Connecticut Newborn Screening For Severe Combined Immunodeficiency (SCID)

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Presented to the Advisory Committee on Heritable Disorders in Newborns and Children
November 9, 2017

CT Newborn Screening

Screening of all CT newborns for select genetic and metabolic disorders is mandated

- Connecticut General Statutes (CGS 19a-55)

The CT State Lab screens for 64 disorders including AA, OA, Urea Cycle, FAO, hemoglobin production, endocrine, autoimmune & peroxisomal disorders

- 37,242 births in 2016
- 99.89% newborns screened
- CF Screening conducted at UCONN and Yale Laboratories
- DPH Family Health Section oversees hearing screening, CCHD screening and birth defect registry

Connecticut NBS Implementation Timeline

1964 | 1979 | 1983 | 1995 | 05/2004 | 09/2004 | 11/2004 | 01/2005 | 09/2010 | 10/2011 | 07/2016

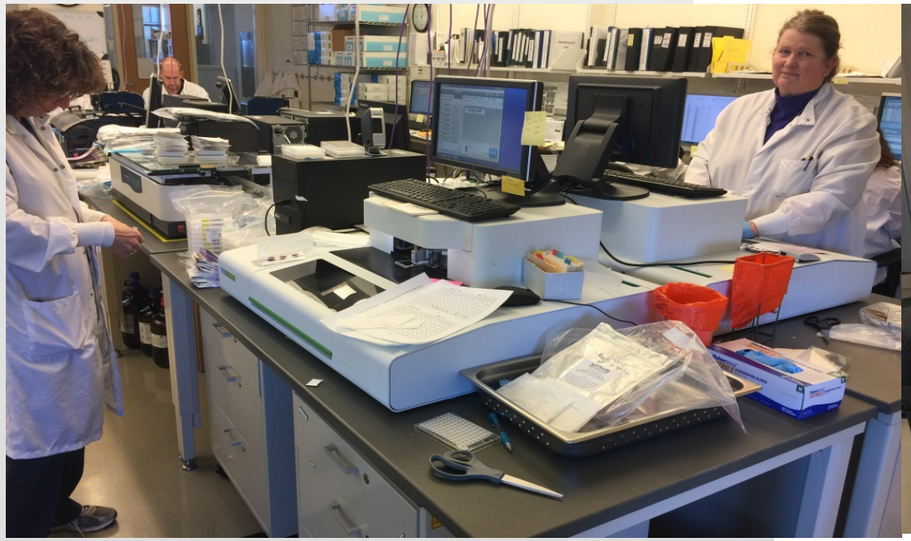
- 1964
  - PKU
- 1979
  - CH
  - GALT
- 1983
  - MSUD
  - HCY
  - BIO
  - HGB S
  - HGB SC
  - Hgb C
  - Hgb SD
  - Hgb D
  - Hgb SE
  - Hgb E
  - Hgb Bart's
  - Hgb Sβ° Thal
  - Variant Hg
- 1995
  - CAH
- 05/2004
  - MSUD
  - HCY
  - MET
  - TYR
  - MCAD
  - LCHAD
  - VLCAD
  - TFP
- 09/2004
  - CPT1
  - GAII
  - CPTII
  - CACT
  - CUD
- 11/2004
  - PPA
  - MMA
  - IVA
  - HMG
  - 3MCC
  - MCD
  - GA I
  - ßKT
- 01/2005
  - ARG
  - CIT
  - ASA
  - OTC
  - SCAD
  - DE RED
  - MMA
  - HHH*
  - NKH*
- 09/2010
  - M/SCAD
  - IBG
  - EME
  - FIGLU
  - 2MBG
  - 2M3HBA
  - 3MGA
  - CPS
  - PC
  - RMD
  - PHE
  - BIOPT (REG)
  - BIOPT (BS)
- 10/2011
  - SCID
  - T-Cell Lymph openia
- 07/2016
  - X-ALD

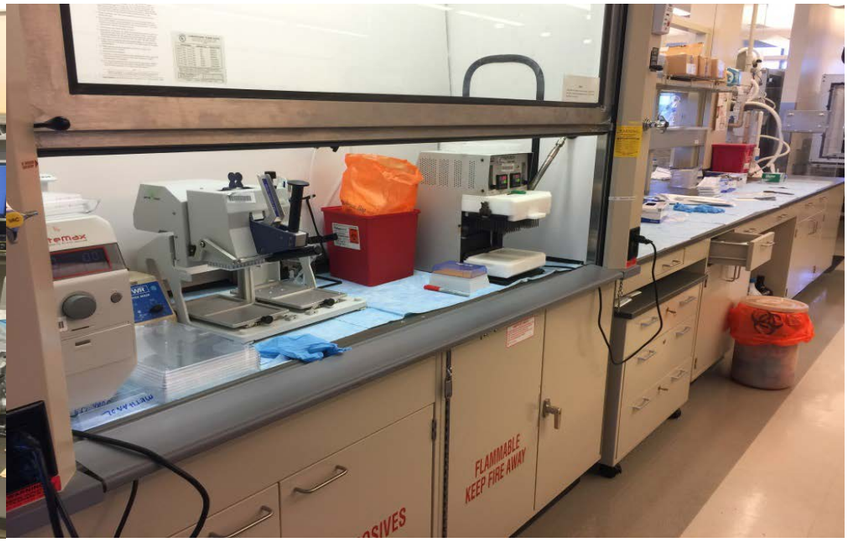
*removed 2016

CT Newborn Screening

Laboratory Responsibilities

- Receipt, login, sample quality evaluation
- Creating worklists, punching of samples into 96-well plates
- Sample preparation
- Instrument maintenance and analysis set-up
- Sample interpretation
- Reporting of sample results





