



## Evaluation of Multiplexing Spinal Muscular Atrophy with a Laboratory Developed Severe Combined Immunodeficiency Assay in Minnesota

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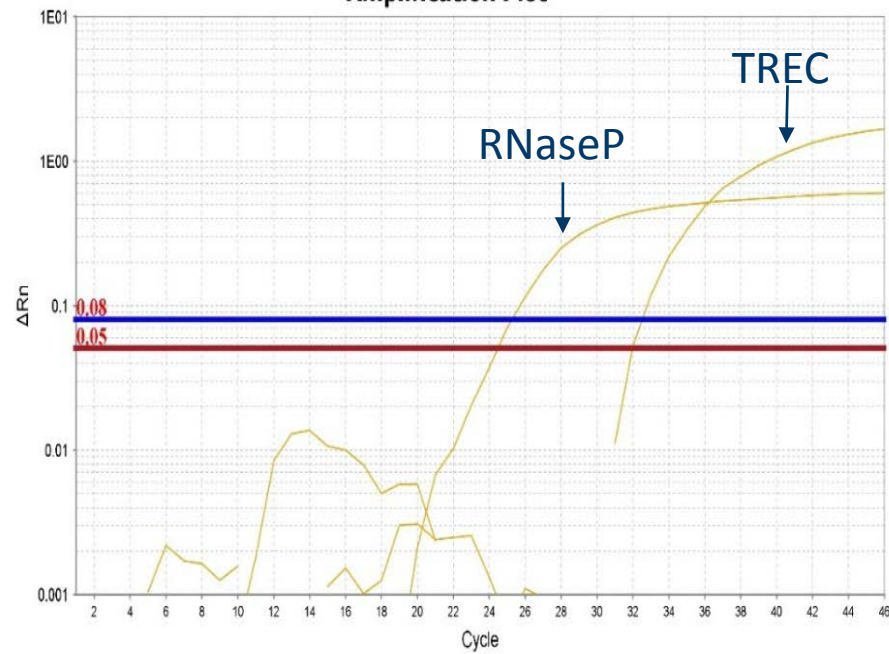
- Minnesota has been screening for SCID since January 2013
- A SMA assay was developed by the CDC's Newborn Screening and Molecular Biology Branch and transferred to the Minnesota Newborn Screening Laboratory
- Minimal changes to the current SCID assay are needed to include SMA screening

- Comparison between current SCID assay (TREC & RNaseP) and a multiplexed SCID/SMA assay (TREC/RNaseP/SMN 1).
- Analysis of 4 SCID/SMA assays
- Comparison of 2 DNA extractions for each assay

