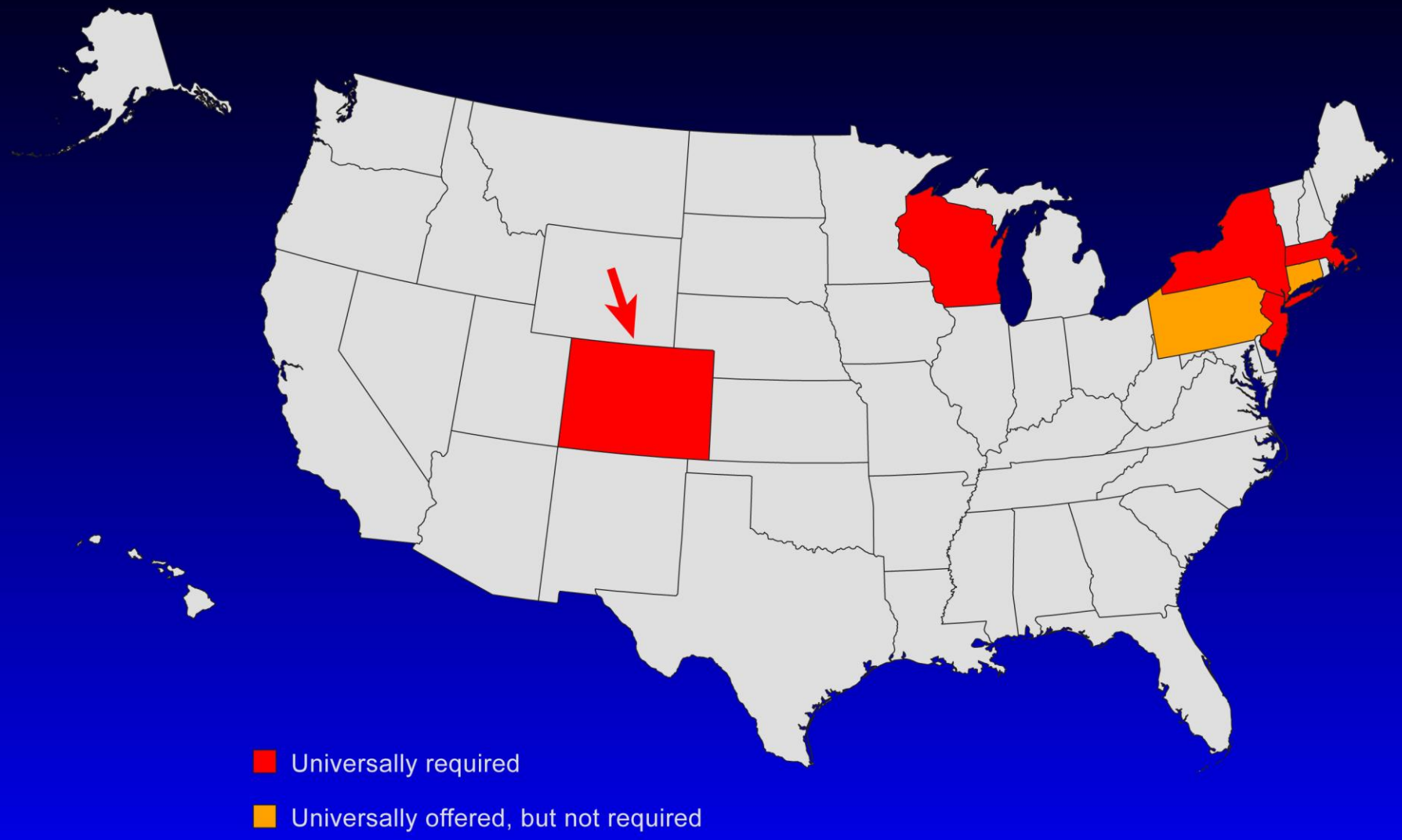
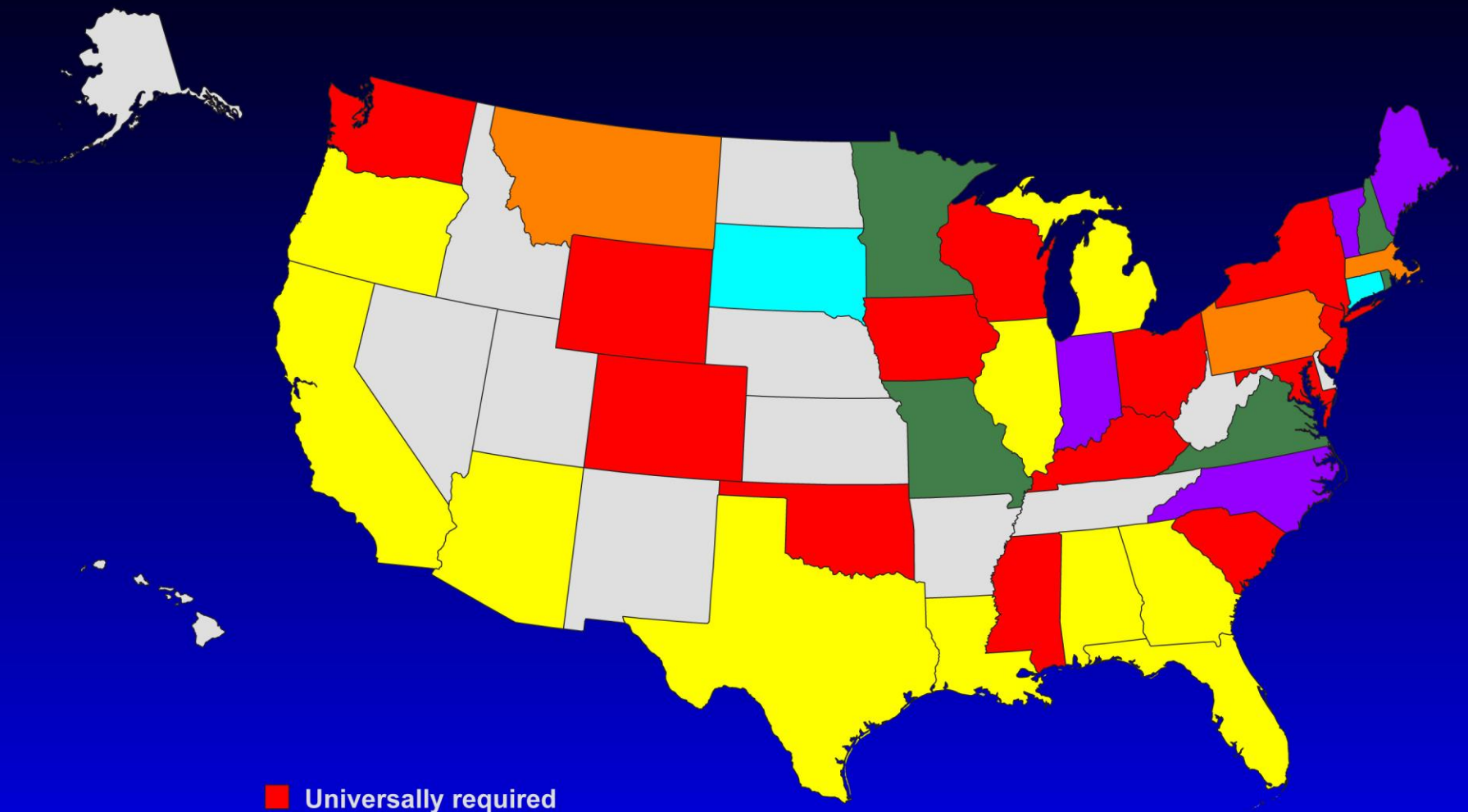
www.jpeds.com