CT Newborn Screening

Short term Follow-up and Tracking Responsibilities

- Using the NBS database, assuring that all infants are screened
- Reporting abnormal results and
  - Requesting a repeat NBS specimen or
  - Referring to a regional diagnostic/treatment center
- Following up through diagnosis or exclusion of a disorder
- Maintaining and reporting of statistics
- Educating stakeholders
- Maintaining and trouble shooting the NBS database
- Collaborating with and supporting hospital and birthing center staff, diagnostic/ treatment center staff, primary care providers and parents

Screening for Severe Combined Immunodeficiency (SCID) In Connecticut

Challenges For Implementation Of Molecular Screening Tests In A Newborn Screening Program

FUNDING **STAFFING** **SPACE**

SCID NBS Implementation in CT

Timeline

- 2008
  - National Level - Grant awarded to two laboratories for SCID testing (MA and WI)
  - Connecticut – Program had 8 Laboratory staff (12 in 2006-2007)
- Financial crisis—budget cuts and union concessions
- December 2009
  - Increased interest in SCID testing from laboratory management
  - Information was gathered from Massachusetts, Wisconsin and CDC
- Evaluation of available methods begins
- 2010 – Training Opportunities
  - February at the CDC
  - April at the New England Newborn Screening Laboratory
  - May at the Wisconsin Newborn Screening Laboratory
- Attempt made to acquire funds to implement SCID newborn screening in April 2010; no funding available

SCID NBS Implementation in CT

History and Advocacy

Jeffrey Modell Foundation
747 Third Avenue
New York, NY 10017
www.info4pi.org
T: 212.819.0200
F: 212.764.4180
Hotline: 866.INFO.4.PI
Curing PI. Worldwide.

Governor M. Jodi Rell
Office of the Governor
State of Connecticut
210 Capitol Avenue
Hartford, CT 06106

Dear Governor Rell,

This communication is a response to your letter dated July 29, 2010. We want to assure you that we offer Connecticut our support to accelerate this process as soon as possible. We work closely with one of the world's most preeminent immunologists, Dr. Eric Meffre, at Yale, New Haven.

The states of Wisconsin and Massachusetts are now in their third year of general population screening for SCID, and several states, including Louisiana, New York, and California, are starting programs at this time. The results of those programs have been published in leading scientific journals including the Journal of Allergy and Clinical Immunology (September 2009), JAMA (December 2009), and Public Health Report (May/June 2010). If you have any difficulty accessing these published articles please let us know and we will send them to you.

We would very much appreciate the opportunity to share the technology and results achieved to date with Connecticut. Several babies have already been saved as a result of the Newborn Screening Program, and together we can save many more precious lives.

Please let us know of your suggested next steps.

Best regards,

Vicki and Fred Modell

Vicki and Fred Modell
Co-founders
Jeffrey Modell Foundation

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November 4, 201(

Dear Ms. Manning,

As you know, Health and Human Services Secretary Kathleen Sebelius, recently acted to add Severe Combined Immune Deficiency (SCID) to the core panel for universal screening of all newborns in the United States. The Jeffrey Modell Foundation has successfully implemented and helped fund this program, utilizing the TREC's Assay as an initial screen and Bone Marrow Transplantation as an effective intervention.

We are pleased to report to you that 8 states are now screening all newborns for SCID and over 1.5 million newborns will be screened for this devastating disorder. There are 17 additional states currently developing programs to screen for SCID and related T-Cell Lymphopenia. Indeed, this program has already saved many babies' lives!

Attached is a recently published article from the Journal of American Medical Association relating to this initiative. We have also included a brief "snapshot" of the Jeffrey Modell Foundation. We hope to pursue a working collaboration with the State of Connecticut pursuant to past communication. Please let us know how we can assist you in implementing this program and whether you require any further information.

With hope for our cause,

Vicki and Fred Modell

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SCID NBS Implementation in CT

Timeline

2010

- **National Level - ACHDNC Recommends SCID Screening to be added to the RUSP**
- **Connecticut - Mid-2010 to 2011: 6 laboratory staff**

2011

- **January: SB543 “An Act Providing Newborn Screening for Severe Combined Immunodeficiency Disease”**
- **July: SCID mandated to start October 1, 2011 via Section 38 of Public Act (PA) 11-48**
- **July: CDC In situ method chosen**
- **July: Equipment requisitions using agency funding for capital equipment procurement placed**
- **July: Method development and testing began July 2011**
- **August: Staff attend training at CDC for preparation of testing calibrator and control reference materials**
- **October: Validation began**

All infants born as of 1st October 2011 were screened for SCID with official start date of January 1, 2012.

