Model Proficiency Evaluation Survey and NSQAP PT Program on T Cell Receptor Excision Circle (TREC) Assay for SCID

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TREC Assay in Newborn Screening for SCID

- Laboratory-developed tests with limited standardization among labs
- Significant variations in major components of assay
TREC Quantitative PCR Assays

**Basic qPCR**
- DBS DNA Extraction
- TREC sequence Amplification
- Single-Point PCR
- Amplicon Quantification

**A**
- DBS DNA Extraction
- Real-time PCR

**B**
- DBS ‘On-Card’
  - One-wash
  - Real-time PCR

**C**
- DBS ‘On-Card’
  - No-wash
  - Single-Point PCR
  - Amplicon Quantification

**Digital PCR**
- DBS DNA Extraction
- Partitioning Sample Reaction Mixture
- TREC Sequence Amplification
- End-point PCR
- Enumeration of Partitions with + or — Reaction
Other Variations in TRECs Assay Protocols

- **DNA Quantity**
  - DNA Extract (from 3 mm punch)
  - Extraction Volume / Reaction Volume
  - DNA on DBS punch
  - 2mm punch / 1.5 mm punch
  - (No wash/Wash 1x or 2x)

- **Materials and Methods**
  - Primers & Probes
    - Singleplex
    - Multiplex
  - 96/384 well format

- **Calibrators**
  - Plasmids
  - Cell-based
Model Performance Evaluation Survey

- Started in February 2010 with three labs (WI, MA, CDC)
- 19 Laboratories currently participating
  - 10 PHL in routine population-based newborn screening for SCID
  - 9 labs in assay development or validation stages
TREC Model Performance Evaluation Survey Program (MPES)

Mission: To support state public health laboratories in

- Assay development and validation
- Accelerated proficiency testing
- Transition to NSQAP PT program
- Data harmonization
Supporting public health laboratories in

- Assay development and validation
  - Consultations on:
    - Physical laboratory layout and practices
    - TREC assay format selection
    - Instruments and reagents
    - Calibrators
    - Assay validation
  - Reference materials
CDC TREC Reference Materials

QC Materials

- SCID-like DBS: mononuclear cells—depleted blood (low/no TREC, normal reference gene level)
- “Unsat” DBS: leukocyte depleted blood (low/no TREC, low reference gene)
- Cord blood DBS: (TREC and reference genes in reference range)
  - High
  - Medium
  - Low
TREC Cq distribution in DBS from newborns with normal birth wt. (N=2000) by on-card real time PCR Assay
Special Reference Materials for TREC Assay evaluation

Serial Dilutions of Cord Blood

- Begin at above median level of expected range for TREC

- Diluted into MNC-depleted blood
  (diluent w No detectable TREC; normal level of reference genes)

- 100%, 50%, 25%, 12.5%, 6.2%, 3.1%

Potential use: Assay development; LOD/LOQ studies;
  Calibration comparison; ‘Cut-off’ placement
Model Performance Evaluation Survey
an accelerated pilot PT program

- Panel sent out at 4-6 week interval
- Five well-characterized DBS with prior consensus categorization for proficiency assessment
- Additional ‘non-scoring’ DBS included for technical or harmonization studies
- All samples blinded
- Reports submitted by participants within 3 weeks
### MPES Report Form

<table>
<thead>
<tr>
<th>Lab # ___</th>
<th>TREC</th>
<th>Final Categorical Result</th>
<th>Reference Gene: ___</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cq Value</td>
<td>Copy Number</td>
<td>No F/U</td>
<td>F/U action required</td>
</tr>
<tr>
<td></td>
<td>per Rxn</td>
<td>per µL Bld</td>
<td>TREC NL</td>
<td>TREC ↓</td>
</tr>
<tr>
<td>Sample ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cutoff**

*If TREC↓ selected, indicate reference gene category*
# Sample Report from MPES Labs

