

Newborn Screening for Severe Combined Immunodeficiency:

NSQAP TREC Proficiency Testing and NSTRI MPES Program

Jennifer L. Taylor, PhD.

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

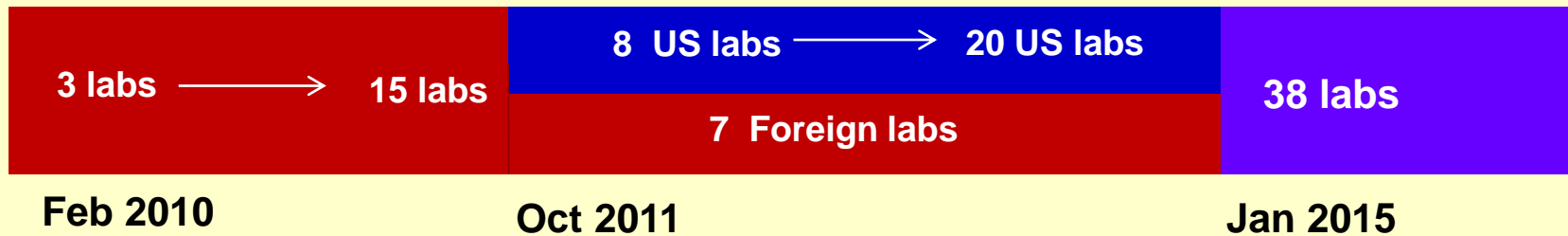
NBS Molecular Biology Training Workshop
CDC, Atlanta, March 9-13, 2015

CDC TREC Proficiency Testing Programs

MPES

NSQAP TREC PT Pilot

NSQAP PT



Total participant labs	38
US	23
Foreign	15

MPES: Model Proficiency Evaluation Survey

NSQAP: Newborn Screening Quality Assurance Program

PROFICIENCY TESTING

- NSQAP PT for TREC
- Please contact:
 - 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

Country	Number of Labs
United States	23
Canada	2
China	2
Finland	1
France	2
Iceland	1
India	1
Japan	1
Spain	1
Switzerland	1
Taiwan	3
Total Laboratories	38
Total Countries	10