SCID NBS Implementation in CT

Selection of Method – CDC In Situ Method

FUNDING

COST:

- **~$80,000 in instrument costs and ~$10,000 in ancillary costs**
- **QC and reference materials prepared at CDC during method training**

STAFFING

MINIMAL STAFFING/SPECIALIZED STAFFING REQUIRED:

- **Only 6 existing NBS staff**
- **No staff familiar with molecular biology/PCR methodology experience available**
- **Master's student intern available from UCONN**
- **No DNA extraction required—easier method**

SPACE

MINIMAL SPACE REQUIRED:

- **No DNA extraction required—less space required, however no space available within the NBS laboratory: NEEDED TO BE CREATIVE**
- **Space was initially provided in another (serology) laboratory: a STORAGE CLOSET was emptied and converted to sample preparation area (dead air box used for preparation of primers, probes and mastermix), this area contained all pre-PCR steps/equipment**
- **~4-feet of bench top space in the serology laboratory marked off for Stratagene PCR equipment**
- **Also were able to share Stratagene PCR equipment from another laboratory to decrease analysis time**

SCID NBS Implementation in CT

Selection of Method – CDC In Situ Method

8-point DBS B-TREC calibration curve

- Prepared using T lymphocyte depleted blood with aliquots of a human EBV (Epstein Barr virus)-transformed B-cell line that contain a single copy of TREC per cell for final a nominal concentration of TREC/µL of blood where a known number of cells have been added.*
- Quantitative and qualitative QC reference materials
- PerfCta Multiplex RT (2.5X) reaction cocktail for PCR amplification
- Qiagen DNA Purification Solution 1 and DNA Elution Solution 2
- Primers and Probes for TREC and RNase P

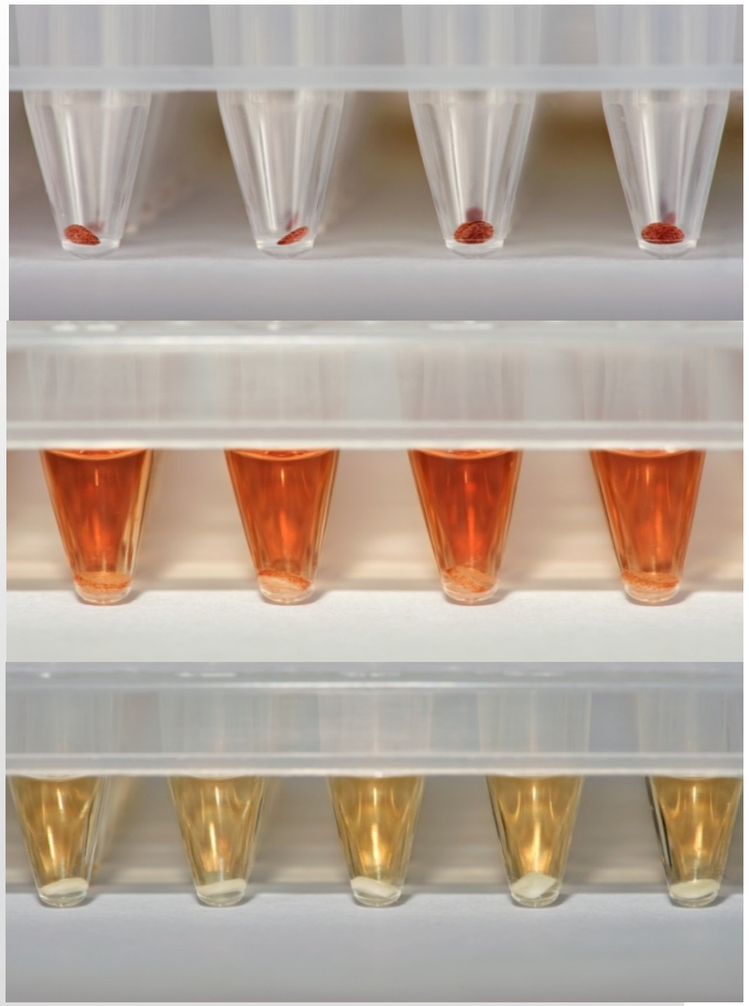
Calibrator ID	TREC copies/µl blood
CT BTREC CAL1	1500
CT BTREC CAL2	750
CT BTREC CAL3	350
CT BTREC CAL4	175
CT BTREC CAL5	75
CT BTREC CAL6	30
CT BTREC CAL7	15
CT BTREC CAL8	8

Sequence Description	Sequence
TREC Primer-Forward	TTTGTAAAGGTGCCCACTCCT
TREC Primer-Reverse	TATTGCAACTCGTGAGAACGGTGAAT
RnaseP Primer-Forward	AGATTTGGACCTGCGAGCG
RnaseP Primer-Reverse	GAGCGGCTGTCTCCACAAGT
TREC Probe FAM	/56-FAM/CGGTGATGCATAGGCACCTGC/3IABlk_FQ/
RnaseP Probe HEX	/5HEX/TTCTGACCTGAAGGCTCTGCGCG/3IABlk_FQ/

*Punwani D, Gonzalez-Espinosa D, Comeau AM, Dutra A, Pak E, Puck J, *Cellular calibrators to quantitate T-cell receptor excision circles (TRECs) in clinical samples.* Molecular Genetics and Metabolism, 2012 Nov;107(3):586-91. doi: 10.1016/j.ymgme.2012.09.018. Epub 2012 Sep 21

