Newborn Screening for Severe Combined Immunodeficiency:

NSQAP TREC Proficiency Testing and NSTRI MPES Program

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Newborn Screening Translational Research Initiative
Newborn Screening and Molecular Biology Branch, CDC

NBS Molecular Biology Training Workshop
CDC, Atlanta, March 9-13, 2015
MPES: Model Proficiency Evaluation Survey
NSQAP: Newborn Screening Quality Assurance Program
PROFICIENCY TESTING

- NSQAP PT for TREC

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- Irene Williams for NQSAP PT
  ial2@cdc.gov 770-488-7024
- Francis Lee for TREC MPES
  icr0@cdc.gov 770-488-7946
# Number of Countries Participating TREC PT Program Expansion

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>23</td>
</tr>
<tr>
<td>Canada</td>
<td>2</td>
</tr>
<tr>
<td>China</td>
<td>2</td>
</tr>
<tr>
<td>Finland</td>
<td>1</td>
</tr>
<tr>
<td>France</td>
<td>2</td>
</tr>
<tr>
<td>Iceland</td>
<td>1</td>
</tr>
<tr>
<td>India</td>
<td>1</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
</tr>
<tr>
<td>Switzerland</td>
<td>1</td>
</tr>
<tr>
<td>Taiwan</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total Laboratories</strong></td>
<td><strong>38</strong></td>
</tr>
<tr>
<td><strong>Total Countries</strong></td>
<td><strong>10</strong></td>
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</table>
NSQAP TREC PT Program Expansion 2011-2015

Enrollment of International Labs
# Newborn Screening Quality Assurance Program

**T-Cell Receptor Excision Circle (TREC) Analysis in Dried-Blood Spots To Detect Severe Combined Immunodeficiency (SCID) Pilot Proficiency Testing (PT) program**

**Issued:** April 6, 2015  
**Data Reporting Deadline:** May 4, 2015

Email your complete worksheet to Irene Williams at iwilliams1@cdc.gov. Phone number is 770-488-7024.

## Specimen Numbers and Analytes

<table>
<thead>
<tr>
<th>Specimen Number</th>
<th>Analyte</th>
<th>Assessment Code</th>
<th>If follow-up is required, select reference gene category</th>
</tr>
</thead>
<tbody>
<tr>
<td>215R1</td>
<td>TREC</td>
<td>1 - No Follow-up required (Screen Negative) 2 - Follow-up required</td>
<td>Reference gene category</td>
</tr>
<tr>
<td>215R2</td>
<td>TREC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>215R3</td>
<td>TREC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>215R4</td>
<td>TREC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>215R5</td>
<td>TREC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Supporting Tables

### LIST OF METHOD CODES
- 63 Real Time PCR
- 70 EnLite™ Neonatal TREC kit
- 19 Other (Please specify name and source)

### LIST OF DNA PREPARATION METHODS
1. In situ/on card (no DNA extraction) with washing step(s)
2. In situ/on card (no DNA extraction) with no washing step
3. DNA extracted at 95°C with washing step(s)
4. DNA extracted with no washing step
5. Other

### LIST OF ASSAY OVERALL ASSESSMENT CODES
- 1 No follow-up required (Screen Negative)
- 2 Follow-up required

### LIST OF REFERENCE GENES
1. RNase P (RPPH1) - Ribonuclease P coding segment
2. Beta-actin
3. Serum albumin
4. TERT - Telomerase Reverse Transcriptase
5. Other

### REFERENCE GENE ASSESSMENT CATEGORY
- 1 Below normal range
- 2 Within normal range
- 3 Above normal range
## Types of TREC PT Specimens

### Assessment Code 1 (No Follow-Up Required) Specimen Type

<table>
<thead>
<tr>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal specimen; below average TREC level, reference gene level within standard reference range</td>
</tr>
<tr>
<td>Normal specimen; medium TREC level, reference gene level within standard reference range</td>
</tr>
<tr>
<td>Normal specimen; TREC level below population average but within reference range, reference gene level within standard reference range</td>
</tr>
</tbody>
</table>

### Assessment Code 2 (Follow-Up Required) Specimen Type

<table>
<thead>
<tr>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte-reduced blood -  TREC and reference gene levels both below standard reference range</td>
</tr>
<tr>
<td>SCID-like specimen; low or no TREC, reference gene level within standard reference range</td>
</tr>
</tbody>
</table>
## Number of Proficiency Testing Misclassification Errors 2011-2015

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Quarters</th>
<th>TREC within reference range identified as “Follow-Up Required”*</th>
<th>TREC below reference range identified as “No Follow-up Required”</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2012</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2014**</td>
<td>4</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>2015</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

*Misclassifications likely due to conservative TREC analytical cutoffs.