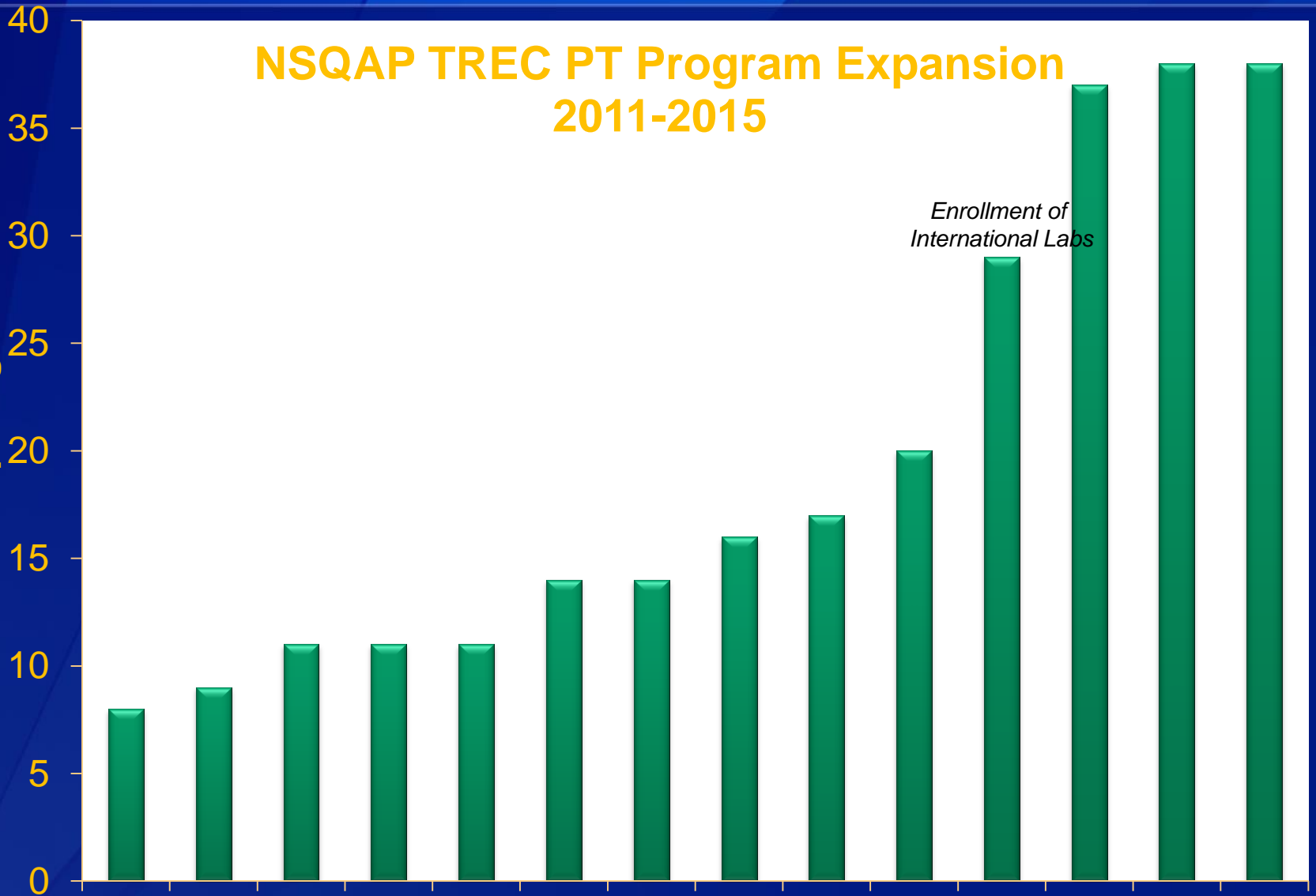
NSQAP TREC PT Program Expansion 2011-2015

Number of Participating Laboratories

*Enrollment of
International Labs*

2011 Q4 2012 Q1 2012 Q2 2012 Q3 2012 Q4 2013 Q1 2013 Q2 2013 Q3 2013 Q4 2014 Q1 2014 Q2 2014 Q3 2014 Q4 2015 Q1

Year and Quarter



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**

Proficiency Testing Quarter 2

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.

NSQAP Laboratory Code

Contact Person:

Phone Number:

Fax Number:

E-mail:

Method Code:

If Other Indicated, please specify Name and Source:

DNA Preparation Method:

If Other Indicated, please describe:

Reference Gene:

If Other Indicated, please specify Name:

Specimen Number	Analyte	Assessment Code	If followup is required, select reference gene category
		1 - No Follow-up required (Screen Negative) 2 - Follow-up required	
215R1	TREC		
215R2	TREC		
215R3	TREC		
215R4	TREC		
215R5	TREC		

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)

5 DNA extracted with no washing step

6 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

Normal specimen; below average TREC level, reference gene level within standard reference range

Normal specimen; medium TREC level, reference gene level within standard reference range

Normal specimen; TREC level below population average but within reference range, reference gene level within standard reference range

Assessment Code 2 (Follow-Up Required) Specimen Type

Leukocyte-reduced blood - TREC and reference gene levels both below standard reference range

SCID-like specimen; low or no TREC, reference gene level within standard reference range

Number of Proficiency Testing Misclassification Errors 2011-2015

Year	Number of Quarters	TREC within reference range identified as "Follow-Up Required"*	TREC below reference range identified as "No Follow-up Required"
2011	4	0	0
2012	4	3	0
2013	4	2	0
2014**	4	11	2
2015	1	1	0
Total		17	2

*Misclassifications likely due to conservative TREC analytical cutoffs.