SCID NBS Implementation in CT

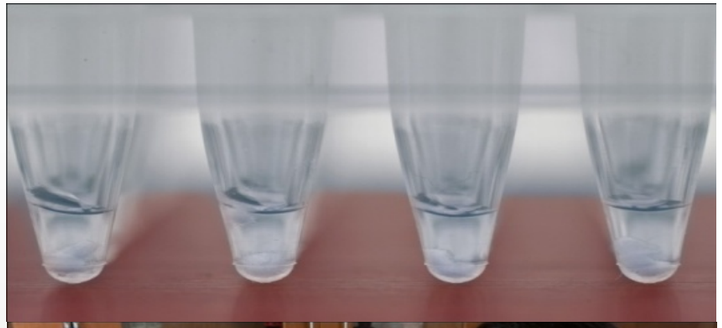
Method Summary

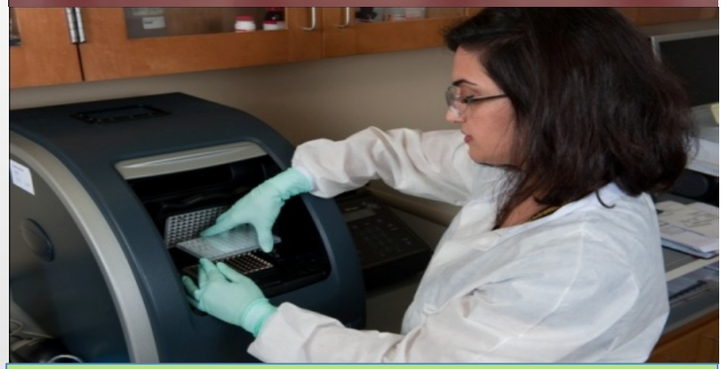


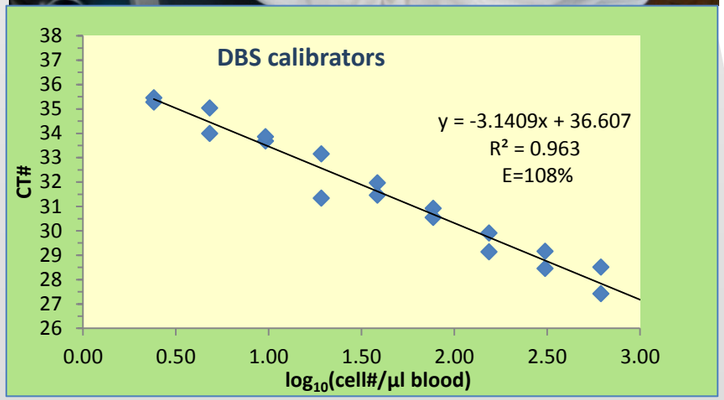
- Punch one 2.0 mm discs from DBS specimen into PCR tubes
- Wash with 125 µl of DNA purification solution S1 (shake for 15 minutes at room temp)
- Wash with 125 µl of DNA elution solution S2 (shake for 5 minutes at room temp)

SCID NBS Implementation in CT

Method Summary







- **Discard S2 wash buffer and add 15 µl of qPCR Master Mix**
- **Run qPCR in Stratagene MX3000p**
  - **UNG Activation (5' @ 45º)**
  - **Denaturation (20' @ 95º)**
  - **Amplification [45 cycles: 15"@ 95º / 1' @ 60º]**
- **Analyze qPCR Data, Check QC Results and Report NBS Results**

SCID NBS Implementation in CT

- Intern from UCONN assisted with method validation process due to major staffing shortages
- Pre-patient analysis meeting held with state clinical immunologist (information about who could fulfill this role obtained through discussions with CDC and Dr. Lisa Kobrynski) to set guidelines for follow-up for possible true abnormal findings; set a lower limit action level for TREC recovery
- Patient sample population analysis commenced following accuracy and precision study (samples received 10/3/11 to 11/15/11, >4400 samples analyzed)
- Massachusetts (New England Newborn Screening) program assisted with second analysis of potentially abnormal results using their well-established and validated method
- Guidance available through Massachusetts, CDC and Wisconsin during the validation process

SCID NBS Implementation in CT

Validation Results

Validation Patient Analysis Results	
Total Analyzed	4457
% Repeated Total (Full Term and Preterm)	105 (2.36%)
1st Unsatisfactory	17 (0.38%)
Not Tested (waiver, expired)	7 (0.167%)
1st Sample Abnormal Retest Specimens Tested	13 Preterm
Sample Results	**TREC copies/µL**
Median (50th Percentile)	252
10% Median (5th Percentile)	25.2
Mean (Average)	281
10% Mean	28.1
Analysis Acceptance Criteria	
Efficiency	85-115%
R-Squared	>=0.93
Validation Cutoff	**TREC copies/µL**
EGA All	55
Post Validation Initial Cutoff	**TREC copies/µL**
EGA >= 37 weeks	40
EGA <37 weeks	25
Current Cutoff	**TREC copies/µL**
EGA >= 37 weeks	30
EGA <37 weeks	25

5 Full Term Patient samples sent to Massachusetts for analysis during validation patient population study (4 normal), 1 CONFIRMED SCID during validation

