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Spinal Muscular Atrophy: Overview of Available Screening Methods

Thursday, June 28, 2018

Dial in: 866.740.1260

(passcode 4852701#)

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Agenda

Moderator: Patricia Hunt, Texas Department of State Health Services

1:00 - 1:05 Welcome and Introduction

1:05 - 1:20 Overview of Available Screening Methods

Francis Lee, PhD, Centers for Disease Control and Prevention

1:20 – 2:00 State Implementation Experiences: NY, MA, UT, MN

Michele Caggana, ScD, FACMG New York State Department of Health, Wadsworth Center
Anne Comeau, PhD, New England Newborn Screening Program
Andy Rohrwasser, PhD, MBA, Utah Department of Health
Carrie Wolf, MBS, Minnesota Department of Health

2:00 – 2:15 Overview of Second Tier Screening Methods

Mei Baker, MD, FACMG, Wisconsin State Laboratory of Hygiene

2:15 - 2:30 Q&A and Closing

Newborn screening for spinal muscular atrophy (SMA) in the US

Francis Lee, MSc, PhD

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

APHL SMA Webinar, June 28, 2019

National Center for Environmental Health
Division of Laboratory Sciences

SMA is the leading genetic cause of death among infants

- A neuromuscular disease caused by progressive degeneration of motor neurons
- Major signs and symptoms include loss of normal motor function and respiratory difficulty/failure; can result in death in severe cases
- 3 clinical types based on age of onset and severity

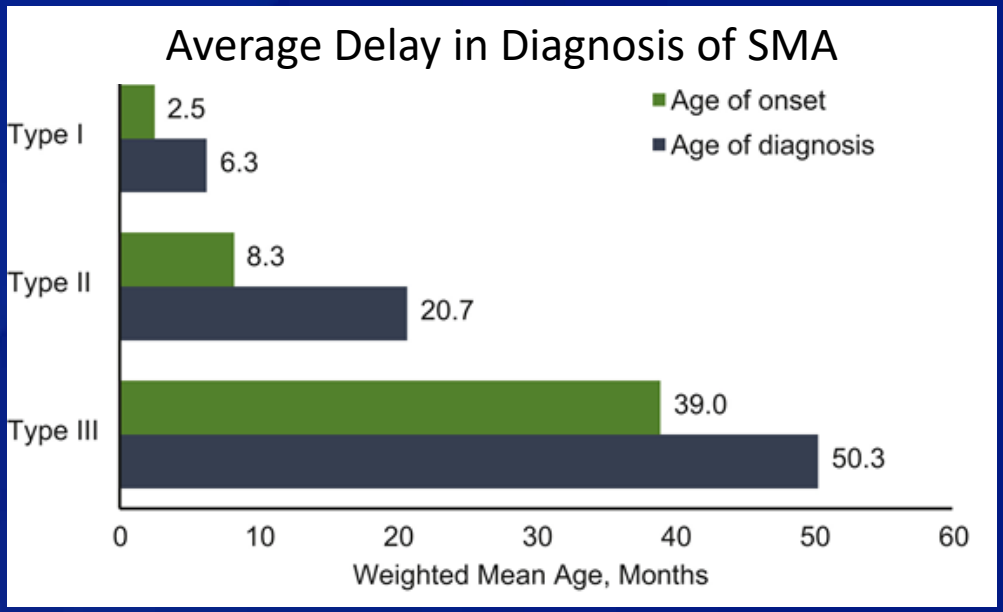
  Type I: Birth – 6 mos.

  Type II: 6 mos. – 2 years

  Type III: 18 mos. – 3+ years
- Birth prevalence ~ 1 : 10,000

Newborn screening for SMA can lead to early diagnosis and treatment

- In SMA type 1, motor neuronal death begins perinatally; >90% loss within 6 months

- 

- FDA approved drug available since December 2016

Advisory Committee on Heritable Disorder in Newborns and Children

- Submitted recommendation to the Secretary of Health and Human Services to "Expand the Recommend Uniform Screening Panel (RUSP) to include the addition of SMA due to homozygous deletion of exon 7 in *SMN1*" Mar 8, 2018
- Deputy HHS Secretary interim response – April 19, 2018 will provide "detailed response regarding actions on the recommendation within 120 days"