**Increased in misclassifications due to international lab participation in TREC PT Program.

DNA Preparation and Method

DNA preparation Method	Number of Laboratories
1. In situ/on card (no DNA extraction) with washing step(s)	5
2. EnLite™ (no DNA extraction)	3
3. DNA extracted at 99°C with washing step(s)	11
4. DNA extracted at 95°C with washing step(s)	2
5. DNA extracted at 70°C with washing step(s)	2
6. DNA extracted with no washing steps	0
7. Other	3
8. Not provided	3

Laboratory Method for TREC Assay

Laboratory Method	Number of Laboratories
1. Real-Time PCR - Singleplex	10
2. Real-Time PCR - Multiplex	15
3. EnLite™ Neonatal TREC Kit	3
4. Other	1

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

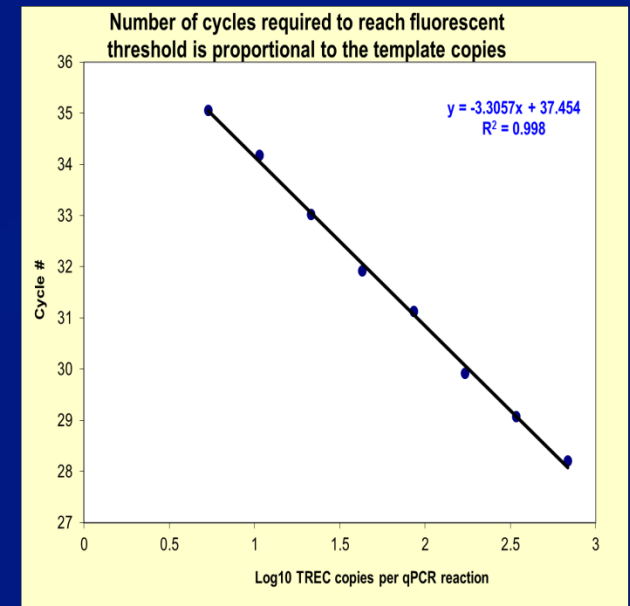
Development of Reference Materials for TREC Assay Evaluation

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

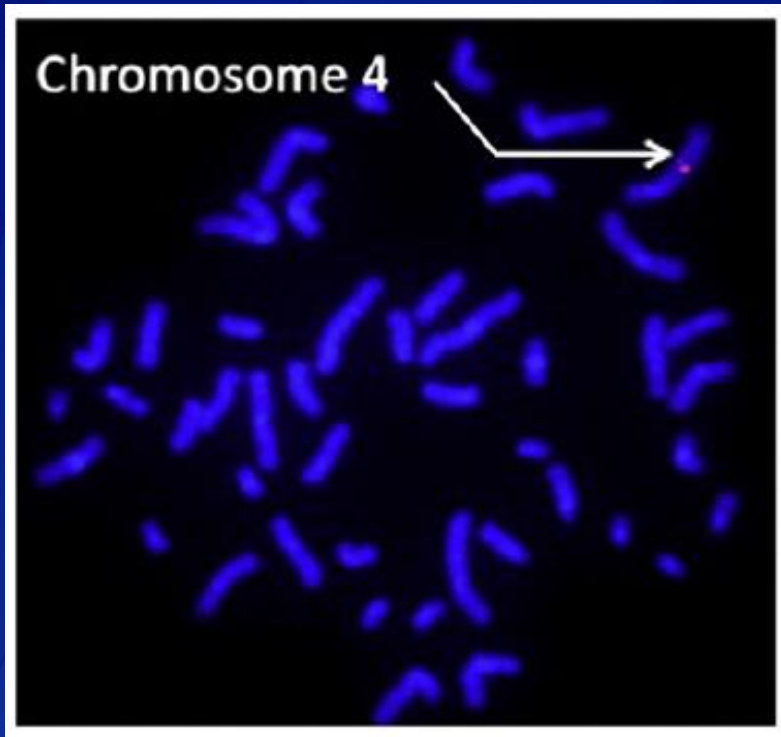


Quantitative Calibration of the TREC Assay

- ❑ 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 C_q value and use archived curve for copy number estimation.