SCID Newborn Screening in CT

Current Testing Information

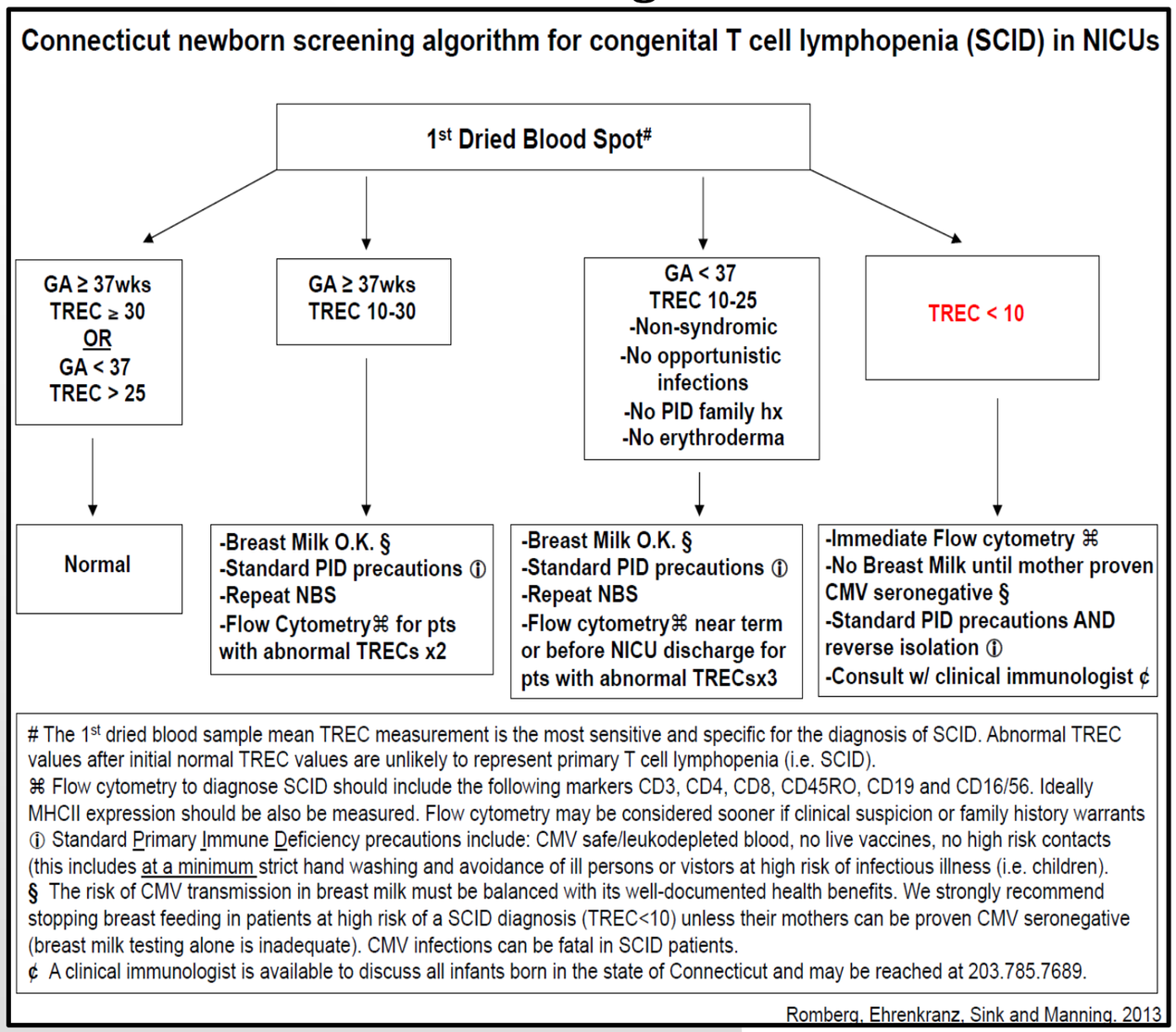
CT Algorithm for reporting sample results:

Gestation Age	TREC (copies/µL)	RNase P (Cq)	Action	Final result
Any	≥ 30	<28	NA	Normal
<37	≥ 25	<28	NA	Normal
Any	Any	≥28	Repeat sample x 2	Invalid, Request Repeat Specimen 2X
≥ 37	≥10, <30	<28	Repeat sample x 2	Abnormal, Request Repeat Specimen 1X
Any	<10	<28	Repeat sample x 2	Abnormal, Immediate Referral
Any	=No Ct	<28	Repeat sample x 2	Abnormal, Immediate Referral
< 37	≥10, <25	<28	Repeat sample x 2	Abnormal, Request Repeat Specimen 2X

SCID Newborn Screening in CT

Current Testing Information

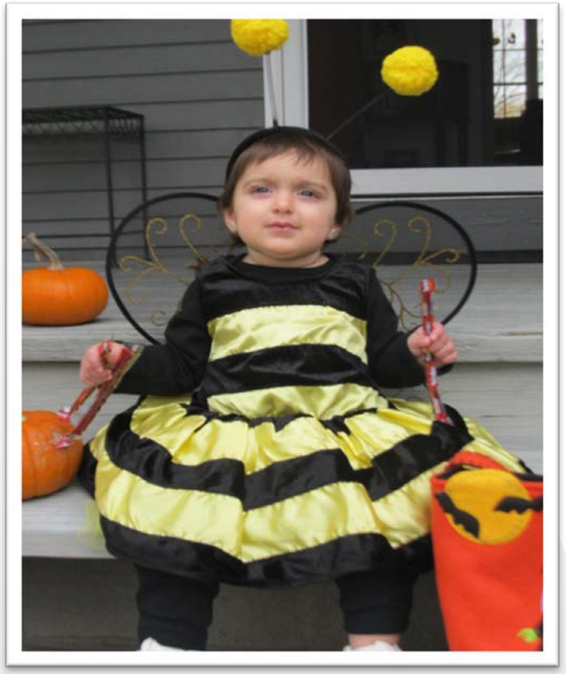
CT NICU Algorithm:



CT Patient Results: Total Infants Screened 221,554 from 2011-2017

Patient #	Description
Patient 1	Moderate T-cell Lymphopenia
Patient 2	SCID
Patient 3	22q11; partial DiGeorge
Patient 4	SCID
Patient 5	SCID
Patient 6	T & B-cell lymphopenia
Patient 7	T-cell Lymphopenia
Patient 8	T-cell Lymphopenia
Patient 9	DiGeorge Syndrome
Patient 10	CLOVES Syndrome
Patient 11	T-cell Lymphopenia
Patient 12	T-cell Lymphopenia
Patient 13	T-cell Lymphopenia
Patient 14	T and B cell lymphopenia
Patient 15	T-cell Lymphopenia; 7q32 deletion including TCR beta gene
Patient 16	Moderate T-cell Lymphopenia
Patient 17	T-cell Lymphopenia
Patient 18	Sepsis, prematurity
Patient 19	Chronic Lymphopenia
Patient 20	T-cell Lymphopenia due to prematurity
Patient 21	T-cell Lymphopenia due gastroschisis and prematurity
Patient 22	DiGeorge Syndrome
Patient 23	DiGeorge Syndrome
Patient 24	T-cell Lymphopenia due to prematurity. Bronchopulmonary dysplasia, chromosomal abnormalities with duplication at 19q13.33 and 8q13.3
Patient 25	DiGeorge Syndrome with CCHD
Patient 26	Lost to f/u
Patient 27	Sepsis, prematurity

SCID Newborn Screening in CT



Research

Original Investigation

Newborn Screening for Severe Combined Immunodeficiency in 11 Screening Programs in the United States

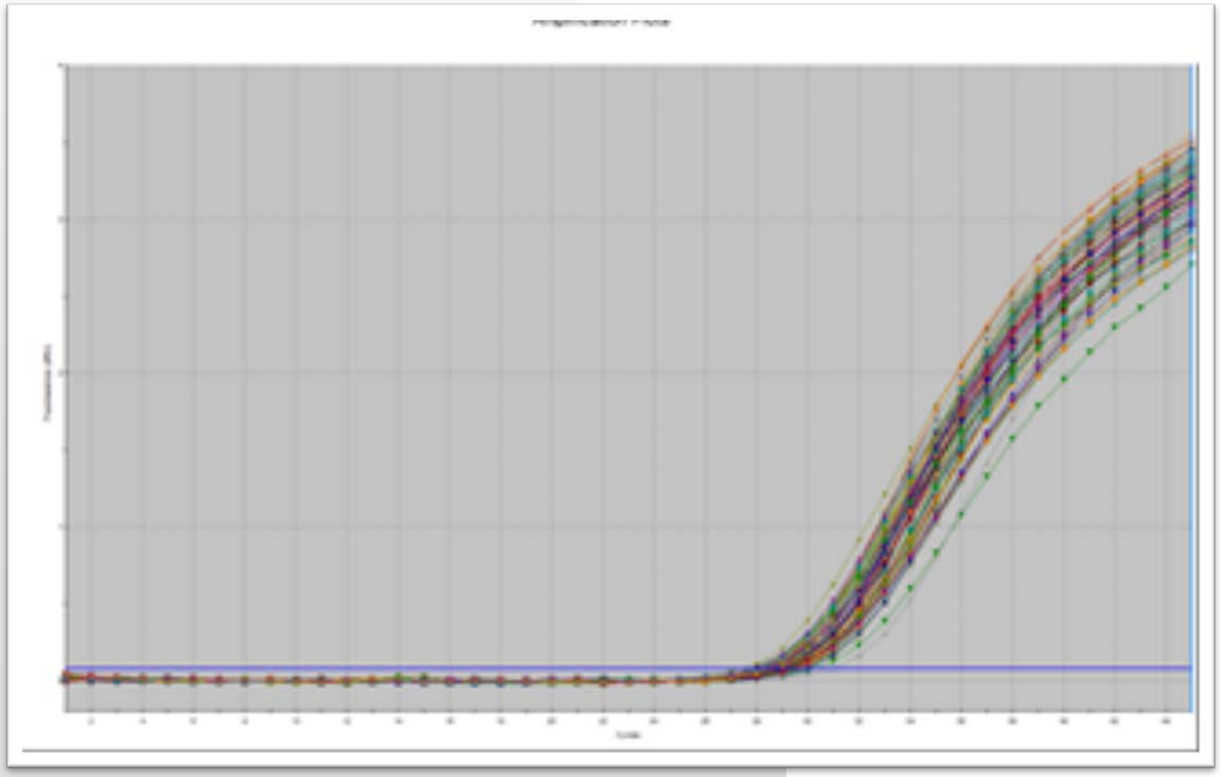
Antonia Kwan, PhD, MRCPCH; Roshini S. Abraham, PhD; Robert Currier, PhD; Amy Brower, PhD; Karen Andruszewski, BS; Jordan K. Abbott, MD; Mei Baker, MD; Mark Ballow, MD; Louis E. Bartoshesky, MD; Francisco A. Bonilla, MD, PhD; Charles Brokopp, DrPH; Edward Brooks, MD; Michele Caggana, ScD; Jocelyn Celestin, MD; Joseph A. Church, MD; Anne Marie Comeau, PhD; James A. Connelly, MD; Morton J. Cowan, MD; Charlotte Cunningham-Rundles, MD; Trivikram Dasu, PhD; Nina Dave, MD; Maria T. De La Morena, MD; Ulrich Duffner, MD; Chin-To Fong, MD; Lisa Forbes, MD; Debra Freedenberg, MD; Erwin W. Gelfand, MD; Jaime E. Hale, BS; I. Celine Hanson, MD; Beverly N. Hay, MD; Diana Hu, MD; Anthony Infante, MD, PhD; Daisy Johnson, BSN; Neena Kapoor, MD; Denise M. Kay, PhD; Donald B. Kohn, MD; Rachel Lee, PhD; Heather Lehman, MD; Zhili Lin, PhD; Fred Lorey, PhD; Aly Abdel-Mageed, MD, MBA; Adrienne Manning, BS; Sean McGhee, MD; Theodore B. Moore, MD; Stanley J. Naides, MD; Luigi D. Notarangelo, MD; Jordan S. Orange, MD; Sung-Yun Pai, MD; Matthew Porteus, MD, PhD; Ray Rodriguez, MD, JD, MPH, MBA; Neil Romberg, MD; John Routes, MD; Mary Ruehle, MS; Arye Rubenstein, MD; Carlos A. Saavedra-Matiz, MD; Ginger Scott, RN; Patricia M. Scott, MT; Elizabeth Secord, MD; Christine Seroogy, MD; William T. Shearer, MD, PhD; Subhadra Siegel, MD; Stacy K. Silvers, MD; E. Richard Stiehm, MD; Robert W. Sugerman, MD; John L. Sullivan, MD; Susan Tanksley, PhD; Millard L. Tierce IV, DO; James Verbsky, MD, PhD; Beth Vogel, MS; Rosalyn Walker, MD; Kelly Walkovich, MD; Jolan E. Walter, MD, PhD; Richard L. Wasserman, MD, PhD; Michael S. Watson, MS, PhD; Geoffrey A. Weinberg, MD; Leonard B. Weiner, MD; Heather Wood, MS; Anne B. Yates, MD; Jennifer M. Puck, MD