# Old Versus New

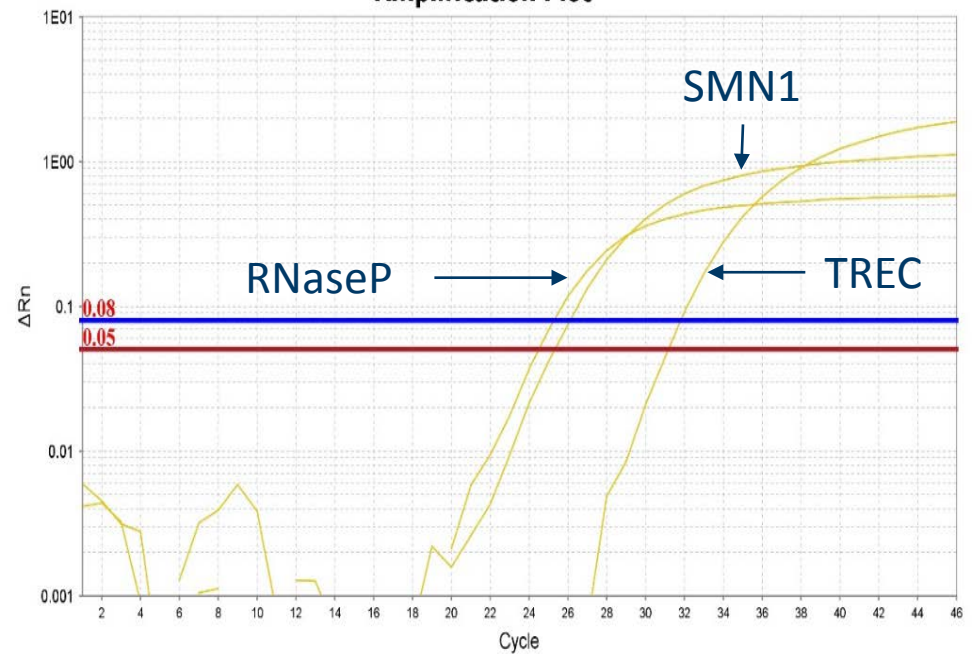
## SCID Assay

Amplification Plot



## SCID/SMA Assay

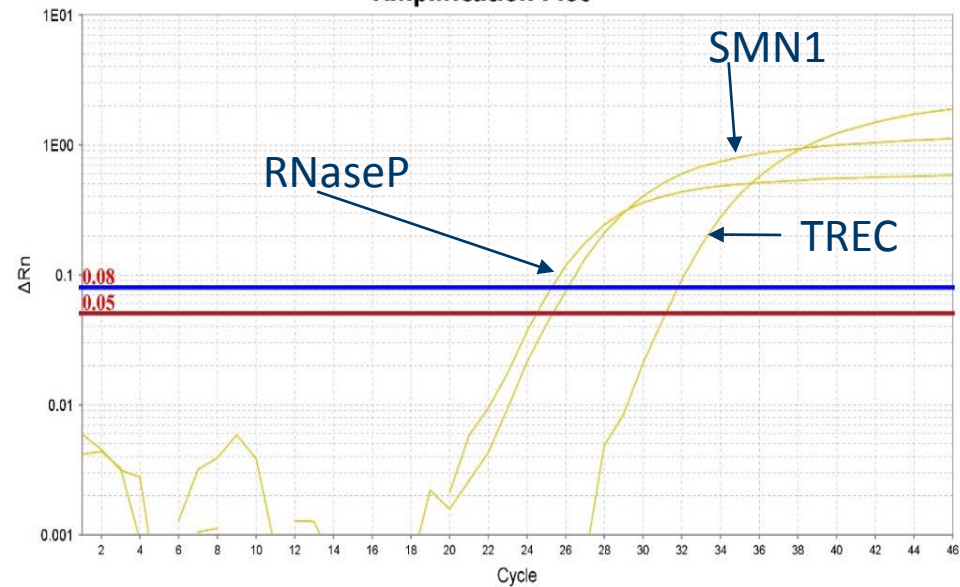
Amplification Plot



# Unaffected Versus Affected SMA Patient

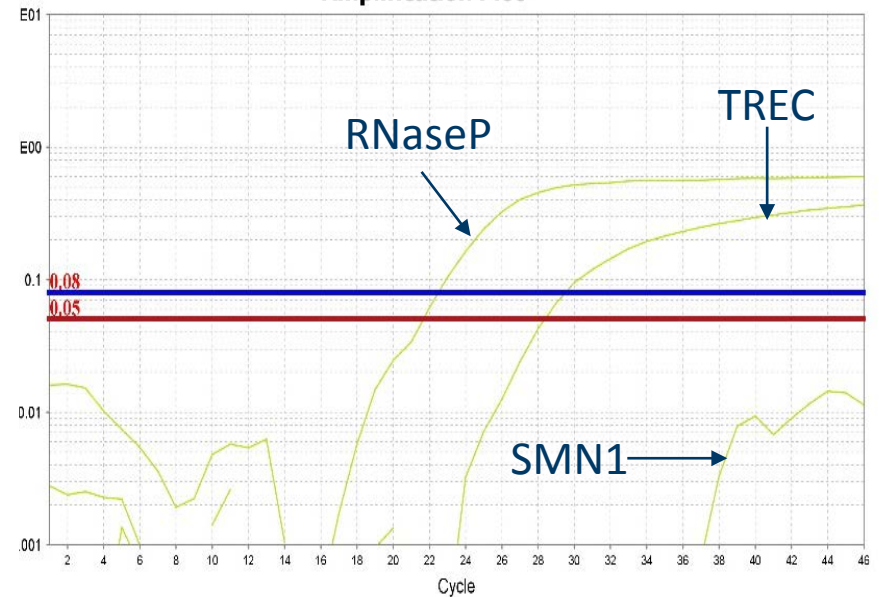
## SMA Negative

Amplification Plot



## SMA Positive

Amplification Plot



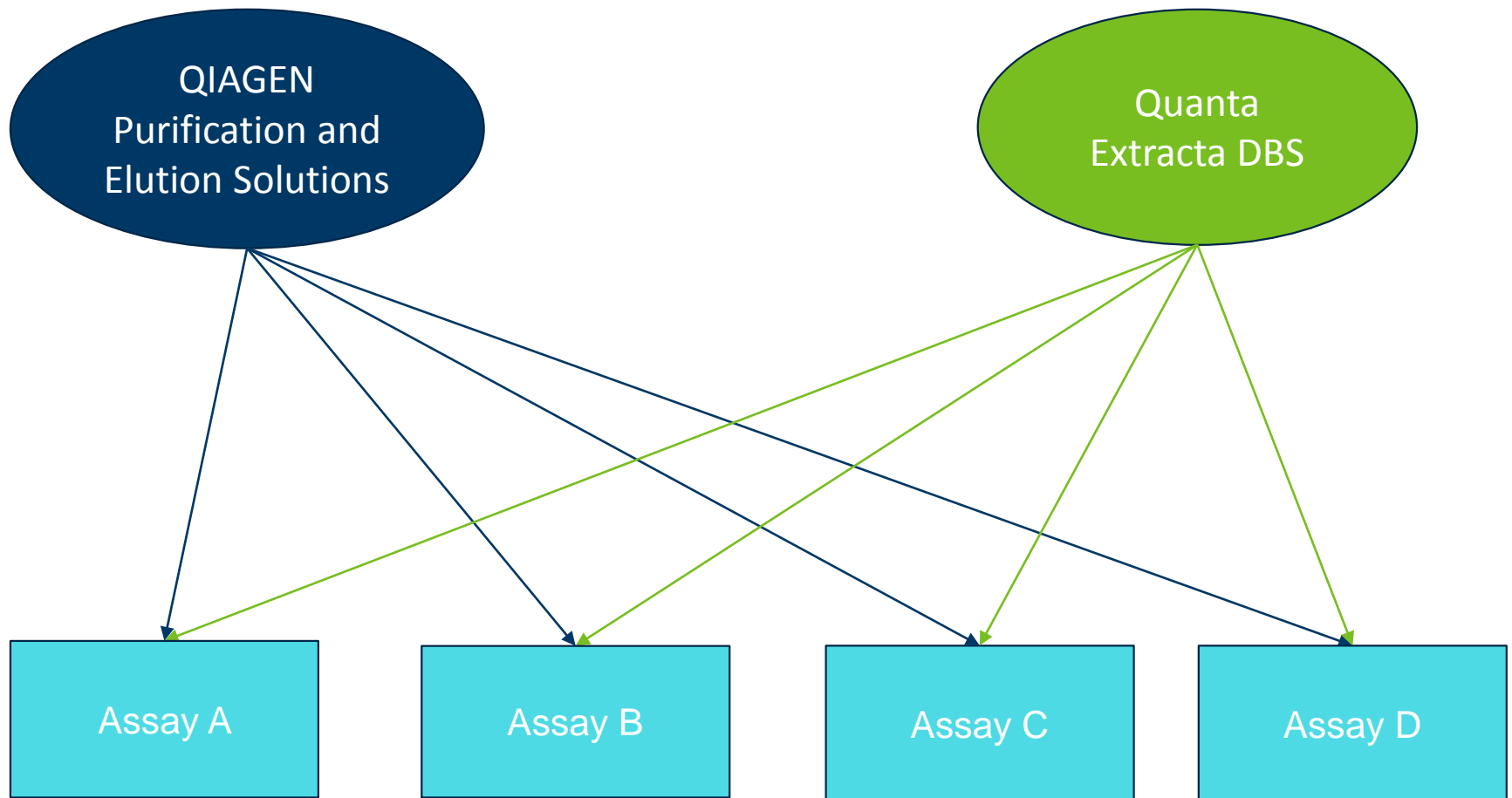
## 4 SCID/SMA Assay Comparison

Assay A	Assay B	Assay C	Assay D
TREC/RNaseP Reagents	TREC/RNaseP Reagents	TREC/RNaseP Reagents	TREC/RNaseP Reagents
SMN Exon Forward	SMN Exon Forward	SMN Exon Forward	PE Forward
SMN Exon Reverse	SMN1 Exon-Intron <b>LNA</b> Reverse	SMN Exon Reverse	PE Reverse
SMN 1 Exon <b>RS</b> Probe	SMN1 Exon <b>RS</b> Probe	SMN1 Exon <b>FS</b> Probe	PE Probe
Annealing Temp. 65 <sup>0</sup> C	Annealing Temp. 65 <sup>0</sup> C	Annealing Temp. 63 <sup>0</sup> C	Annealing Temp. 60 <sup>0</sup> C