Status of CF NBS in 2004



Current Status of CF NBS (2006)



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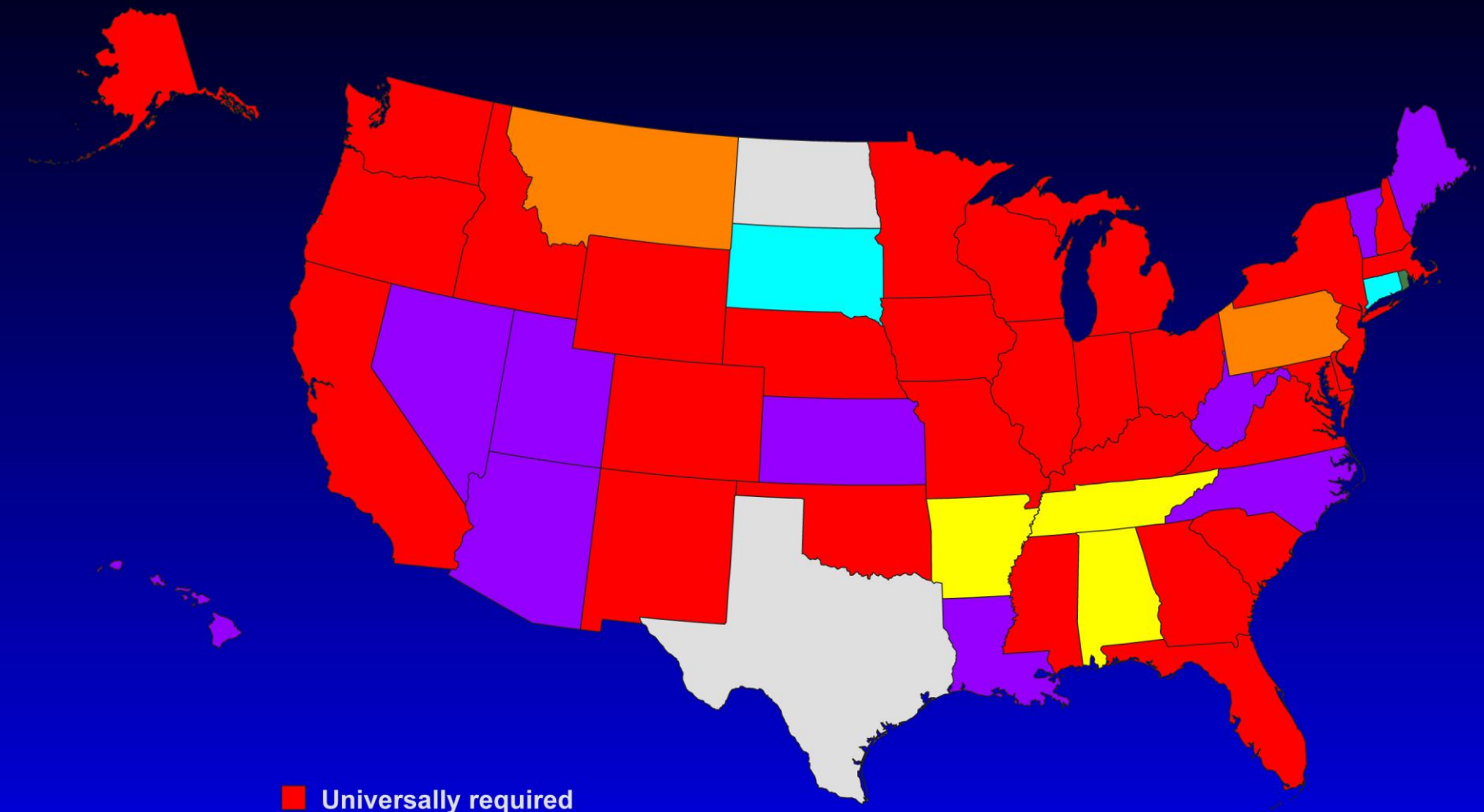