JAMA. 2014;312(7):729-738. doi:10.1001/jama.2014.9132

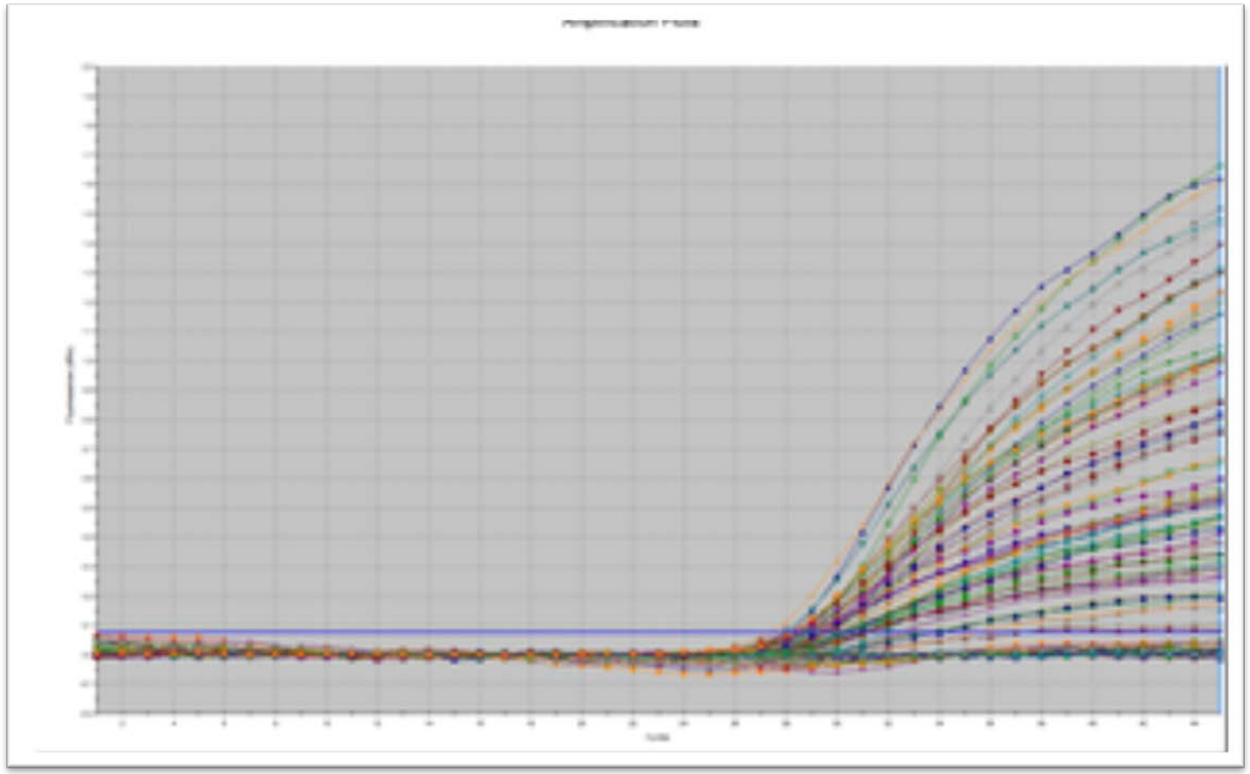
SCID Newborn Screening in CT

Mid-2014 SCID Assay Troubleshooting

What the amplification plots <u>should</u> look like for TREC



What the amplification plots <u>actually</u> looked like for TREC



SCID Newborn Screening in CT

Mid-2014 SCID Assay Troubleshooting

PROBLEM

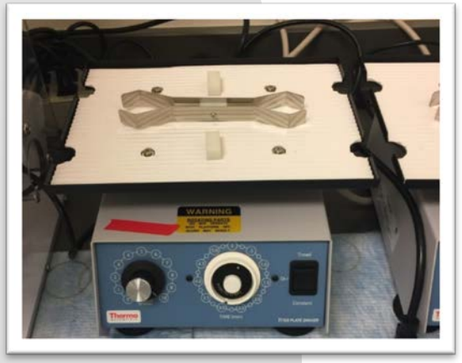
- Multiple plate analysis failures
- 14 days of sample analysis backlog