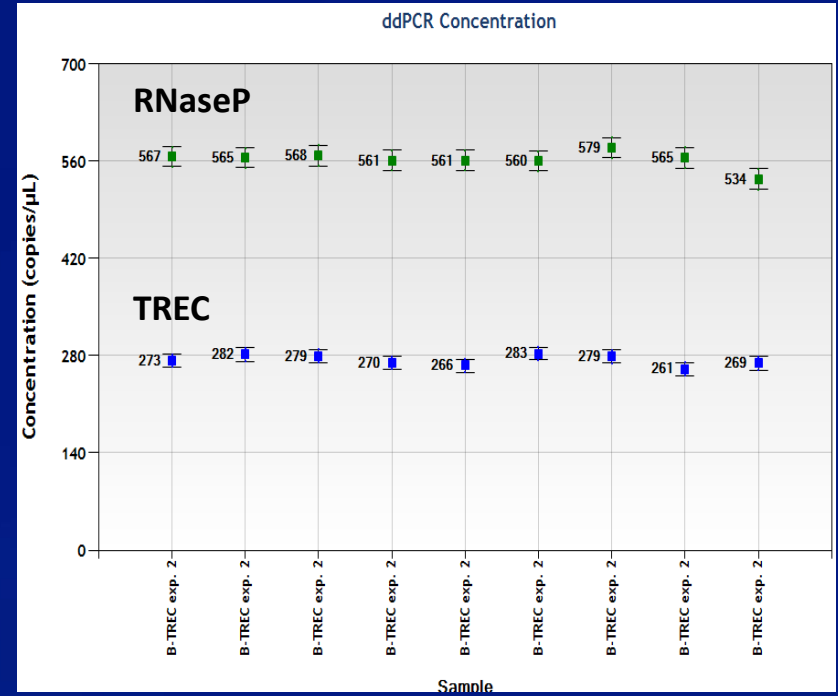
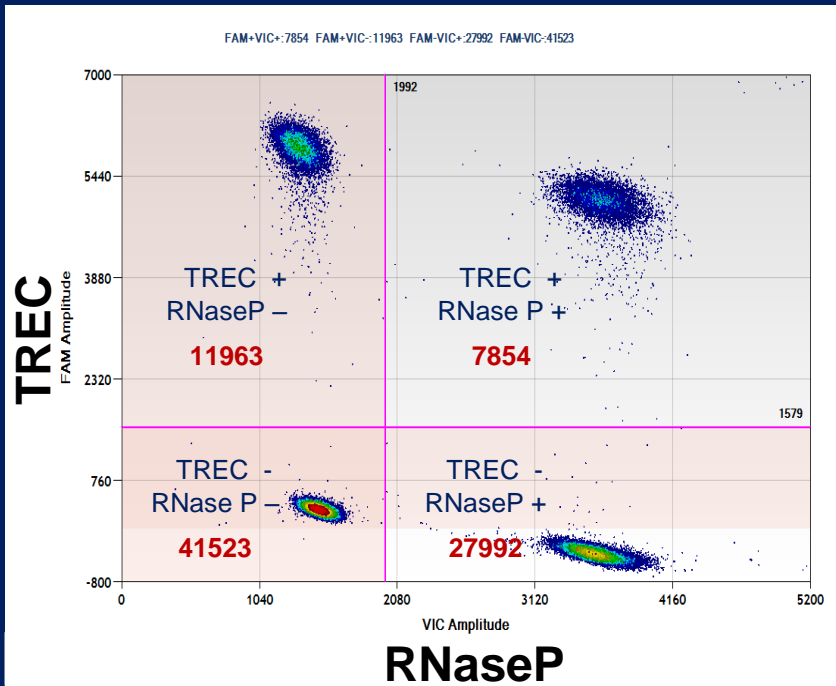
Primary DBS Calibrator based on TREC-Transfected B Cell Line from USCF/NENSP

