

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

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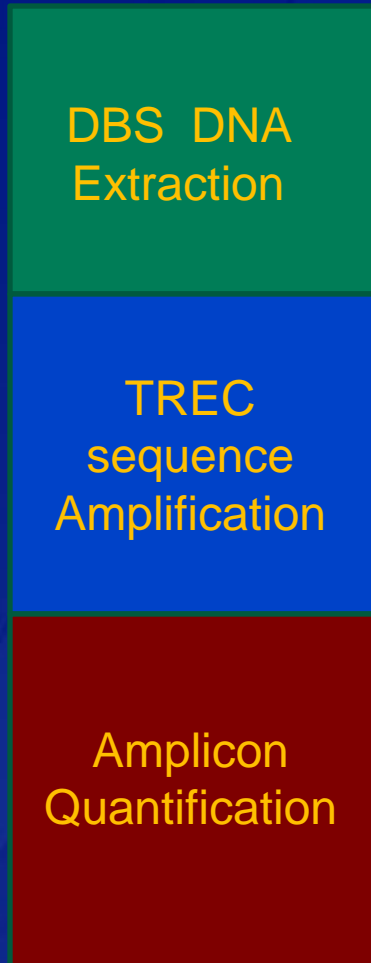


T Cell Receptor Excision Circle (TREC) Assay in Newborn Screening for SCID

- ❑ Laboratory-developed tests with limited standardization among labs**
- ❑ Significant variations in major components of assay**

TREC Quantitative PCR Assays

Basic
Quantitative PCR



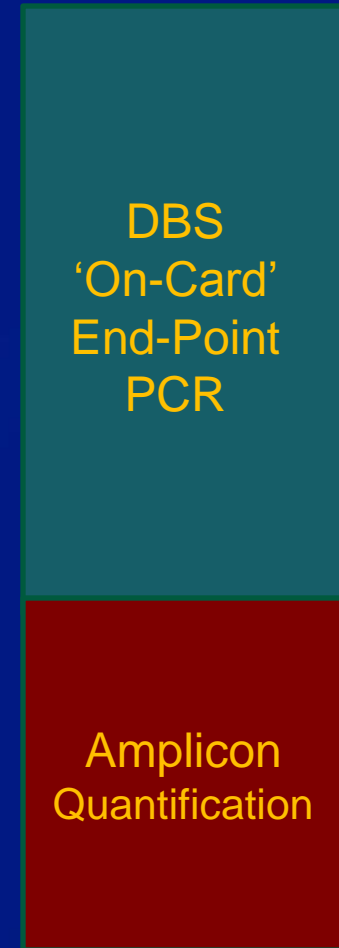
A



B



C



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
2mm 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

Lab # ____	TREC			Final Categorical Result				Reference Gene: ____			Comments
	Cq Value	Copy Number		No F/U	F/U action required			Cq Value	Copy Number		
Sample ID		per Rxn	per μ L Bld	TREC NL	TREC \downarrow	Ref gene NL	Ref gene \downarrow		per Rxn	per μ L Bld	
A											
B											
C											
D											
E											
F											
G											
		Cutoff			<i>If TREC\downarrow selected, indicate reference gene category</i>				Cutoff		

Sample Report from MPES Labs

Lab #300	TREC			Final Categorical Result				Reference Gene: RNase P			Comments
	Cq Value	Copy Number		No F/U	F/U action required			Cq Value	Copy Number		
		per Rxn	per µL Bld	TREC NL	TREC ↓	Ref gene NL	Ref gene ↓		per Rxn	per µL Bld	
A	35.1	0	0		√	√		23.7			SCID-like
B	29.7	132	132	√		√		23.9			Normal
C	No Ct	0	0		√		√	30.5			Unsat
D	31.5	46	46	√		√		25.6			Normal
E	37.0	1	1		√	√		23.8			SCID-like
F	29.3	180	180	√		√		24.2			Normal
G	33.7	12	12		√	√		23.5			SCID-like
		Cutoff	25		<i>If TREC↓selected, indicate reference gene category</i>			27.5	Cutoff		

