Secretary's Advisory Committee on Heritable Disorders in Newborns and Children

Newborn Screening for SCID:
Experiences of State Laboratories Using the TREC Assay

Carla Cuthbert, PhD, FACMG, FCCMG
Chief, Newborn Screening and Molecular Biology Branch,
Division of Laboratory Sciences
NCEH, CDC

Thursday 27th January 2011

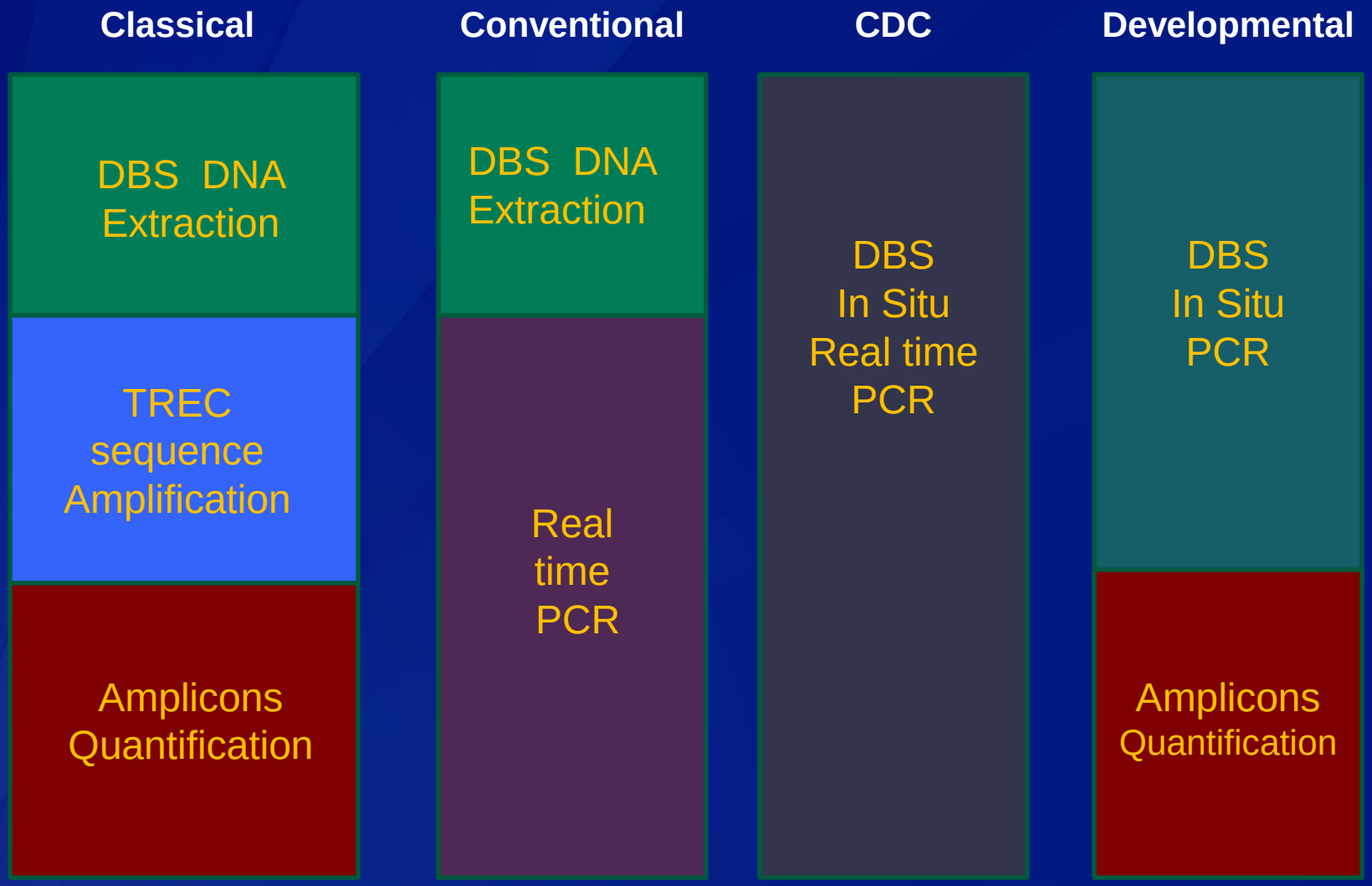
Screening for SCID Using the TREC Assay

General Principles and Assay Development

- **SCID Screening Marker: T cell receptor excision circles (TREC)**
  - By-products of rearrangement of T cell receptor genes during T cell maturation in the thymus
  - Are episomal DNA, TREC does not replicate during mitosis – diluted by cell divisions
  - Peripheral blood level reflects T cell production in the thymus
- **TREC assay – now adapted to detect SCID and other lymphopenia in newborns**
  - Originally developed to assess thymic function in HIV-infected infants
  - Real Time PCR
  - Variations in TREC Assay procedures can be based on choice of primers/probes and DNA extraction procedures