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

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

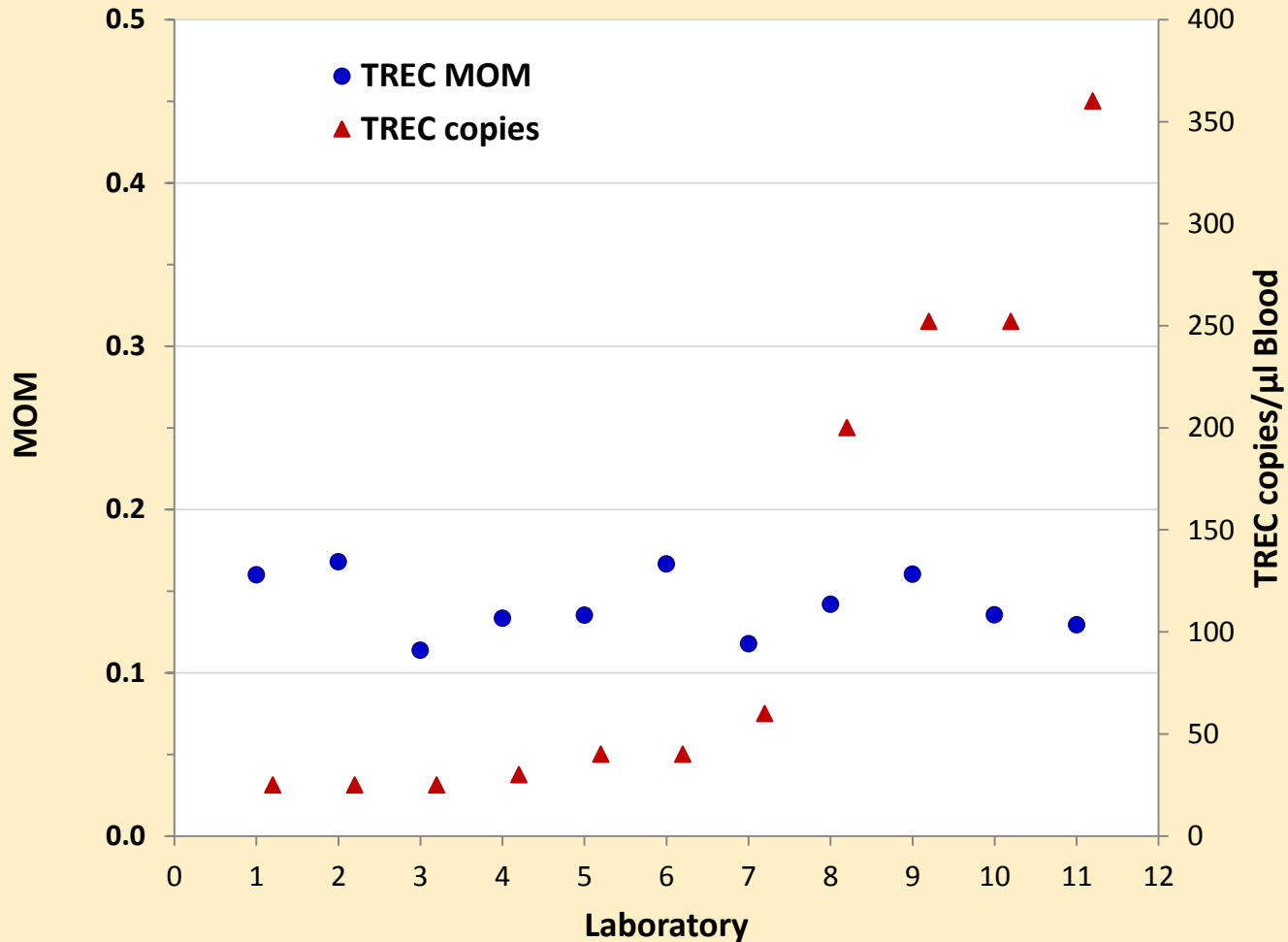
While the categorical results have been generally consistent among laboratories, the quantitative results in TREC copy number on any particular specimen can differ extensively.