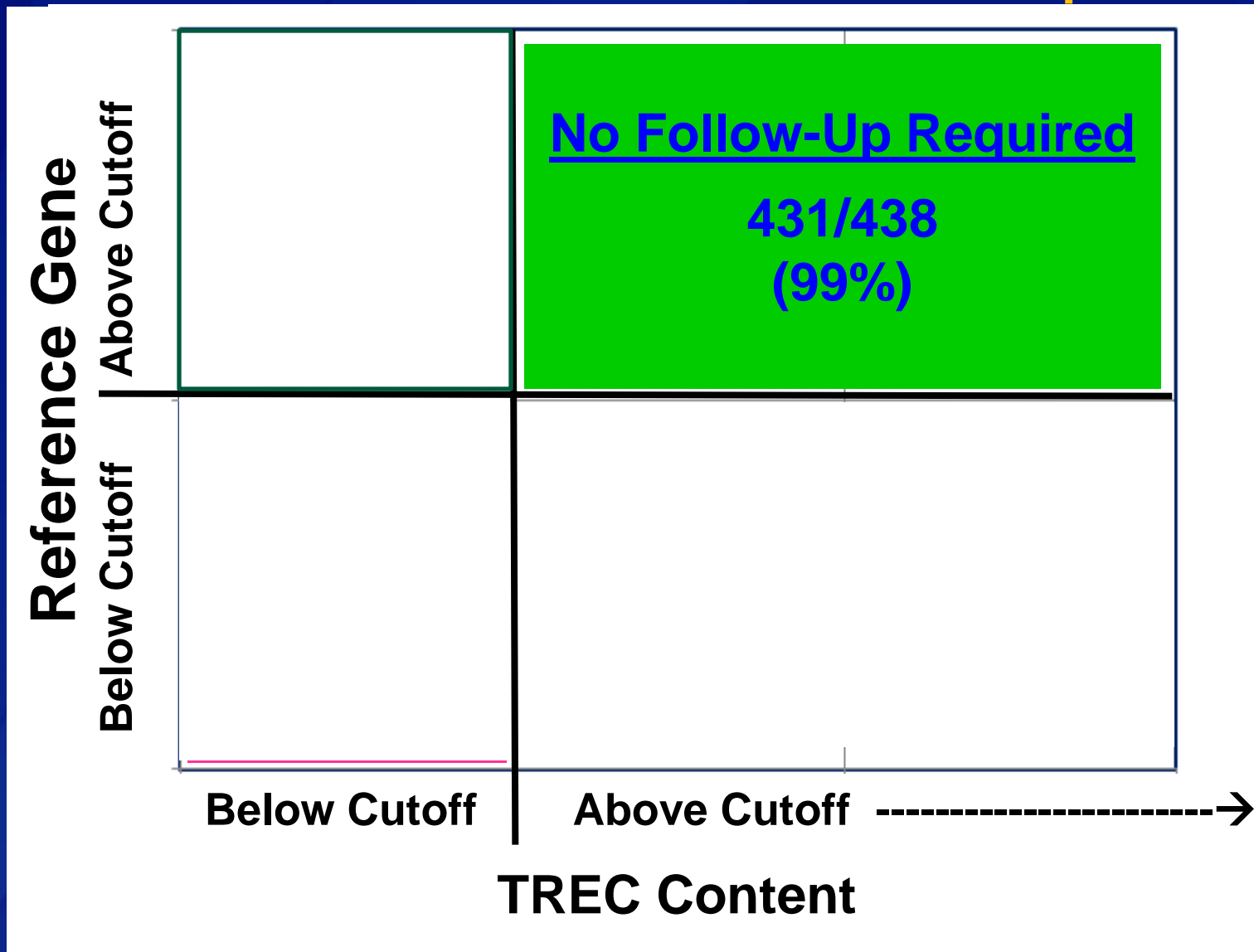
Lab #999	TREC			Final Categorical Result				Reference Gene: RNase P			Comments
	Cq Value	Copy Number		No F/U	F/U action required			Cq Value	Copy Number		
		per Rxn	per µL Bld	TREC NL	TREC ↓	Ref gene NL	Ref gene ↓		per Rxn	per µL Bld	
A	Undeterm.	0	0		√	√		26.4	2144	13833	in report. range
B	32.7	146	943	√		√		26.7	3559	22960	in ref. range
C	Undeterm.	0	0		√		√	33.5	39	250	Unsat
D	34.6	46	296	√		√		27.7	2101	13554	in ref. range
E	Undeterm.	0	0		√	√		26.8	4095	26418	in report. range
F	32.3	195	1261	√		√		27.7	1754	11313	in ref. range
G	Undeterm.	0	0		√	√		26.8	3997	25785	in report. range
		Cutoff	250		<i>If TREC↓selected, indicate reference gene category</i>				Cutoff	5000	

MPES#26 CDC Report - Summary of Results

Sample ID	Sample Code	No F/U	F/U required		
		TREC NL	TREC ↓	Ref gene NL	Ref gene ↓
MNC-depleted blood 1*	A		14	14	
Normal Cord blood med*	B	14			
Leukocyte-depleted bld*	C		14		14
Normal cord blood low*	D	14			
MNC-depleted blood 2	E		14	14	
Normal Cord blood high*	F	14			
Old adult blood	G		14	14	