Different molecular assays have been used to detect SMA

- Restriction Fragment Length Polymorphism (RFLP) analysis
- High Resolution Melting (HRM) analysis
- Multiplex Ligation-Dependent Probe Amplification (MLPA)
- Luminex Genotyping
- DNA sequencing
- Quantitative Real-time PCR (qPCR)

Real-time PCR emerges as the preferred method in newborn screening for SMA

- Real-time PCR allows for high throughput screening
- Most state newborn screening labs are already using this method to detect Severe Combined Immunodeficiency
  - Labs are equipped with the necessary instrumentation
  - Staff is familiar with procedure
- Reactions can be multiplexed into current SCID assay
  - Reduced the cost of adding SMA
  - Does not require added labor cost to run

SMA Real time PCR Taqman assays used in state newborn screening labs

- **New York (hospital-based project) : target *SMN1* Exon 7 (MGB probe; Maranda et al, Clin Chem 45: 88, 2012)**
- **CDC ver. 1* : target *SMN1* Exon 7 – Intron 7 (LNA probe and LNA rev primer)**
- **CDC ver. 2** : target *SMN1* Exon 7 (LNA probe)**
- **Perkin Elmer : target *SMN1* Exon 7 (LNA probe)**

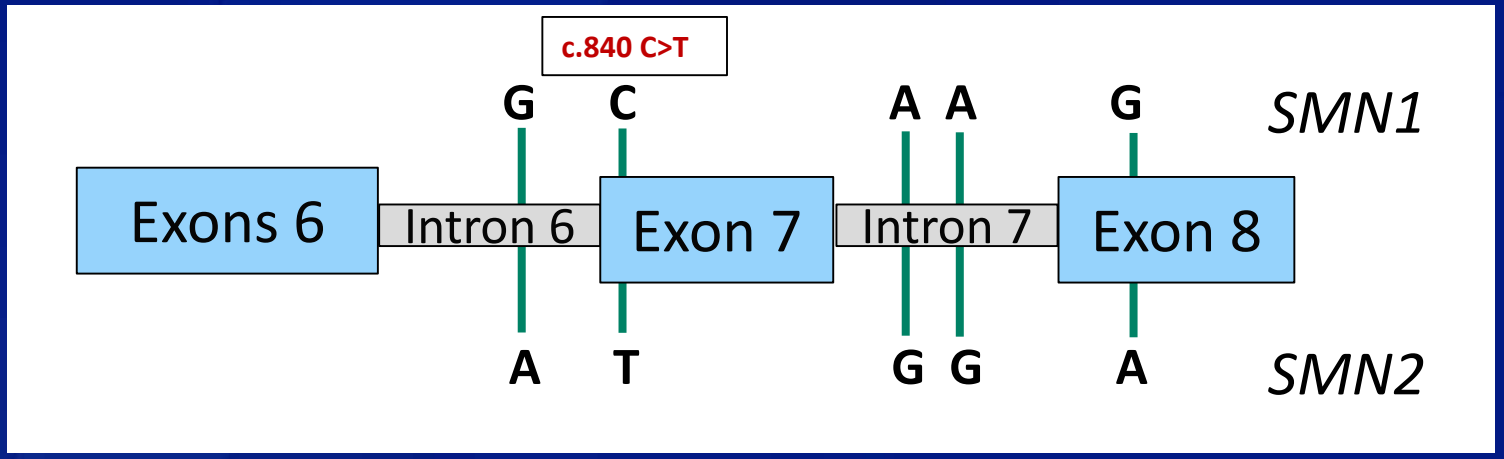
* adopted by New England NBS lab in stand-alone assay

** adopted by UT and MN NBS labs in multiplex assay with TREC

What are the challenges in designing a real-time PCR assay to screen for SMA?

