Newborn Screening for SCID: An Overview

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Why Consider NBS for SCID?

- Lethal disorder – not detectable at birth by routine examination

- Reliable screening assay now available
  - Quantification of TREC (marker for T cell production)

- Confirmatory test readily available (FLOW cytometry)

- Effective curative therapy
  - BMT/HSCT: 95% successful if performed within the first 3.5 months of life
  - PEG-ADA enzyme replacement
  - Gene therapy for X-linked and Artemis
SCID: recommended and adopted to the Uniform National NBS panel

• Jan 2010: SCID was the **FIRST disorder** to be recommended to the HHS Secretary for addition to the expanded uniform national newborn panel

• Early May 2010: **FIRST infant** with SCID was presymptomatically identified in Wisconsin

• Late May 2010: HHS Secretary agrees to add SCID to the Uniform National NBS Panel – **A Milestone**

• May 2011: Report due to HHS Secretary from ACHDNC on the Status of the States’ implementation of this recommendation
SCID: recommended and adopted to the Uniform National NBS panel

“Adopting these SACHDNC recommendations will provide the federal guidance necessary to assist States to voluntarily bring their newborn screening programs into alignment with the most up-to-date research, technology, laboratory and public health standards and practices.”

DHHS Secretary Sebelius
To SACHDNC Chair Dr Rodney Howell
May 21, 2010
Early NBS Pilots for SCID
Wisconsin’s Experience

- **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

- **Winter and Spring 2007**
  - Announcement of the WI NBS SCID Program
  - Optimization of TREC assay & screen on 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
Early NBS Pilots for SCID
Massachusetts’ Experience

- **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
Newborn Screening for SCID

CDC’s Role
CDC Funding Opportunities for SCID through the Newborn Screening and Molecular Biology Branch

- Funding and administration of FIRST public health pilot studies for newborn screening for SCID
  - Public Health Labs: Massachusetts and Wisconsin (~ $500,000 each)
  - A total of $3 million over three years: Fall 2008, Fall 2009, Fall 2010

- Funding and support of SCID Newborn Screening pilot study among Native Americans
  - Pilot studies: Fall 2008, Fall 2009 (~ $100,000 each year)

- CURRENT  CDC SCID funding opportunities for FY11
  - Funding Opportunity Number: RFA-EH-11-001
  - Current Closing Date: May 31, 2011
CDC Funding Opportunities for SCID

through the Newborn Screening and Molecular Biology Branch

Goals of Funding Opportunity

- To expand laboratory capacity for SCID newborn screening in the US
- To increase the pool of laboratory scientists with knowledge and skills in conducting newborn screening for SCID
- To provide training for the public health community about NBS tests for SCID and to foster its integration into the standard of care for communities

Other details

- Full announcement on the grants.gov website (RFA-EH-11-001)
- Estimated funding date is September, 2011
- Additional Questions: Paul Mehta, at 770-488-0556, pum4@cdc.gov
CDC has been mandated by Congress to provide QA materials for NBS Laboratories

- Provide quality assurance for laboratories involved in screening newborns and children for heritable disorders
  - Quality assurance for newborn-screening tests
  - Performance evaluation services
  - Technical assistance and technology transfer to newborn screening laboratories
  - Assistance to ensure analytic validity and utility of screening tests

- Provide appropriate quality control and other performance test materials to evaluate the performance of new screening tools

Newborn Screening Saves Lives Act of 2008
CDC as an instrumental service provider to Public Health Labs for SCID screening

- Only provider of national reference materials for SCID testing in dried blood spots
  - QC materials made available to labs as SCID screening becomes implemented

- Developed a novel laboratory method for detection of patients with SCID

- Provided workshops, training and updates on SCID testing
  - Public health lab representatives from CA, CT, GA, MA, MN, WI, NY, TX; Updates to Secretary’s Advisory Committee
NSTRI Workshop on SCID Reference Materials
February 2010

Public Health Laboratory representatives from CA, CT, GA, MA, MN, WI, NY, TX
APHL and NNSGRC were pleased to host:

Newborn Screening for Severe Combined Immunodeficiency: Implementation, Challenges, and Successes

Target Audience:
- State Newborn Screening Laboratorians
- Newborn Screening Follow-up Program Personnel and Physicians
- Newborn Screening Stakeholders

Goals of Meeting:
- Outline basic information regarding SCID and its detection through the newborn screening process
- Describe the basic testing methodologies for detecting SCID and state implementation experiences
- Discuss the treatment and clinical management of SCID patients

October 27 – 28, 2010
Newborn Screening for SCID

CDC’s TREC Assay and Preparation of Reference Material
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, DNA extraction procedures, calibrators
CDC’s DBS *In Situ* Real Time PCR Assay for TREC

Punch one 2.0 mm discs from DBS specimen into PCR tubes

Wash with 125 µl of DNA purification solution S1
(shake for 15 minutes at room temp)

Wash with 125 µl of DNA elution solution S2
(shake for 5 minutes at room temp)
CDC’s DBS *In Situ* Real Time PCR Assay for TREC

- **Discard S2 wash buffer**
- **Add 15 μl of qPCR mastermix** (contains complete mix of primers & probe)

**Run qPCR in Stratagene MX3000p:**
- 45 deg for 5 min, 95 deg for 20 min
- 45 cycles of [ 95 deg x 15 sec + 60 deg x 1 min ]

![Graph showing TREC-HeLa DBS calibrators](image)

y = -3.1409x + 36.607
R² = 0.963
E=108%

![Graph showing Cord Bloods TREC Amplification Plots](image)
Three Types of DBS Reference Materials for the TREC Assay

1. SCID-like Reference Material
   - Screen Positive for SCID
   - Abnormal TREC result; Normal Control Gene result

2. Normal Reference Material
   - Screen Negative for SCID
   - Normal TREC result; Normal Control Gene result

3. Indeterminate Reference Material
   - Poor real time PCR assay; Assay failure
   - Abnormal TREC result; Abnormal Control Gene result
Preparation of DBS Reference Materials

“Indeterminate RM”

- Order Leukocyte-depleted blood
- Adjust hematocrit to 50%
- Spot onto filter paper

POINT:
Reference material will be deficient for both TREC and the control gene
Preparation of DBS Reference Materials “SCID-like RM”

• Order adult blood which has low TREC content (varies from adult to adult)
• Remove mononuclear cell fraction
• Adjust hematocrit to 50%
• Spot onto filter paper

POINT:
Reference material will be deficient for TREC only.
Control gene levels will be in the normal range.
Preparation of DBS Reference Materials

“Normal RM”

- Acquire units of cord blood
  - Reconstitute to 50% hematocrit
  - Spot samples from each unit onto filter paper and assess for TREC content
  - Pool units with similar TREC levels

- Acquire fresh cord blood; harvest mononuclear cells
  - Mononuclear cells used to enrich cord blood pools to create blood pools with desirable TREC content

- Three NORMAL levels are created
  - Span the linear range of the assay: high, mid-range and low
Preparation of DBS Reference Materials

“Calibration Material”

• Develop HeLa cell line with transfected “TREC” sequence
  – One “TREC” sequence per transfected cell

• Use the “SCID-Like” Reference Material as the base pool, add known quantities of HeLa cells containing TREC sequence
  – Generates Reference Material with quantifiable TREC content

• Calibrator Reference Material
  • Adjust hematocrit to 50%, spot on filter paper
CDC Model Performance Evaluation Survey
(Pilot Proficiency Testing)

- Monthly Sendouts
- Five Blinded Reference DBS

- Ten enrolled Participants
  - Wisconsin NBS
  - Massachusetts NBS
  - California NBS
  - New York NBS
  - University of California San Francisco
  - PerkinElmer Genetics
  - PerkinElmer Life & Analytical Sciences
  - Minnesota NBS
  - Texas NBS
  - Taiwan NBS
Newborn Screening for SCID