# Control Results

	<b>Assay A</b>	<b>Assay B</b>	<b>Assay C</b>	<b>Assay D</b>
	<b>Avg Cq</b>	<b>Avg Cq</b>	<b>Avg Cq</b>	<b>Avg Cq</b>
<b>SMA Normal</b>	23.55	24.50	25.65	22.87
<b>SMA Positive 1</b>	30.16	38.19	No Amplification	No Amplification
<b>SMA Positive 2</b>	30.53	38.48	No Amplification	No Amplification
<b>SMA Positive 3</b>	34.06	36.43	No Amplification	No Amplification
<b>Carrier</b>	22.60	23.36	23.51	21.30

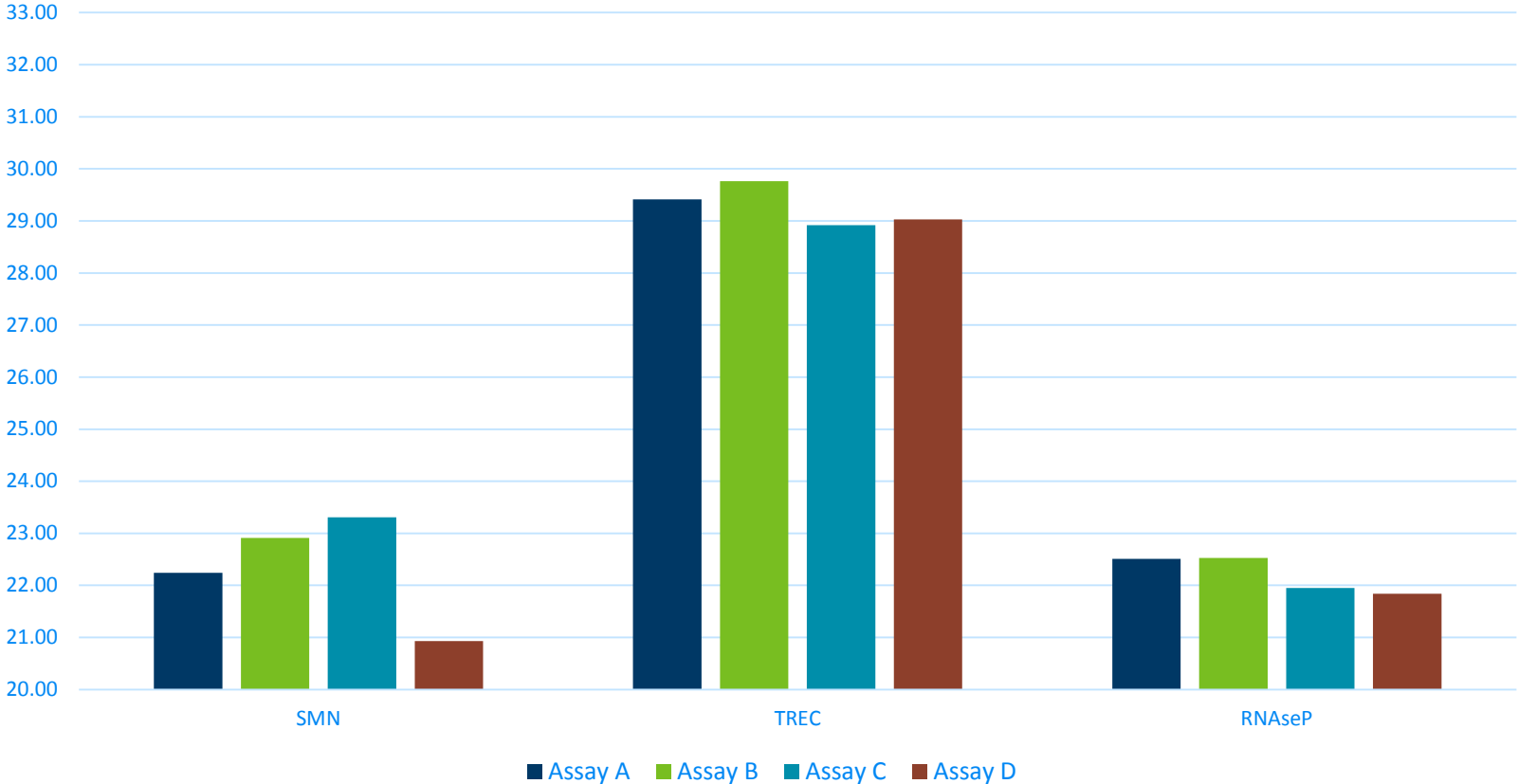
# DNA Extractions





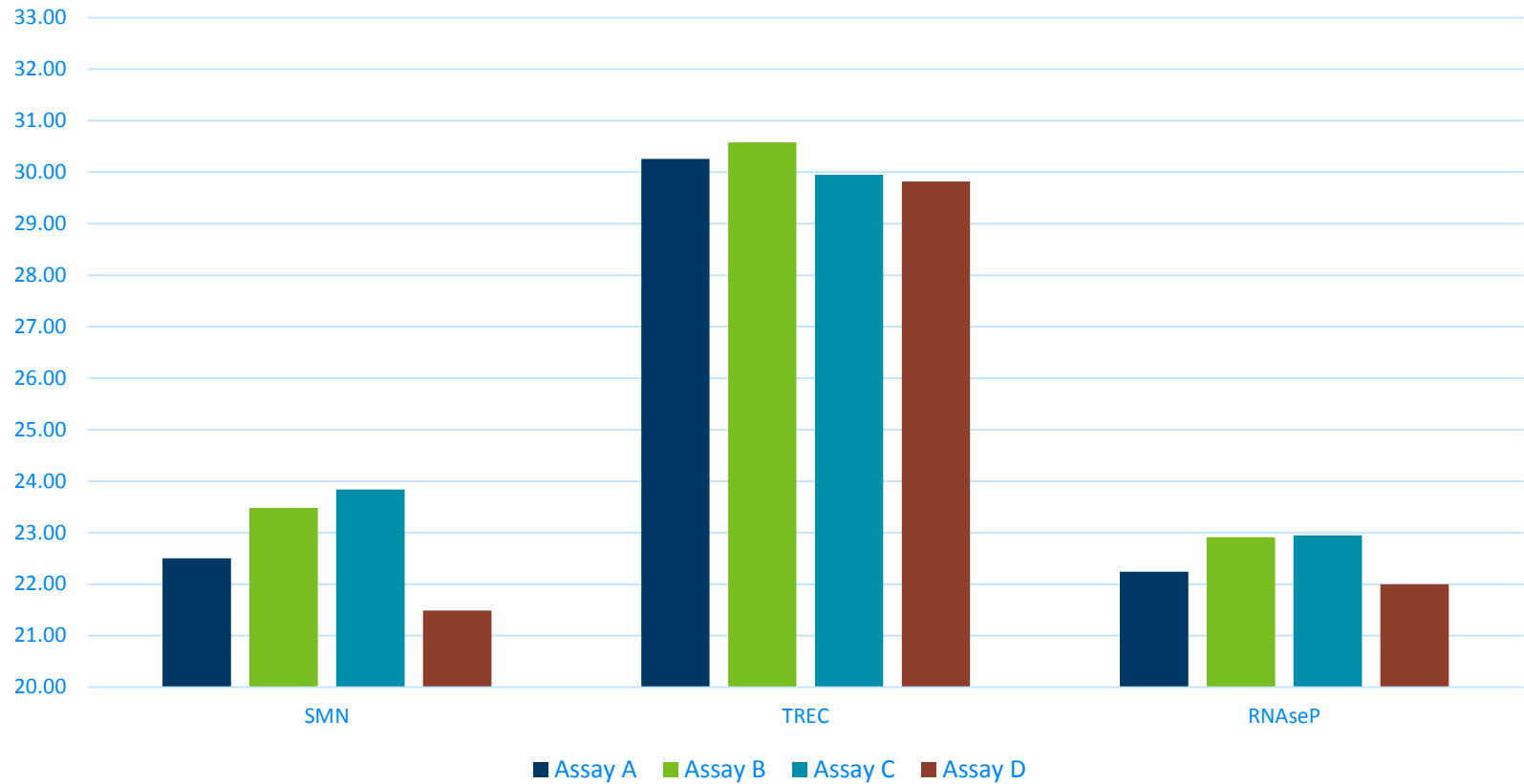
# QIAGEN Extraction

## QIAGEN Extraction Median Cq



# Extracta DBS Extraction

Extracta DBS Extraction  
Median Cq



- SMA can be added to the current SCID assay with minimal changes
- All assay conditions (A,B,C and D) give very comparable results
- The assay will NOT detect carriers
- No additional laboratory analysts will be needed to run the assay
- By multiplexing the assay, the cost will be increased by a little as \$0.15 per reaction

## Next Steps

- A validation of the SCID/SMA assay is in process
- SMA as a candidate condition is currently under review by the Minnesota Advisory Committee on Heritable and Congenital Disorders for recommendation of the addition of the disorder.
- Minnesota Department of Health will be activating a SMA workgroup comprised of specialists, pediatricians, and community advocates this Fall.

# Acknowledgments

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Thank you!