Challenge #1:

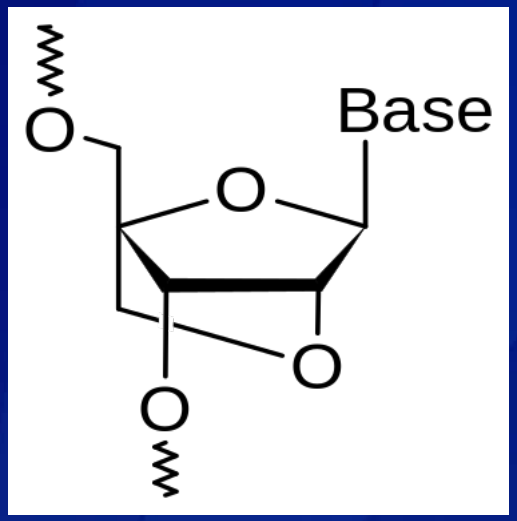
SMN1 has a paralog, the *SMN2* gene, which has nearly identical genomic sequence



- Only 5 nucleotide differences between the two genes in this region
- It is critical to avoid cross signal from *SMN2* when trying to identify the loss of *SMN1*

Need to be able to discriminate single nucleotide polymorphism

Use of LNA (locked nucleic acid) nucleotides can distinguish single nucleotide polymorphism



LNA : A modified RNA nucleotide with extra bridge connecting the 2' oxygen and 4' carbon

"locks" the ribose in the 3'-*endo* conformation

- PCR primers and probes with some nucleotides substituted by LNAs can differentiate single nucleotide mismatch
- LNA primers and probes can be ordered from multiple commercial sources

Initial SMA assay developed at CDC targeted intron 7 sequence

CTTGTGAAACAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATA
GCTATCTATG/ATCTATATAGCTATTTTTTTTAACTTCCTTTATTTTCCT
TACAGGGTTTC(T)AGACAAAATCAAAAAGAAGGAAGGTGCTCACATTCCT
TAAATTAAGGAGTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTTG
TAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACATTTAAAAAGT
TCAGATGTTAA(G)AAAGTTGAAAGGTTAATGTAAAACAATCAATATTAAA
GAATTTTGATGCCAAAACTATTAGATAAAAGGTTAATCTACATCCCTACT

Characters in red = SMN 1(2) exon 7

- The LNA modified probe (in green) was designed to selectively bind *SMN1* by discriminating between the mismatch nucleotides of *SMN1* and *SMN2*
  - *SMN1* nucleotide A and *SMN2* nucleotide (G)
- Forward and reverse primers (in grey) will amplify both *SMN1* and *SMN2* sequences