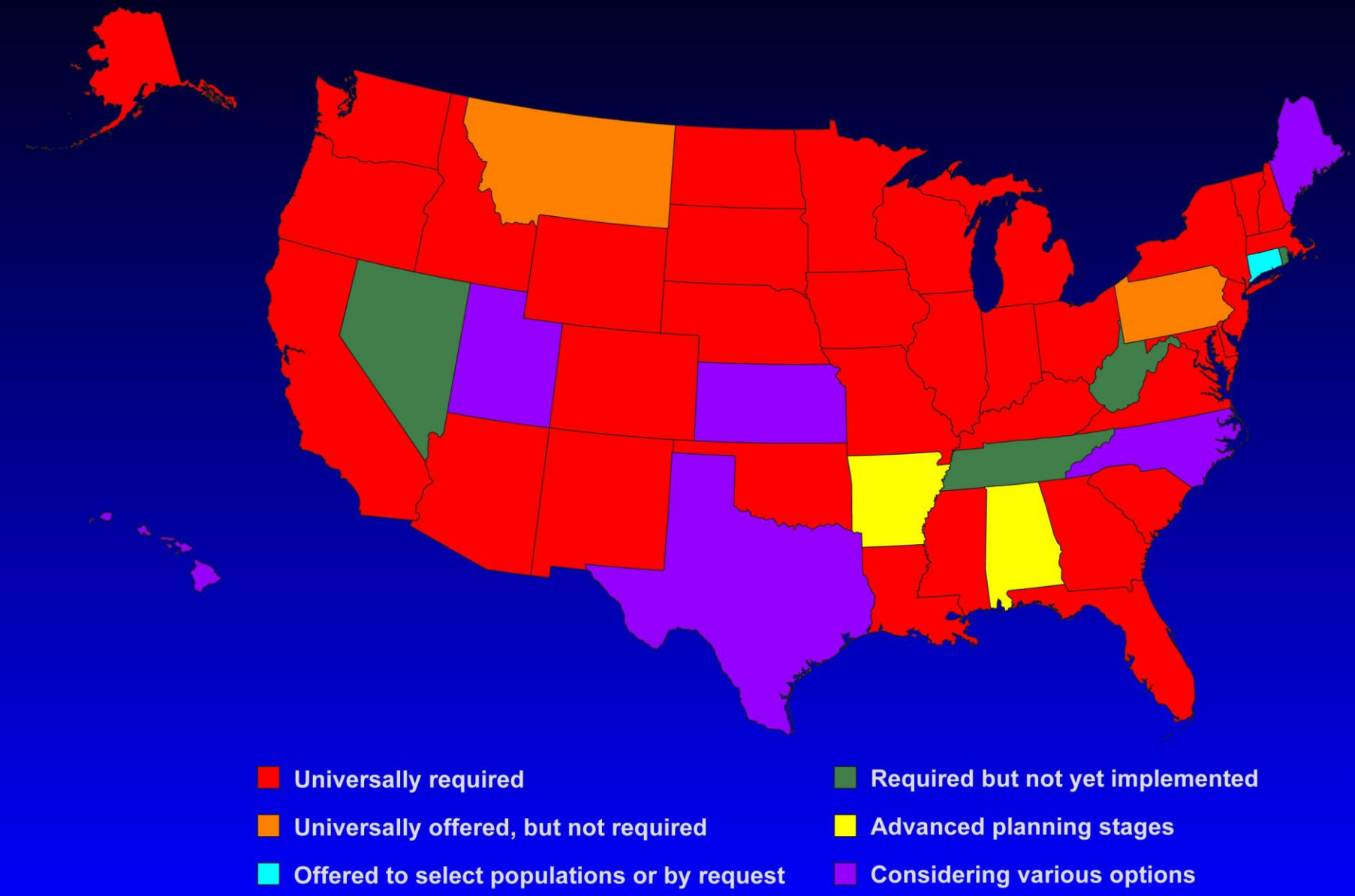
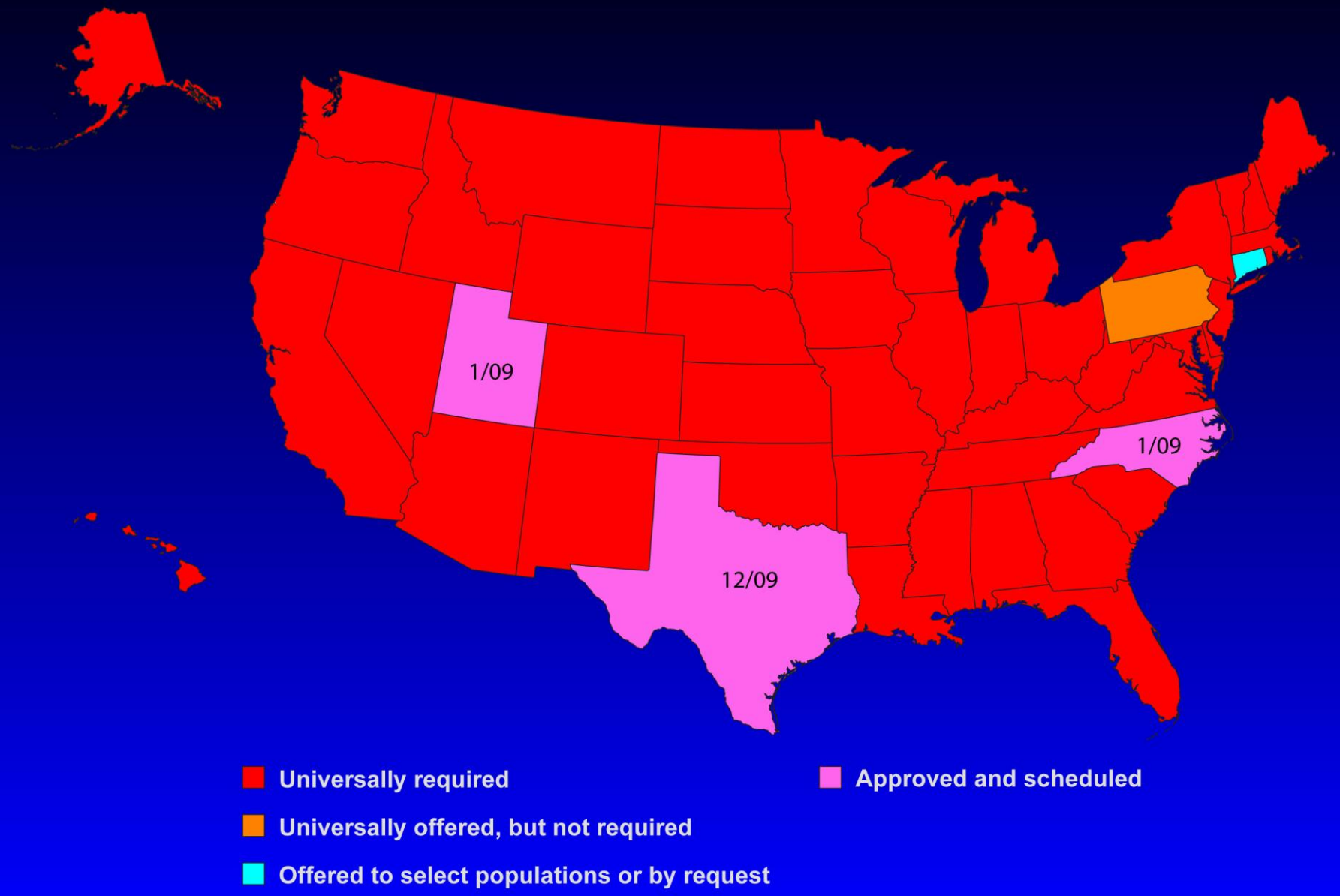
Current Status of CF NBS (2007)