Expanded Pilots - The National Institutes of Health
Expansion of SCID Newborn Screening Pilots

• NIH initiated project to enable additional states to pilot screening (2010 -2011)

• Key Features
  – Initiated pilots New York and California
  – Regionalization model
    • Puerto Rico → Massachusetts
    • Louisiana → Wisconsin
  – CDC quality assurance program
  – SCID data portal
  – Monthly conference calls to share expertise
## Interim Pilot Study Results

<table>
<thead>
<tr>
<th>Pilot</th>
<th>Annual Births/Pilot Target</th>
<th>Date: Start of Screening</th>
<th>Months Screening</th>
<th>Number of Infants Screened as of March 31, 2011</th>
<th>SCID</th>
<th>SCID Variant</th>
<th>Non SCID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wisconsin</td>
<td>69,232</td>
<td>1/1/2008</td>
<td>39</td>
<td>225,004</td>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>77,022</td>
<td>2/1/2009</td>
<td>26</td>
<td>161,707</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Navajo Nation</td>
<td>2,000</td>
<td>2/1/2009</td>
<td>26</td>
<td>1,297</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>California</td>
<td>510,000</td>
<td>8/1/2010</td>
<td>8</td>
<td>340,000</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>45,620</td>
<td>8/1/2010</td>
<td>8</td>
<td>29,115</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>New York</td>
<td>236,656</td>
<td>9/30/2010</td>
<td>6</td>
<td>118,328</td>
<td>2</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Louisiana</td>
<td>65,268</td>
<td>10/1/2010</td>
<td>6</td>
<td>32,634</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>119</strong></td>
<td></td>
<td><strong>914,557</strong></td>
<td></td>
<td><strong>12</strong></td>
<td><strong>7</strong></td>
<td><strong>35</strong></td>
</tr>
</tbody>
</table>

*Courtesy: Amy Brower & SACHDNC*
Emerging Findings

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Incidence</th>
<th>CA</th>
<th>NY</th>
<th>MA</th>
<th>WI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCID</strong></td>
<td>1 in 68,000</td>
<td>1 in 59,164</td>
<td>1 in 161,707</td>
<td>1 in 56,251**</td>
<td></td>
</tr>
<tr>
<td><strong>SCID Variant</strong></td>
<td>1 in 68,000</td>
<td>1 in 59,164</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>SCID + SCID Variant</strong></td>
<td>1 in 34,000</td>
<td>1 in 29,582</td>
<td>1 in 161,707</td>
<td>1 in 56,251**</td>
<td></td>
</tr>
</tbody>
</table>

*Incidence is generally higher than previously reported*

*LA and PR have not had a case

**Rate calculated with an additional SCID case identified in April, 2011

Courtesy: Amy Brower & SACHDNC
Emerging Findings

- Zero TREC with normal copy number for genomic PCR control consistently means the infant is at risk for profound T-cell lymphocyte deficiency.

- Majority of classic SCID cases have zero TREC.

- Molecular etiology of low TREC cases is varied.
Status of Nationwide Implementation

State-wide Screening
Partial Screening
Targeted Pilots
Screening Approved
Fact Finding
Regional Partner

Courtesy: Amy Brower & SACHDNC
All states surveyed have actively considered SCID newborn screening.

Twenty states have presented SCID newborn screening to their state advisory boards and all have recommended implementation.

Over 35% of states participate in a monthly conference call to share expertise and information.

Pilot states have played a key role in educating interested states and stakeholders.

Nine states rely on regional partners to adopt SCID newborn screening.

Three states report a requirement for an FDA cleared or approved kit.
## Acknowledgements

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Association of Public Health Laboratories

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Immune Deficiency Foundation

PerkinElmer Genetics

Newborn Screening Translational Research Network

Newborn Screening Regional Collaborative Groups and National Coordinating Center

American College of Medical Genetics
Thank you for your attention!

For more information please contact Centers for Disease Control and Prevention
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E-mail: cdcinfo@cdc.gov    Web: www.cdc.gov

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