## Lab #300

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Cq Value</th>
<th>Copy Number</th>
<th>No F/U</th>
<th>F/U action required</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35.1</td>
<td>5</td>
<td>5</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>29.7</td>
<td>132</td>
<td>132</td>
<td>✓ ✓</td>
<td>SCID-like</td>
</tr>
<tr>
<td>C</td>
<td>No Ct</td>
<td>0</td>
<td>0</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>31.5</td>
<td>46</td>
<td>46</td>
<td>✓ ✓</td>
<td>Normal</td>
</tr>
<tr>
<td>E</td>
<td>37.0</td>
<td>1</td>
<td>1</td>
<td>✓ ✓</td>
<td>SCID-like</td>
</tr>
<tr>
<td>F</td>
<td>29.3</td>
<td>180</td>
<td>180</td>
<td>✓ ✓</td>
<td>Normal</td>
</tr>
<tr>
<td>G</td>
<td>33.7</td>
<td>12</td>
<td>12</td>
<td>✓ ✓</td>
<td>SCID-like</td>
</tr>
</tbody>
</table>

Cutoff 25

If TREC↓ selected, indicate reference gene category 27.5 Cutoff

## Lab #999

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Cq Value</th>
<th>Copy Number</th>
<th>No F/U</th>
<th>F/U action required</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Undeterm.</td>
<td>0</td>
<td>0</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>32.7</td>
<td>146</td>
<td>943</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Undeterm.</td>
<td>0</td>
<td>0</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>34.6</td>
<td>46</td>
<td>296</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Undeterm.</td>
<td>0</td>
<td>0</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>32.3</td>
<td>195</td>
<td>1261</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Undeterm.</td>
<td>0</td>
<td>0</td>
<td>✓ ✓</td>
<td></td>
</tr>
</tbody>
</table>

Cutoff 200

If TREC↓ selected, indicate reference gene category 200 Cutoff

Cutoff 5000
## Sample CDC Report - Summary of Results

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample Code</th>
<th>No F/U</th>
<th>F/U required</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TREC NL</td>
<td>TREC ↓</td>
</tr>
<tr>
<td>High Normal</td>
<td>E</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Low Normal</td>
<td>A</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>SCID -like</td>
<td>F</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Leuko-depleted</td>
<td>B</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>CB-cal 5 (12.5%)</td>
<td>C</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>CB-cal 6 (6.3%)</td>
<td>H</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>CB-cal 7 (3.1%)</td>
<td>D</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>
Cumulative PT Results from 17 MPES Sample Panels

<table>
<thead>
<tr>
<th>Reference Gene</th>
<th>Below Cutoff</th>
<th>Above Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-Up Required</td>
<td>158/158 (100%)</td>
<td>136/136 (100%)</td>
</tr>
<tr>
<td>No Follow-Up Required</td>
<td>431/438 (99%)</td>
<td></td>
</tr>
</tbody>
</table>
NSQAP TREC Assay PT Program

- Currently restricts enrollment to domestic laboratories performing routine population-based SCID screening
- Quarterly panel of five DBS samples
- Report categorical results (f/u required or not required) only
- 10 labs currently enrolled
TREC Model Performance Evaluation Survey Program (MPES)

Data harmonization for result comparison

Development of consensus cell-based calibrators currently underway
Discussion

- Despite differences in assay format and reagents, all participating laboratories consistently identified samples with SCID-like phenotype correctly.
- Results on the cord blood dilution series indicated good agreement on F/U requirement for samples across a full range of TREC levels, even as the absolute TREC copy numbers detected vary among laboratories.
- UCSF / MA NBS program has developed a TREC-transfected B-cell line currently under evaluation.
- Consensus calibration for TREC in DBS will evolve quickly and may be achieved in the near future.
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Michigan Newborn Screening Laboratory
Connecticut Dept. Public Health Laboratory
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Rachel Lee        Susan Tanksley
Heather Wood      Kevin Cavanagh
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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.