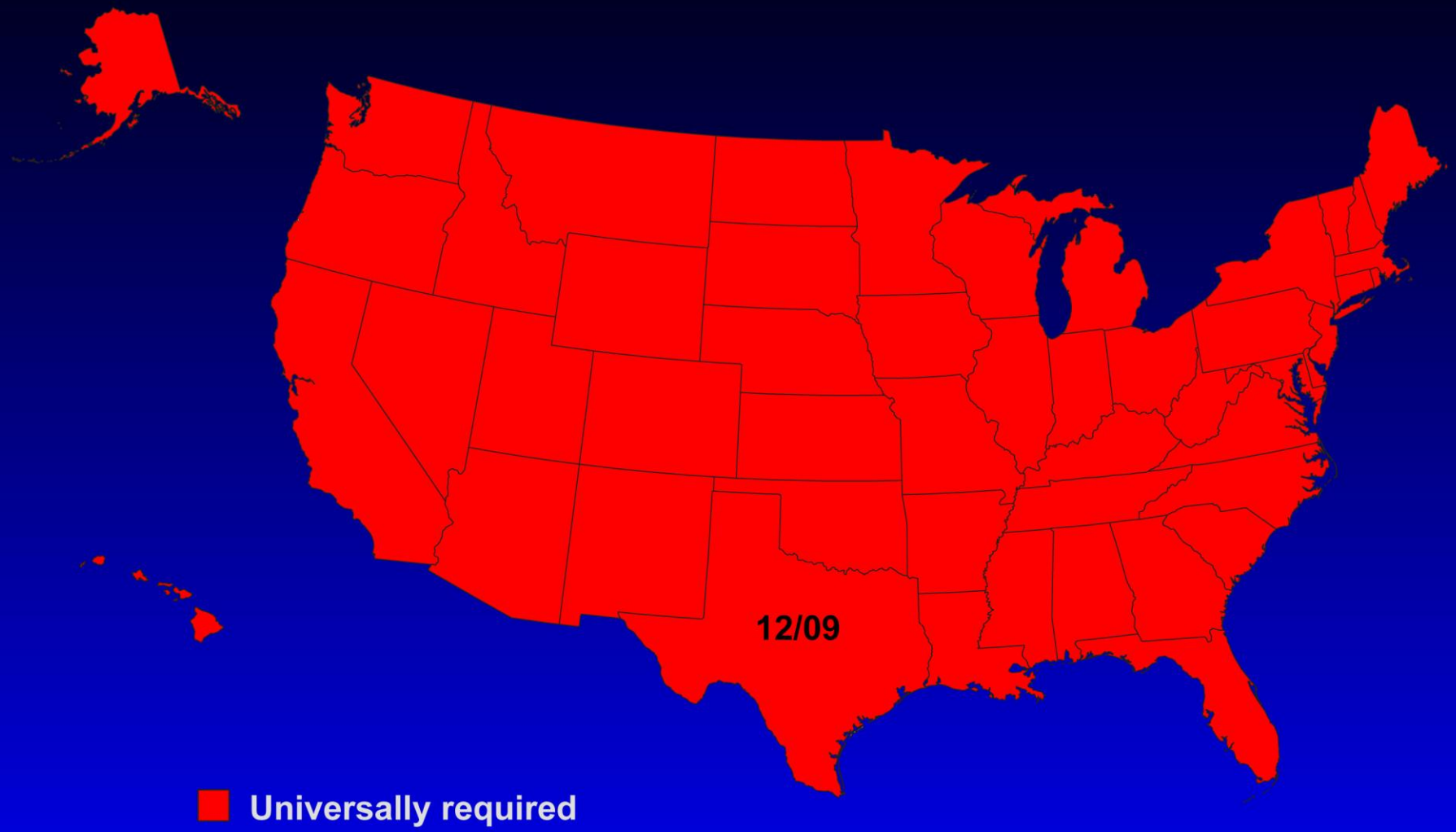
Current Status of CF NBS (2008)



Current Status of CF NBS (2009)



Current Status of CF NBS (2010)