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)

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

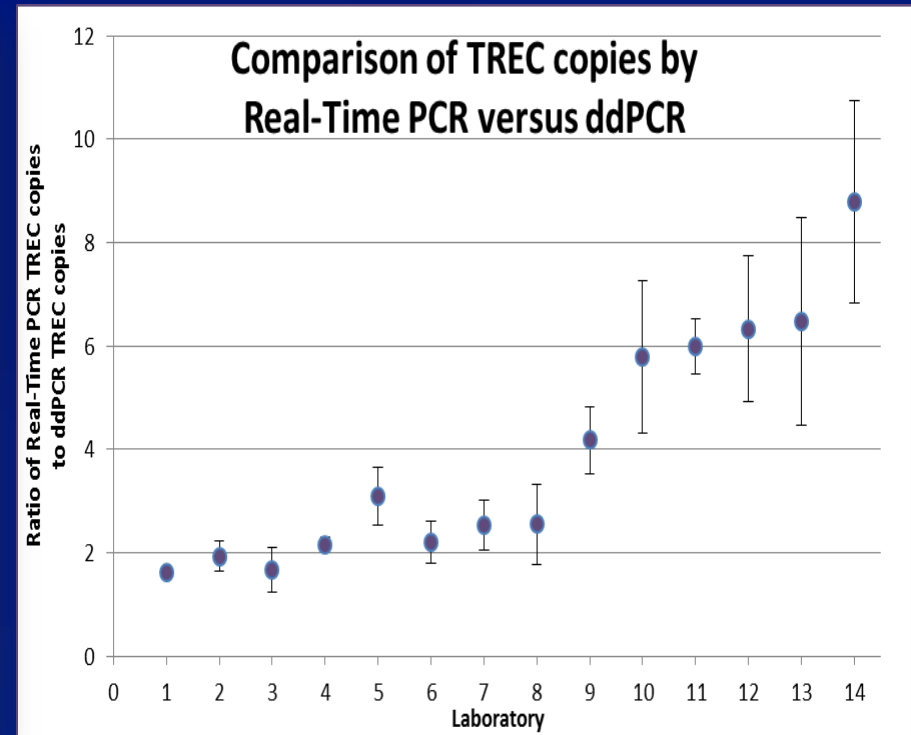
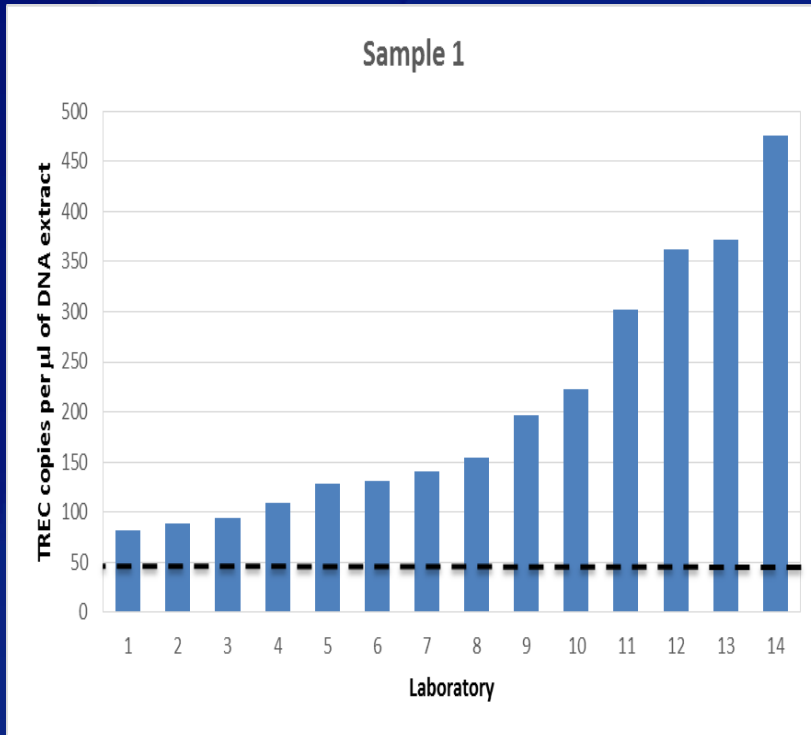
A. Cutoff Values in MOM and TREC Copies



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 are 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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