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

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T Cell Receptor Excision Circle (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 Quantitative PCR

- DBS DNA Extraction
- TREC sequence Amplification
- Amplicon Quantification

A

- DBS DNA Extraction
- Real time PCR

B

- DBS ‘On-Card’ Real-time PCR

C

- DBS ‘On-Card’ End-Point PCR
- Amplicon Quantification
Other Variations in TREC Assay Protocols

- **DNA Quantity in each reaction**
  - DNA Extract (from 3 mm punch)
    - Extraction Volume / Reaction Volume
  - DNA on DBS punch
    - 2 mm punch / 1.5 mm punch

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

- **Calibrators**
  - Plasmids
  - Cell-based
Model Performance Evaluation Survey (MPES)

- Started in February 2010 with three core labs (WI, MA, CDC)
- 15 Laboratories currently participating
  - 7 labs performing population based screening for SCID routinely
  - 7 labs in assay development or validation
  - 1 R & D lab of a major kit manufacturer
TREC Model Performance Evaluation Survey (MPES)

Mission

to support state public health laboratories in

- Pilot proficiency testing
- Data harmonization
- Assay development and validation
- Pilot proficiency testing
Model Performance Evaluation Survey
Procedure

• Panel sent out at 4-6 week intervals
• Five well-characterized dried blood spots (DBS’s) for proficiency assessment
• Additional 2-4 ‘non-scoring’ DBS for research or training objectives
• All samples blinded
• Reports submitted by participants within 2 weeks
# MPES Report Form

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

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**Comments:**
- If TREC↓ selected, indicate reference gene category
- Cutoff 25

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**Comments:**
- If TREC↓ selected, indicate reference gene category
- Cutoff 250
- Cutoff 5000
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* Scored for PT evaluation
Cumulative PT Results from 17 MPES Sample Panels

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<tr>
<td>Above Cutoff</td>
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- **No Follow-Up Required**
  - 431/438 (99%)
Data Harmonization
Reference Materials for Data Harmonization

Serial Dilutions of Cord Blood

– Selected cord blood with TREC level at the median of the population

– Diluted into mononuclear cell-depleted blood to contain 100%, 50%, 25%, 12%, 6%, 3% cord blood

Assess Comparability of Decision Ranges
CDC NSTRI SCID supports NBS labs in early stages of TREC Assay development and validation

- Technical consultation
- Well-characterized reference materials
- Enrollment in MPES program as associate member
  - Receives monthly MPES panels
  - Share summary of results
  - Use of cord blood dilution series and other “challenging samples” for
    - Setting provisional cutoff values
    - LOD/LOQ comparison
    - Calibrator evaluation
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 within a year.
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Minnesota Newborn Screening Program
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Michigan Newborn Screening Laboratory
Connecticut Dept. Public Health Laboratory
Delaware Newborn Screening Laboratory

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E-mail: cdcinfo@cdc.gov    Web: www.cdc.gov

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.