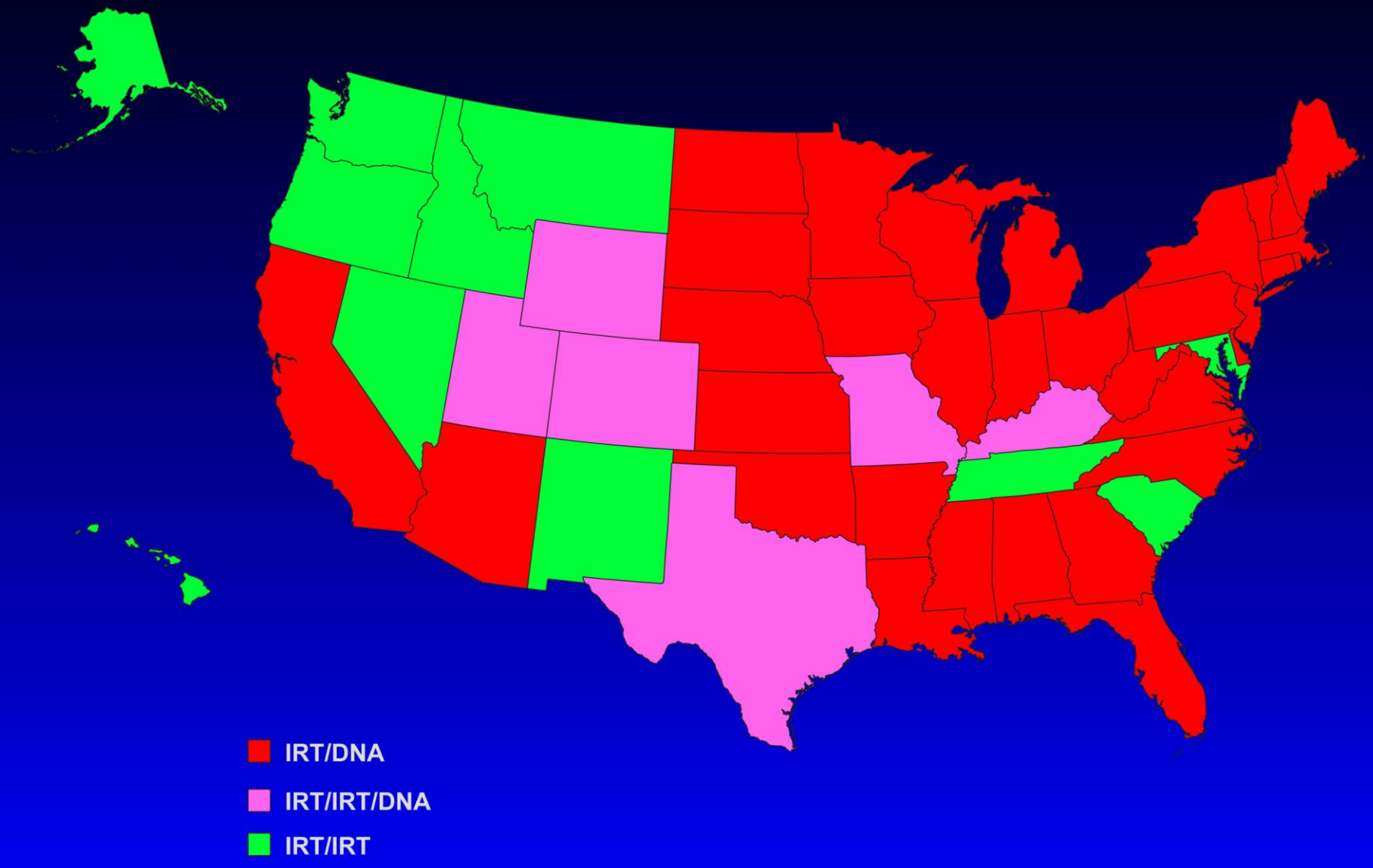


Two Strategies and Four U.S. Methods for CF NBS: All Begin with IRT and End with a Sweat Test for Diagnosis