* Scored for PT evaluation

Cumulative PT Results from 17 MPES Sample Panels



□ Data Harmonization

Reference Materials for Data Harmonization

Serial Dilutions of Cord Blood

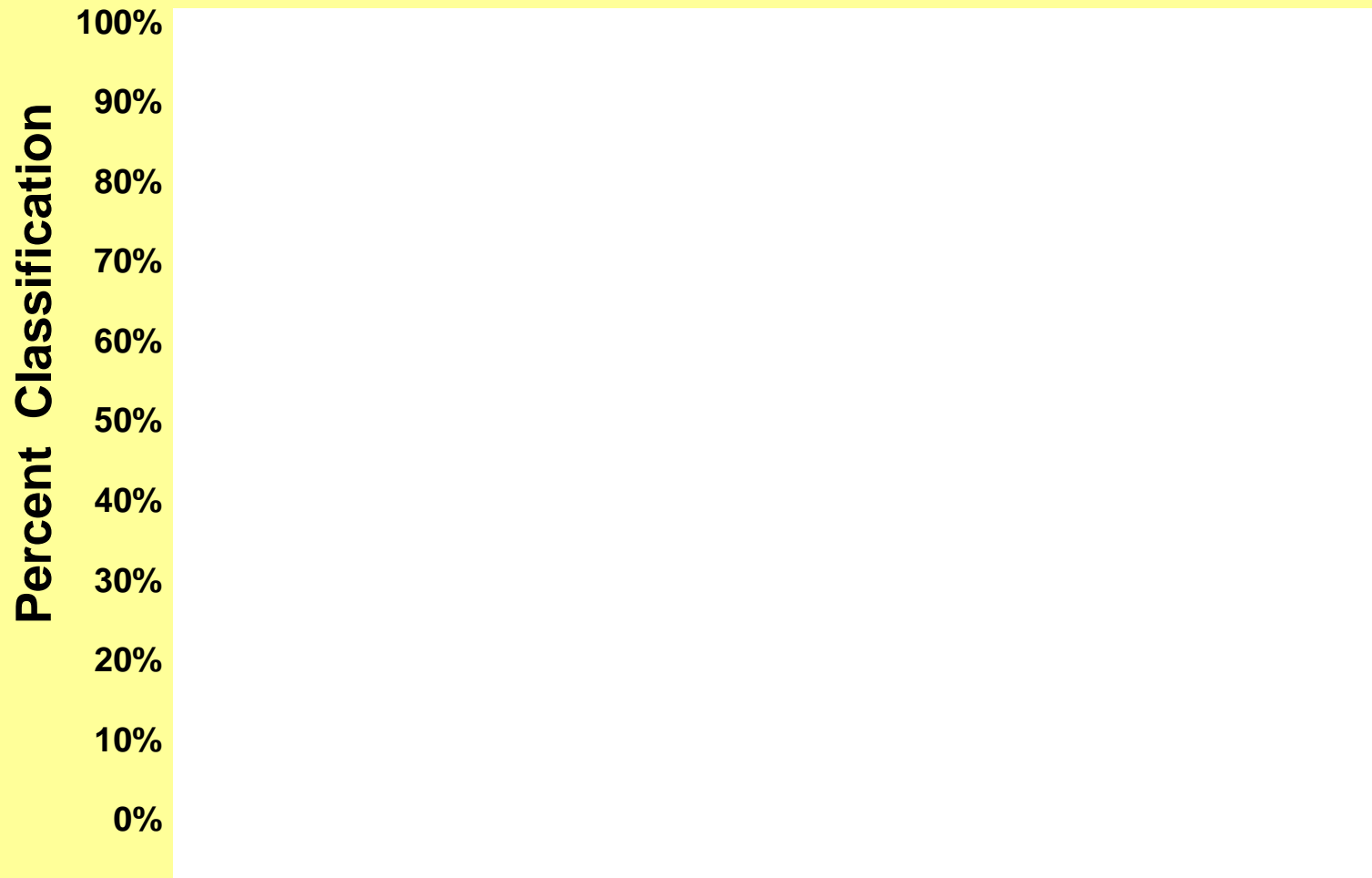
- Selected cord blood with TREC level at the median of the population
- Diluted into mononuclear cell-depleted blood to contain 100%, 50%, 25%, 12%, 6%, 3% cord blood

Assess Comparability of Decision Ranges

Categorical TREC Assay Results on Cord Blood Serial Dilution Samples

■ Follow Up Required

■ No Follow-up



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 years

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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.