**Increased in misclassifications due to international lab participation in TREC PT Program.
## DNA Preparation and Method

<table>
<thead>
<tr>
<th>DNA preparation Method</th>
<th>Number of Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. In situ/on card (no DNA extraction) with washing step(s)</td>
<td>5</td>
</tr>
<tr>
<td>2. EnLite™ (no DNA extraction)</td>
<td>3</td>
</tr>
<tr>
<td>3. DNA extracted at 99°C with washing step(s)</td>
<td>11</td>
</tr>
<tr>
<td>4. DNA extracted at 95°C with washing step(s)</td>
<td>2</td>
</tr>
<tr>
<td>5. DNA extracted at 70°C with washing step(s)</td>
<td>2</td>
</tr>
<tr>
<td>6. DNA extracted with no washing steps</td>
<td>0</td>
</tr>
<tr>
<td>7. Other</td>
<td>3</td>
</tr>
<tr>
<td>8. Not provided</td>
<td>3</td>
</tr>
</tbody>
</table>
## Laboratory Method for TREC Assay

<table>
<thead>
<tr>
<th>Laboratory Method</th>
<th>Number of Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Real-Time PCR - Singleplex</td>
<td>10</td>
</tr>
<tr>
<td>2. Real-Time PCR - Multiplex</td>
<td>15</td>
</tr>
<tr>
<td>3. EnLite™ Neonatal TREC Kit</td>
<td>3</td>
</tr>
<tr>
<td>4. Other</td>
<td>1</td>
</tr>
</tbody>
</table>
Model Performance Evaluation Survey

- Started in February 2010 with three labs (WI, MA, CDC)
- Initially as an accelerated pilot program for proficiency testing for TREC assay
- 31 Laboratories currently participating
  - All NBS labs in routine population-based newborn screening for SCID
  - Additional labs in assay development or validation
- Evolved into a collaborative project to address issues of common interests to SCID screening
MPES activities

- Proficiency Testing for labs not ready for NSQAP PT
- QA Materials development and distribution
- Training (individualized)
  - Assay performance
  - Reference materials and calibrators preparation
- Collaborative Projects
  - Extraction efficiency
  - TREC Copy number harmonization
NSTRI Provides Technical and Scientific Support on SCID Newborn Screening and TREC assays

Pre assay development consultation
- Laboratory set-up
- Assay platform options
- Equipment choices
- Reagents (primers, probes, qPCR mix) and supplies

Post assay development consultation
- Cutoff determination
- Precision (CV%) improvement
- Assay validation
Three Types of DBS Reference Materials for the TREC Assay

1. Normal Reference Material
   - Screen Negative for SCID
   - TREC level (H, M, L) and Reference Genes within range

2. SCID-like Reference Material
   - Screen Positive for SCID
   - TREC result out of range; Reference Genes within range

3. Unsatisfactory/Inconclusive Reference Material
   - Undetermined SCID assay result
   - Both TREC and Reference Genes out of range
Development of Reference Materials for TREC Assay Evaluation

Serial Dilutions of Cord Blood

- Cord blood dilution into Mononuclear Cell-depleted blood (no detectable TREC)
- Create equal-volume serial dilutions 100%, 50%, 25%, 12%, 6%, 3%

Utility of Reference Materials

- Assay Development: Linearity, LOD/LOQ
- Secondary Calibrator
- Cutoff determination
Quantitative Calibration of the TREC Assay

- Many NBS programs currently use plasmid solutions containing a known amount of DNA to calibrate TREC copy number in DBS
- CDC and other labs use DBS calibrators containing a known number of TREC-containing cells
  - Primary calibrator (transfected cell line)
  - Secondary calibrator (cord blood dilutions)
- UCSF / MA has developed a TREC-transfected B-cell line currently under evaluation
- Establish cutoff based on Cq value and use archived curve for copy number estimation.
Primary DBS Calibrator based on TREC-Transfected B Cell Line from USCF/NENSP

Clone #2

- B-cells immortalized by transforming with EBV
- TREC sequence was integrated into gDNA using a lentivirus
- Fluorescent *in situ* hybridization test identified cell population clone #2 with 1 insertion site of TREC sequence

Punwani, D etc. Molecular Genetics and Metabolism 107 (2012) 586-591
Cord blood DNA extracts Calibrator (values determined by digital PCR)

Ratio of TREC to Reference Gene was consistently 1:2

1 copy of TREC per cell
Data Harmonization

The TREC assays employed by different laboratories may vary in procedures, primers, probes and calibrators.

While the categorical results have been generally consistent among laboratories, the quantitative results in TREC copy number on any particular specimen can differ extensively.

Approaches to transform quantitative results in TREC copy number from different laboratories to a common scale of measurement were examined at CDC.
By converting TREC copy into Multiples of Median (MOM)

$$\text{MOM}_x = \frac{x}{\text{Median}}$$

A. Cutoff Values in MOM and TREC Copies

- TREC MOM
- TREC copies
Quantitative Comparison of DNA Extract by NBS Laboratories

- DNA was extracted from 4 cord blood units
- Samples were diluted so TREC copies fall into a range in the NBS standard curve
- Samples were analyzed for TREC copy number using the Bio-Rad ddPCR system
- Samples of DNA extract was sent to 14 laboratories
  - All domestic laboratories that extract DNA from blood spots
  - Eliminate differences in extraction procedure.
- All laboratories had higher estimated TREC copies than ddPCR
Comparison of DNA extract by ddPCR and Real-time PCR

- Real-time PCR results were 1.7 to 10.2 fold higher than ddPCR results.
- Quantitative differences observed is likely due to PCR procedure and/or standard curve used.
Take Home Messages

- CDC Proficiency Testing Programs are available for domestic and international laboratories

- NSQAP TREC PT only available for routine NBS laboratories

- MPES provides support for NBS laboratories developing TREC assay and collaborates with all NBS laboratories

- Reference materials are available for assay development, comparison with current standards, or establishing TREC copy number
  - Quality Control Materials (Normal, SCID-like, Inconclusive)
  - B-TREC cell line
  - DNA extracted from cord blood and quantified using ddPCR
Thank you for your attention!

Newborn Screening

Saving Lives.
Promoting Healthier Babies.
Protecting the Future.

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

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