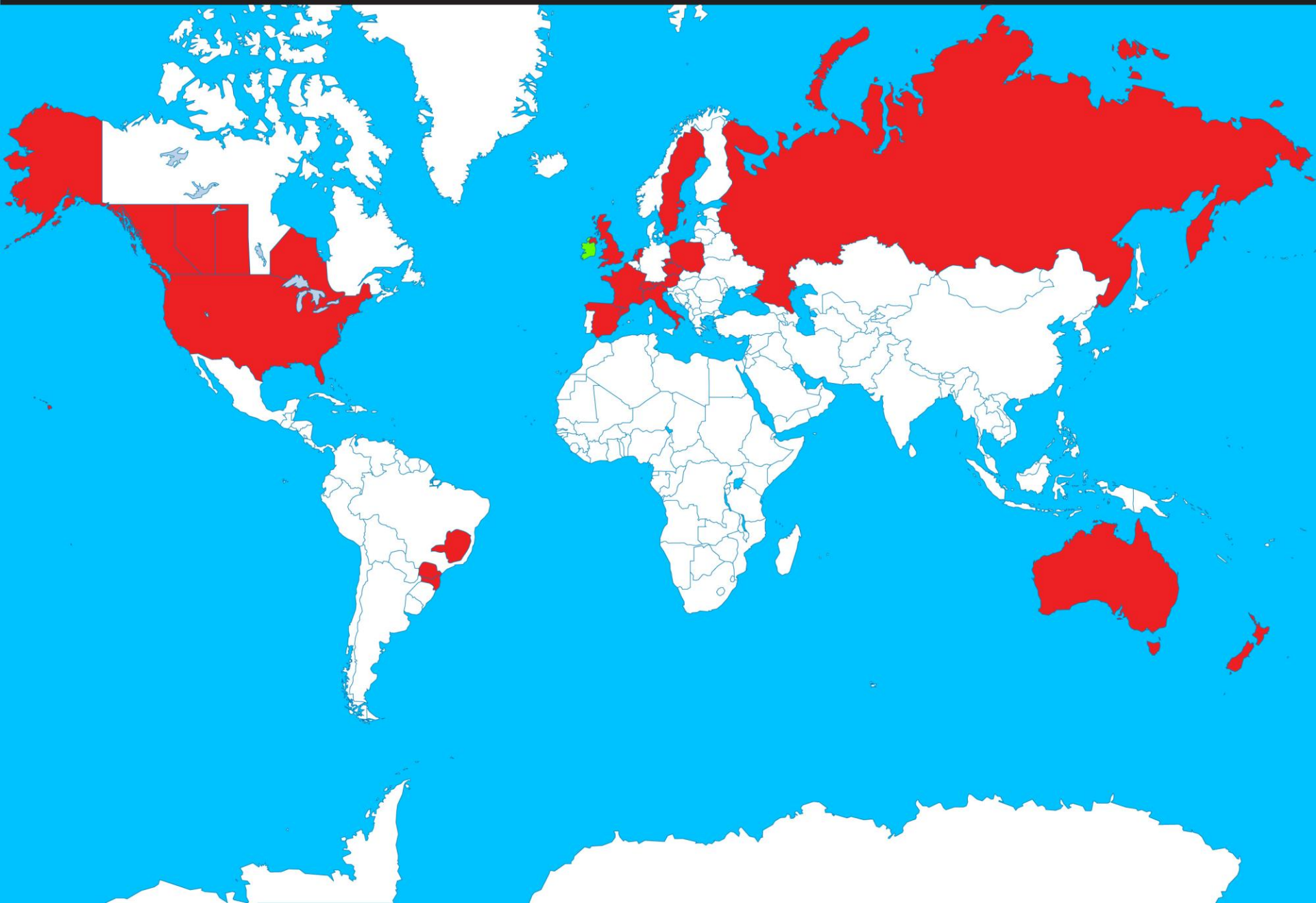
1. **IRT/IRT** (need 2 specimens & longer time)
2. **IRT/DNA** (most states – CFTR panels)
3. IRT/IRT/DNA (method requiring confirmed, persistent hypertrypsinogenemia)
4. IRT/DNA-EGA method (used only in CA with gene scanning and sequencing)

Marty Kharrazi

Current Status of CF NBS by Test (2012)



Global Distribution of CF Newborn Screening in 2012



Shift to an Emphasis on Quality Improvement*

1. Need for system-wide quality assurance to ensure “more good than harm”

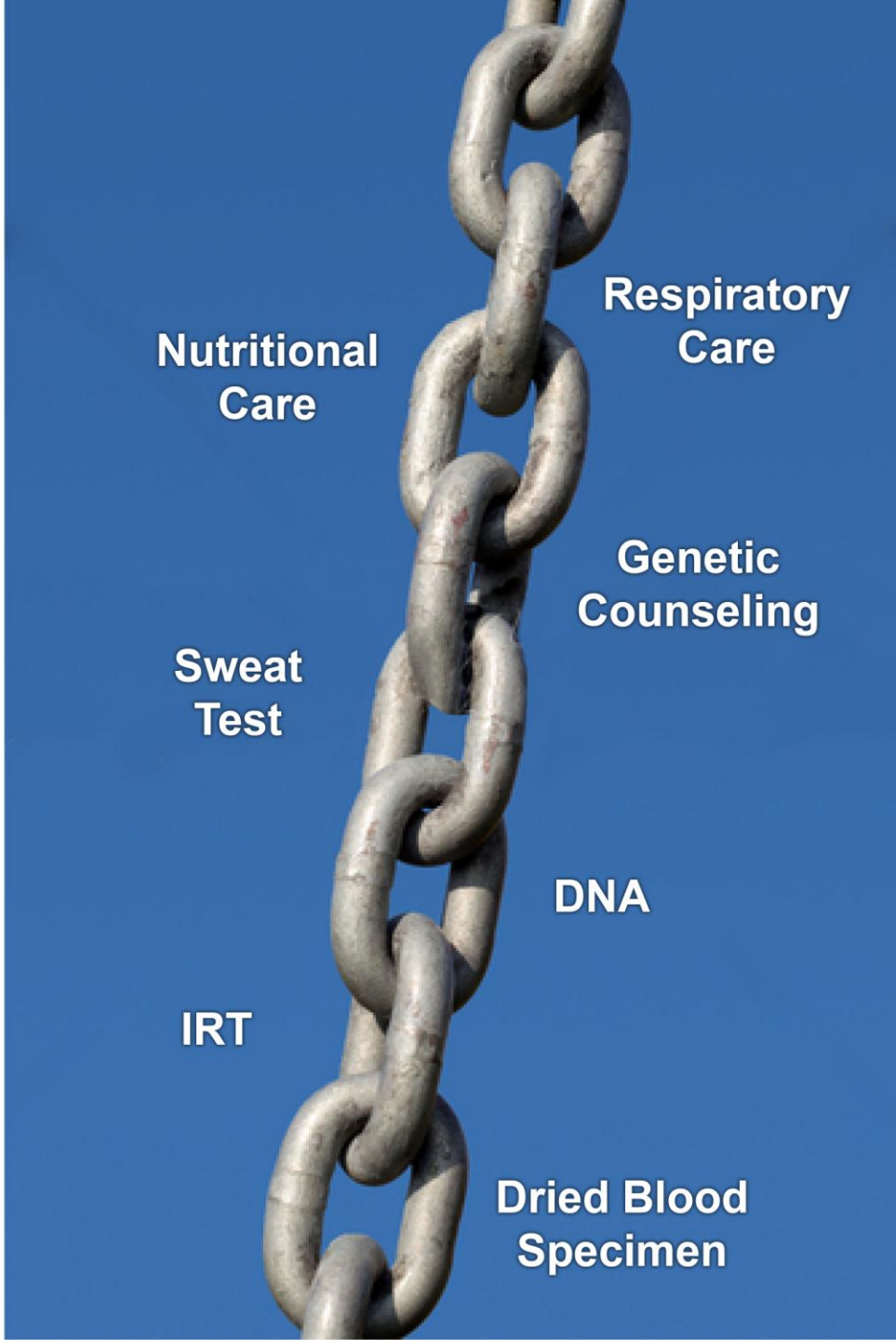
2. Enhancement of screening tests and follow-up nationwide (including sweat test performance)