Screening for SCID Using the TREC Assay

General Principles and Assay Development



Slide courtesy of Francis Lee

States Currently Screening for SCID

Performed Within State Laboratories

Wisconsin

Massachusetts

California

New York

Wisconsin's Laboratory Experience

History and Current Status

- **November – December 2006**
  - November: JMF provides $250,000 matching contribution to fund WI NBS SCID Program
  - December: CHW matches JMF $250,000 donation and WSLH in-kind contribution
- **January 2007**
  - Announcement of the WI NBS SCID Program
- **Winter and Spring 2007**
  - Optimization of TREC assay & screen anonymized NBS cards
- **January 2008**
  - WI Launched routine NBS for SCID
- **2008 - Current**
  - Demonstrate efficacy of TREC assay to detect SCID
  - Supported by a CDC grant which started in Oct. 2008)

Wisconsin's Laboratory Experience

Results of Testing

Number Screened:	**206,982**	
– Premature (< 37 wks)	18,861	
– Full term	188,121	
Abnormal results:	**159**	
– Premature (<37 wks)	93	(0.04%)
– Full term	66	(0.03%)
Inconclusive Results:	**288**	
– Premature (<37 wks)	240	(0.12%)
– Full term	48	(0.02%)

Wisconsin's Laboratory Experience

Results of Testing

Severe Lymphopenia Cases

- Idiopathic Lymphopenia
  - Regular IVIG, planning BMT
- Rac 2 mutation
  - Successful BMT
- Idiopathic Lymphopenia
  - BMT
- T-, B-, NK+ SCID
  - Successful BMT—normal TRECs !!!
- ADA SCID
  - Possible gene therapy

Wisconsin's Laboratory Experience

TREC Assay Performance in Full Term Babies

- Sensitivity: 100% (No known false negatives reported)
- Positive Predictive Value: 40% (based on Flow results)
- Specificity: > 99%
- Detection Rate on Severe T-cell Lymphopenia (BMT needed) in Wisconsin population

  1/41,396 (5 cases in 206,982 screened newborns)

Funding Support

Jeffrey Modell Foundation

Children's Hospital of Wisconsin

Wisconsin State Laboratory of Hygiene

Centers for Disease Control and Prevention

Massachusetts' Laboratory Experience

History and Current Status

- **March 2007**
  - Massachusetts SCID NBS Working group
- **July 2007**
  - Development of multiplex TREC Assay began
- **May 2008 and onward**
  - IRB submissions: statewide pilot updates CDC award
- **February 2009 and onward**
  - Statewide screening for SCID in MA
- **September 2010 and onward**
  - Screening for SCID in parallel in MA and TX

Massachusetts' Laboratory Experience

Results of Testing

143,172 initial specimens*

833	declined SCID NBS	0.6%
872	no recorded consent SCID NBS	0.6%
1,743	Program-wide unsatisfactory	1.2%

139,724 valid specimens

160	total SCID-specific unsatisfactory	0.1->.03%
139,219	screen negative	99.6
345	screen positive	0.26
	29 referred to flow cytometry	

Through guthrie date 12/31/10

*by current algorithm

Massachusetts' Laboratory Experience

Results of Testing

Abnormal SCID NBS & Referred to Flow Cytometry: 29

– Abnormal Flow result	18
– Pending Flow / Rpt NBS	7
– Flow within normal limits	1
– Closed	1
– Expired	2

Massachusetts' Laboratory Experience

Results of Testing

Abnormal SCID NBS & Abnormal Flow Cytometry: 18

- **SCID** **1**
- **DiGeorge Syndrome** **4**
- **Multiple Congenital Anomalies** **1**

- **T-cell Lymphopenia** **3** ***(Not SCID, no further testing needed)***
- **T-cell Lymphopenia** **6** ***(Not SCID, final diagnosis pending)***
- **T-cell Lymphopenia** **3** ***(SCID unlikely, pending further work-up)***

Sensitivity: 100% ***(no known missed cases)***

Funding Support

Centers for Disease Control and Prevention

California's Laboratory Experience

History and Current Status

- **July 2010**
  - NIH provides $480,000 for CA NBS SCID Pilot Program. CA will provide data to NIH.
  - JMF agrees to provide up to $800,000 matching contribution to fund CA NBS SCID Pilot Program.
- **August 2010**
  - Pilot begins 8/16/2010 with Perkin Elmer staff testing CA NBS specimens at Genetic Disease Laboratory facility (lab within a lab concept).
- **September 2010**
  - TREC Cut-off dropped from 60 to 25.
- **January 2011**
  - Actin assay refined and nursery (ie regular nursery vs. NICU) evaluation added to flow chart.