TROUBLESHOOTING

- Contacted and collaborated with CDC Newborn Screening and Molecular Biology Branch: (Dr. Francis Lee, Dr. Jennifer Taylor and Golriz Yazdanpanah)
- Identified and eliminated potential causes

- PCR Instrument
- Mastermix
- Primers/Probes
- S1 & S2 Reagents
- Calibration Reference Material

CULPRIT



SOLUTION

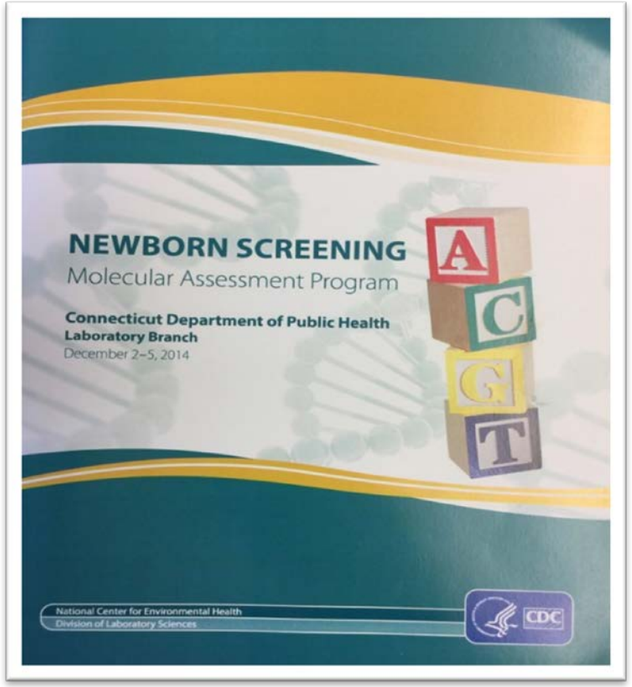
SCID Newborn Screening in CT

FURTHER IMPROVEMENTS

- New Laboratory Space (as of 2012)
- Additional Instrumentation
- Additional Staff

MOLECULAR ASSESSMENT PROGRAM

- Reconfiguration of laboratory SCID testing setup/space



Implementation of SCID NBS in CT

Summary

Connecticut SCID NBS launch was successful (6th state in the country to start screening for SCID), however it was not without CHALLENGES

METHOD CHOICE

- **In 2011, limited choices and no commercial method available, choice was between DNA extraction methods or In situ method**
- **Currently both commercial kits and LDTs are available for laboratories to choose from: MORE CHOICES enable laboratories to choose between FDA approved kits or LDTs based upon technical expertise, convenience, etc.**
- **In 2011 CT had no expertise with PCR, least complicated method was chosen, has worked very well**

STAFFING

- **No experience with PCR methods, but lots of SUPPORT/HELP available and given by other NBS laboratories (Massachusetts and Wisconsin) and the CDC to assist CT to start SCID testing**
- **Immunologist identified through assistance given by an immunologist in another state—one with contacts around the country**
- **Critically low staffing at time of mandate, however, methodology used was easier and required very little time to complete (~30minutes sample preparation, 2hours analysis)**

SPACE

- **Necessary to be CREATIVE/INNOVATIVE to identify and set up the minimal amount of space (Pre-PCR, Post-PCR) for carrying out the procedure (initially we used a storage closet and ~4feet of bench space in another laboratory)**

FUNDING

- **For the types of assays available (commercial kits or LDTs), LDTs generally are less expensive**
- **Sharing of equipment with another laboratory reduced the initial amount of $$ needed to start SCID testing**

ACKNOWLEDGEMENTS

Connecticut Newborn Screening	**Molecular Assessment Program**
Joseph Ubaike	Suzanne Cordovado (CDC Molecular Quality Improvement Program)
Corina Boluk	Christopher Greene (CDC Molecular Quality Improvement Program)
Mary Jo Guiliano	Rachel Lee (Biochemistry and Genetics Branch Manager, Texas DSHS)
Debra Studwell	Tim Davis (Lead Microbiologist, Hemoglobin and Molecular, Washington DOH)
Leslie Mills	Guisou Zarbalian (Senior Specialist, Newborn Screening and Genetics, Association of Public Health Laboratories)
Edith Zimmermann	**CDC Newborn Screening and Molecular Biology Branch**
Kathryn Holden	Carla Cuthbert
Agnieszka Bouthot	Robert Vogt
Anna Filipkowska	Francis Lee
Barbara Szupryczynski	Golriz Yazdanpanah
Julie Riccio	Jennifer Taylor **(RTI International)**
Ryan Richard	**CDC Foundation**
Marie Burlette	**Dr. Lisa Kobrynski**
Rose Marie Mitchell	**New England Newborn Screening**
Linda Bailey	Anne Comeau
Yale New Haven Hospital	Jackie Gerstel-Thompson
Neil Romberg	**Wisconsin Newborn Screening**
Odelya Pagovich	Mei Baker
Jason Catanzaro	**University of Connecticut**
Tamar Rubin	Lia Ribustello
CCMC Pediatric Infectious Diseases and Immunology	Nicholas Bennett

Thank You!