3. National guidelines/standards are needed from CFF, ECFS, CDC, and CLSI

"A system is no stronger than its weakest link"
(Harry Hannon, PhD)

CF NBS

Only as strong as its weakest link



Clinical and Laboratory Standards Institute (CLSI)

MISSION: To develop best practices in clinical and laboratory testing and promote their use throughout the world, using a consensus-driven process that balances the viewpoints of industry, government, and the healthcare professions.

CLSI Document Development Process

1. Idea for a new document/project

2. Approval of the new project proposal
   - Includes open nominations for Document Development Committee of the new project

3. Project Development Stages
   - includes five voting stages by different committees
   - includes public review and commenting period
   - document development is conducted through meetings and teleconferences

CLSI Document Development Committee

By definition, CLSI Document Development Committee:

- **Responsible for drafting the document**
- **Resolving comments received on all stages of review**
- **Ensures that the document is technically accurate, globally applicable, and reflect its scope statement**

CLSI I/LA35 Document Development Committee

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- Voting Members
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  - Marie Early, PhD (CDC)
  - Gary Hoffman (Wisconsin State Laboratory)
  - Martin Kharrazi, PhD (California Department of Public Health)
  - Richard Parad, PhD (Harvard Medical School)
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– Dianne R. Webster, PhD, FHGSA (Auckland District Health Board) – *also a voting member*

CLSI Document on *Newborn Screening for Cystic Fibrosis* (I/LA35)

Document Scope Statement

- **Describes the use of newborn screening (NBS) laboratory tests for detecting risk for CF, especially immunoreactive trypsinogen**
  [Note: ~all methods begin with IRT and end with a sweat Cl test]
- **Addresses the detection of specific *CFTR* mutations that cause CF in second-tier screening with the strategy of applying immunoreactive trypsinogen/deoxyribonucleic acid (IRT/DNA).**
- **Presents the various strategies [IRT/IRT, IRT/DNA but not PAP] and methods used for CF NBS [IRT/IRT, IRT/DNA, IRT/IRT/DNA]**

CLSI document on *Newborn Screening for Cystic Fibrosis* (I/LA35)

Document Content Overview

- Pathophysiology of CF and Importance of Early Diagnosis Through NBS
- IRT as primary screening test
- CFTR mutations
- Current Strategies and Methods for CF NBS
- Laboratory Methods for DNA Analysis
- Guidelines for CFTR Panels in IRT/DNA Screening
- CF NBS Follow Up Information, Program Evaluation and Quality Assurance

CLSI I/LA35 Significant Milestones

- 02 September 2009 – project proposal and the Document Development Committee were approved
- 18 September 2009 – Document Development Committee first teleconference
- 13-14 October 2009 – Document Development Committee first meeting conducted at 2009 NACF meeting in Minneapolis, Minnesota, USA.

CLSI I/LA35 Significant Milestones

- 19-20 October 2010 - Document Development Committee second meeting conducted at 2010 NACF meeting in Baltimore, Maryland, USA
  - During this meeting, draft was completed and approved to move forward for voting

CLSI I/LA35 Voting Results

- Voting Stage 1 (first vote of the Document Development Committee)
  - Approved with 300 comments
- Voting Stage 2 (public review, first vote of the CLSI Consensus Committee on Immunology and Ligand Assay, CLSI Board of Directors and Delegates)
  - Approved with 174 comments
- Voting Stage 3 (final vote of the Document Development Committee)
  - Approved with 76 comments

CLSI I/LA35 Status Update

As of 24 October 2011:

- **Reviewed and approved by the Cystic Fibrosis Foundation**
- **Two remaining Voting Stages**
  - **Voting Stage 4 (the CLSI Consensus Committee on Immunology and Ligand Assay for technical review)**
  - **Final Draft Vote (vote to publish the document and ensure the CLSI Consensus Process was correctly followed)**
- **The draft is on track for publication soon and certainly before the end of 2011**

Immunoreactive Trypsinogen

- It should be noted that IRT is not a single analyte, but is made up of IRT1 and IRT2. In fact, there is some evidence that the IRT elevated in CF may be predominantly different from that found in infants without CF.
- IRT method validation protocols and quality control methods are described, along with proficiency testing recommendations using for instance the CDC program.
- IRT variations associated with seasonal exposures and kit changes, and their significance, are described, along with the advantages of using a floating cutoff value for adjustments.

CLSI on IRT (continued)

- It is emphasized that the IRT results from multiple specimens, especially infants in the NICU, can be quite variable, e.g., from initially negative to abnormal, or vice versa. When an abnormal IRT result is encountered (initial or on a subsequent specimen), the appropriate follow-up action is recommended depending upon the screening algorithm.

- Other variables affecting IRT levels are discussed such as the observed higher levels in low birthweight, premature infants and decreases of IRT with increasing postnatal age after 2 weeks.

Recommendations on CFTR Panel

- The ACMG recommended panel of 23 CF-disease causing mutations provides a high degree of sensitivity in many newborn populations. **Therefore, it is recommended that the ACMG-23 mutations be used in IRT/DNA screening methods as the core and preferred *CFTR* panel.**
- However, if special circumstances such as a significant population of minorities susceptible to CF exist in a regional CF NBS program, it is recommended that other mutations beyond the ACMG-23 list be added to the *CFTR* panel based on compelling data.
- In addition, the data available in *CFTR2* and other information…may be useful to guide decisions regarding the composition of expanded *CFTR* panels.