California's Laboratory Experience

Results of Testing

(August 16 – December 31, 2010)

Number Screened: 217,515 (initial NBS specimen)

- **Positive*: 12 (.01%)**
  - SCID 4
  - DiGeorge Syndrome 1
  - Non-SCID T Cell Lymphopenia 1
  - Negative Flow Cytometry 3
  - Expired 3
- **Inconclusive Results: 229 (.11%)**
  - Positive* 10
  - Inconclusive* 3
  - Negative 127
  - Expired 23
  - Lost to Follow-up 7

* Positives and inconclusives on 2nd heelstick go on to Flow Cytometry

California's Laboratory Experience

Results of Testing

(August 16 – December 31, 2010)

From Second Heelstick

- **Positive: 10**
  - **DiGeorge Syndrome 1**
  - **Non-SCID T Cell Lymphopenia 1**
  - **Negative Flow Cytometry 4**
  - **Expired 1**
  - **Pending 3**

- **Inconclusive 3**
  - **SCID * 1**
  - **Negative Flow Cytometry 2**

California's Laboratory Experience

Evaluation of Screened Positive Infants

(August 16 – December 31, 2010)

217,515 initial specimens

157 Second Heelsticks

(Total 217,672)

26 Referred for Flow Cytometry

• SCID	5
• Di George Syndrome	2
• Non-SCID T Cell Lymphopenia	2
• Negative	10
• Expired	4
• Pending	3

Funding and Support

Jeffrey Modell Foundation

National Institutes of Health

DBS Reference Materials

Available for the TREC Assay

Screen Normal

Cord Blood Pools: Highest, Lower, Lowest

Individual Cord Bloods (~50)

Screen Positive

Two Pools

Indeterminate

Two Pools

~ 4000 DBS in each category

CDC Model Performance Evaluation Survey (Pilot Proficiency Testing)

- Monthly Sendouts
- Five Blinded Reference DBS
- Additional Prototype DBS

- Seven enrolled Participants
  - Wisconsin NBS
  - Massachusetts NBS
  - California NBS
  - New York NBS
  - University of California San Francisco
  - PerkinElmer Genetics
  - PerkinElmer Life & Analytical Sciences

Publications

Identification of an infant with severe combined immunodeficiency by newborn screening.
Hale JE, Bonilla FA, Pai SY, Gerstel-Thompson JL, Notarangelo LD, Eaton RB, Comeau AM.
J Allergy Clin Immunol. 2010 Nov;126(5):1073-4. Epub 2010 Oct 8.

A multiplex immunoassay using the Guthrie specimen to detect T-cell deficiencies including severe combined immunodeficiency disease.
Janik DK, Lindau-Shepard B, Comeau AM, Pass KA.
Clin Chem. 2010 Sep;56(9):1460-5. Epub 2010 Jul 21.

High-throughput multiplexed T-cell-receptor excision circle quantitative PCR assay with internal controls for detection of severe combined immunodeficiency in population-based newborn screening.
Gerstel-Thompson JL, Wilkey JF, Baptiste JC, Navas JS, Pai SY, Pass KA, Eaton RB, Comeau AM.
Clin Chem. 2010 Sep;56(9):1466-74. Epub 2010 Jul 21.

Guidelines for implementation of population-based newborn screening for severe combined immunodeficiency.
Comeau AM, Hale JE, Pai SY, Bonilla FA, Notarangelo LD, Pasternack MS, Meissner HC, Cooper ER, DeMaria A, Sahai I, Eaton RB.
J Inherit Metab Dis. 2010 Oct;33(Suppl 2):S273-81. Epub 2010 May 20.

Publications

Development of a routine newborn screening protocol for severe combined immunodeficiency.
Baker MW, Grossman WJ, Laessig RH, Hoffman GL, Brokopp CD, Kurtycz DF, Cogley MF, Litsheim TJ, Katcher ML, Routes JM.
J Allergy Clin Immunol. 2009 Sep;124(3):522-7. Epub 2009 May 31.

Statewide newborn screening for severe T-cell lymphopenia.
Routes JM, Grossman WJ, Verbsky J, Laessig RH, Hoffman GL, Brokopp CD, Baker MW.
JAMA. 2009 Dec 9;302(22):2465-70.

Implementing routine testing for severe combined immunodeficiency within Wisconsin's newborn screening program.
Baker MW, Laessig RH, Katcher ML, Routes JM, Grossman WJ, Verbsky J, Kurtycz DF, Brokopp CD.
Public Health Rep. 2010 May-Jun;125 Suppl 2:88-95.