CFTR Mutant Alleles in U.S. Patients*

(Cystic Fibrosis Foundation Registry, 1998)

	% mutations
ΔF508	68.6
G542X	2.4
G551D	2.1
W1282X**	1.4
N1303K	1.3
R553X**	0.9
621+1G → T	0.9
3849+10kbC → T**	0.7
1717-1G → A	0.7
R117H***	0.7

	% mutations
Δ I507	0.3
2789+5G → A	0.3
G85E	0.3
R347P	0.2
R334W	0.2
R1162X	0.2
R560T	0.2
A455E	0.2
2184delA	0.1
711+1G → T	0.1a

* Bobadilla et al, Human Mutation 2002; 19:575-606. These 20 alleles and 3 others are included in the 23 mutation ACMG panel.

** Found in specific ethnic populations.

*** Associated with CF when the 5T variation is present.

CLSI on CFTR Panel: R117H

- One mutation included in the ACMG-23 panel, R177H, is especially challenging and receives special attention the CLSI Guideline document because not all R117H alleles are pathogenic.
- Consequently, inclusion of R117H in primary NBS panels is controversial. The difficulty in interpreting a finding of R117H without knowing the poly-T status of the R117H allele makes reflex testing for poly T essential, and is recommended whenever R117H is included in the *CFTR* panel.
- If R117H-7T is reported as the second mutation with another that is a CF-causing allele, it is recommended that NBS programs perform a sweat chloride test, etc.

Other CLSI Recommendations

- It is strongly recommended that designers of CF NBS programs ensure that any mutations that they include in their panel are of proven pathogenicity.
- This is especially important with methods that employ EGA.
- The *CFTR*2 project will provide ongoing objective functional evidence on the pathogenicity of the most common *CFTR* mutations. This will aid the development of a larger, panethnic panel that will provide greater screening test sensitivity and better coverage of genetically diverse populations.

Immunoreactive Trypsinogen (IRT) as a Biomarker for Cystic Fibrosis: Technical Issues and Challenges for Newborn Screening

May 23-24, 2011
Annapolis MD

IRT Workshop Goals and Publication Plans

- **Goal of the Work Shop is to improve CF newborn screening results at the initial analytical phase and publish results and recommendations.**

  “To ensure the Newborn Screening principle of equity, each child should have exactly the same chance to screen positive or negative.”

- **Focus of work shop: discussion of analytical issues related to IRT assays.**

IRT Questions Addressed (1):

- What have been the clinical consequences of IRT results (positive and negative)?
- What are the laboratory issues centered around the use of IRT as the initial screening test?
- To what extent are the IRT issues kit dependent?
- To what extent do the laboratory issues relate to non-kit issues?

IRT Questions Addressed (2):

- What are the IRT experiences in older programs in the US and other countries?
- Do protocol variations result in similar or different experiences?
- What are newcomers to IRT testing experiencing?
- What clinical consequences are being encountered?

IRT Questions Addressed (3):

- What strategies are being used to address the issue of high IRT levels in premature infants and other newborns requiring intensive care?

- Should the IRT cutoff be adjusted to cope with persistently high levels in premies?

- What issues have been encountered in applying IRT to African American infants?

- What quality control measures are needed to address IRT variations related to season and kit/lot changes?

IRT Questions Addressed (4):

- What quality improvement principles/practices apply to the challenge of enhancing the value of IRT as a first tier screening test?
- Can IRT assay kits be manufactured with more consistent performance?
- What analytical strategies are promising?
- Are there mathematical methods that might be better than the current practices such as using the 95th-99th percentile cutoff values in IRT/DNA algorithms?

The majority of false-negative cases result from IRT values falling below cutoff.

Potential variables affecting IRT assays:

- Birth weight of newborn/infant
- Age of newborn/infant at specimen collection
- Age of specimen at assay
- Stability of IRT
- Season of birth impact/biological
- Lot to lot variation in reagents/assay kit performance
- Cutoff selection: fixed/floating
- Algorithm impact: IRT/IRT; IRT/DNA; IRT/IRT/DNA

Concluding Comments

Goal and focus of the Work Shop was identify ways to improve CF newborn screening results at the initial IRT analytical phase.

Harmonize!

Comments:

- **Many false-negative cases occur in all screening labs**
- **African-Americans higher IRT levels**
- **Kit lot-to-lot variation is an issues with all labs**
- **Floating cutoffs seems to be the valid choice – still not ideal!!**
- **Use ROC curves/sensitivity and specificity checks**
- **Need nationwide surveillance for “missed” CF cases**
- **10 years or longer before your “missed CF cases show**

Probable Recommendations

- Support the effort of the CFF to develop registry of NBS “missed”/delayed diagnosed CF cases with linked lab and clinical information.
- Manufacturers of assay kits should work diligently to reduce lot-to-lot transition issues.
- Be transparent with the medical community and parents that false-negative cases occur in newborn screening for CF.

Workshop Products

- A critical discussion and review is expected with a targeted outcome, so that by workshop completion, we will identify outstanding IRT testing issues and achieve resolution and harmonization!!
- Develop recommendations for IRT testing improvements.
- A publication will be developed from the content of the work shop that presents recommendations for quality improvements toward elimination of false-negative cases. [Journal of Cystic Fibrosis (1,500 word max)]

The 21st Century is a New Era for Children with Cystic Fibrosis!

- Early diagnosis & therapy through newborn screening
- New opportunities for understanding and prevention
- No longer dominated by intervention in ill individuals
- Prevention of …

early deaths	salt depletion
malnutrition	growth failure
chronic *Pseudomonas*	"cross-infections"
many hospitalizations	lung disease (eventually)