Taylor, J., Lee FK, Yazdanpanah, G., et al., *Clin. Chem*, (2015), 61 (2): 412-9

Challenge #2: Chimeric gene

- Taiwan pilot newborn screening for SMA

  Feasibility trial for pre-symptomatic diagnosis

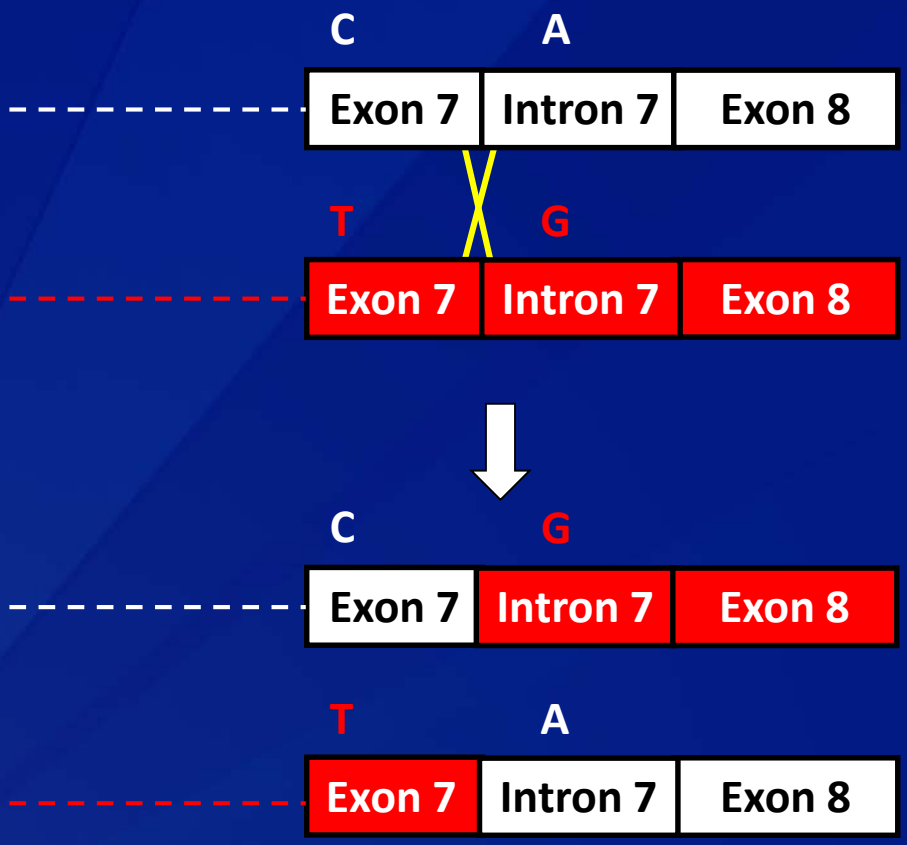
  Nov 2014 – Sept 2016

  Total Screened: 120,267

  - Tier-One Positive: 15 (by absence of *SMN1* intron 7)
  - Tier-Two Positive and Confirmed: 7 (by ddPCR & MLPA)

Yin-Hsiu C. et al., *The Journal of Pediatrics* (2017);

False positive due to recombination between *SMN1* and *SMN2* resulting in a hybrid genotype



*Yin-Hsiu C. et al., *The Journal of Pediatrics* (2017); **Hahnen, E. et al., *Am. J. Hum. Genet.*, (1996), 59: 1057-1065

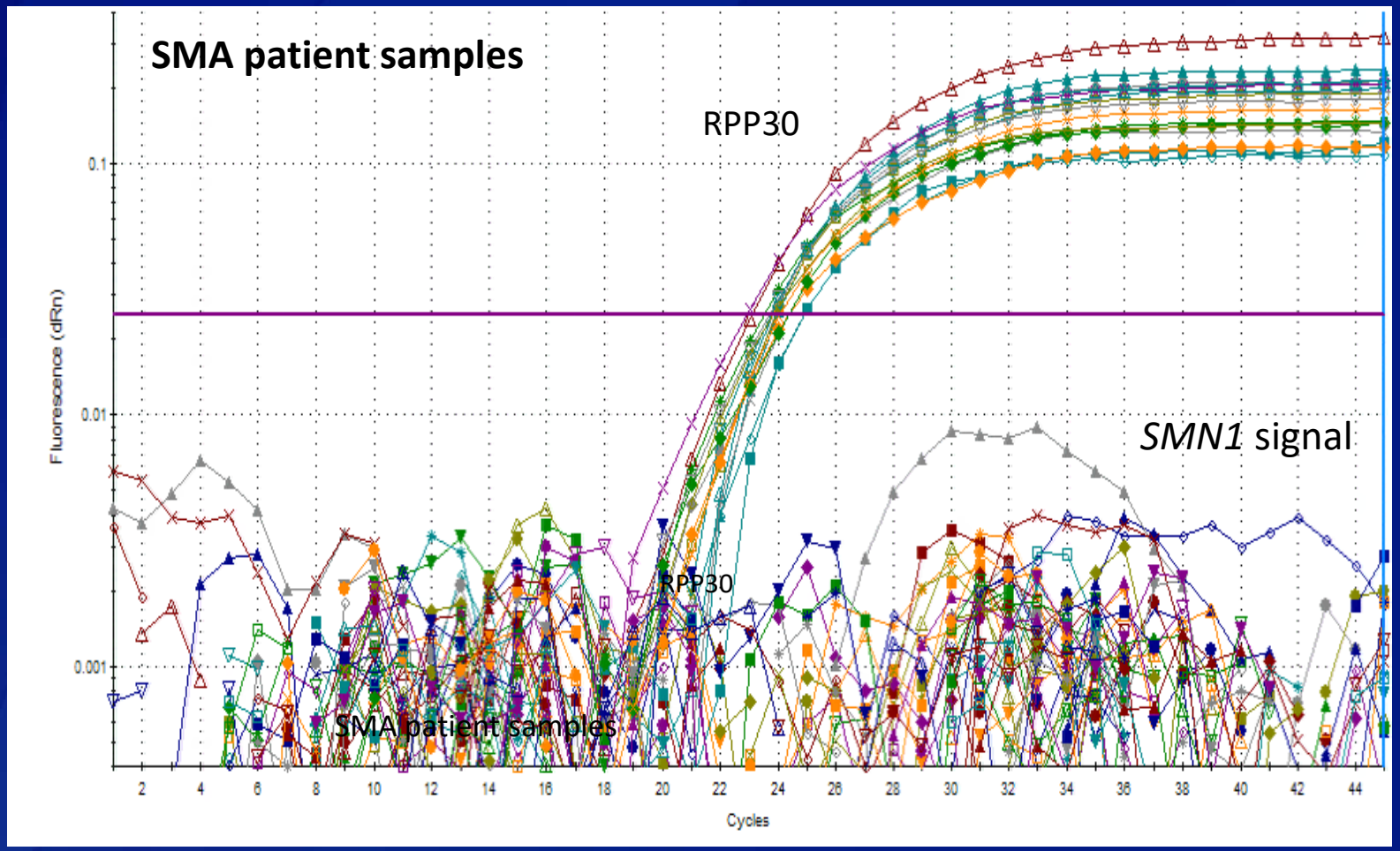
Revised SMA Assay ver. 1 – Target exon 7

CTTGTGAAACAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATA
GCTATCTATG/ATCTATATAGCTATTTTTTTTTAACTTCCTTTATTTTCCT
TACAG*GGTTTC(T)AGACAAAATCAAAAAGAAGGAAGGTGCTCACATTCCT*
*TAAATTAAGGA*GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTTG
TAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACATTTAAAAAGT
TCAGATGTTAA(G)AAAGTTGAAAGGTTAATGTAAAACAATCAATATTAAA
GAATTTTGATGCCAAAACTATTAGATAAAAGGTTAATCTACATCCCTACT

Characters in red = SMN 1(2) exon 7

- We replaced the reverse primer with an *SMN1*-specific LNA primer (in yellow) to eliminate *SMN2* amplification
- The LNA probe targets the exon 7 region with the mismatch between SMN1 *C* and SMN2 *(T)*
- Assay has two layers of specificity to eliminate any X-reaction to *SMN2*

Assay ver. 1 - specificity improves by adding LNA primer



- No background signal from *SMN2* (maximum sensitivity in detecting *SMN1* absence)
- However, no signal if either *SMN1* exon or intron is absent
- Requires confirmation with second tier assay specific for exon 7 or intron 7

Limitations associated with LNA primer

While highly specific, LNA primers are technically more demanding

- Sensitive to quality of DNA extract
- Sensitive to type of Taqman master mix
- Sensitive to temperature accuracy
- PCR efficiency around 90%

Revised SMA Assay ver. 2 – Targeting exon 7

CTTGTGAAACAAAATGCTTTTTAACATCCATATAAAGCTATCTATATATA
GCTATCTATG(A)TCTATATAGCTATTTTTTTTTAACTTCCTTTATTTTCCT
TACAG*GGTTTC(T)AGACAAAATCAAAAAGAAGGAAGGTGCTCACATTCCT*
*TAAATTAAGGA*GTAAGTCTGCCAGCATTATGAAAGTGAATCTTACTTTTG
TAAAACTTTATGGTTTGTGGAAAACAAATGTTTTTGAACATTTAAAAAGT
TCAGATGTTAA(G)AAAGTTGAAAGGTTAATGTAAAACAATCAATATTAAA
GAATTTTGATGCCAAAACTATTAGATAAAAGGTTAATCTACATCCCTACT

Characters in red = SMN 1(2) exon 7

- Reverse primer moved to exon 7 region : the unmodified forward and reverse primer will amplify exon 7 of both SMN1 and 2
- The LNA probe (in green) for exon 7 was further optimized for maximum specificity

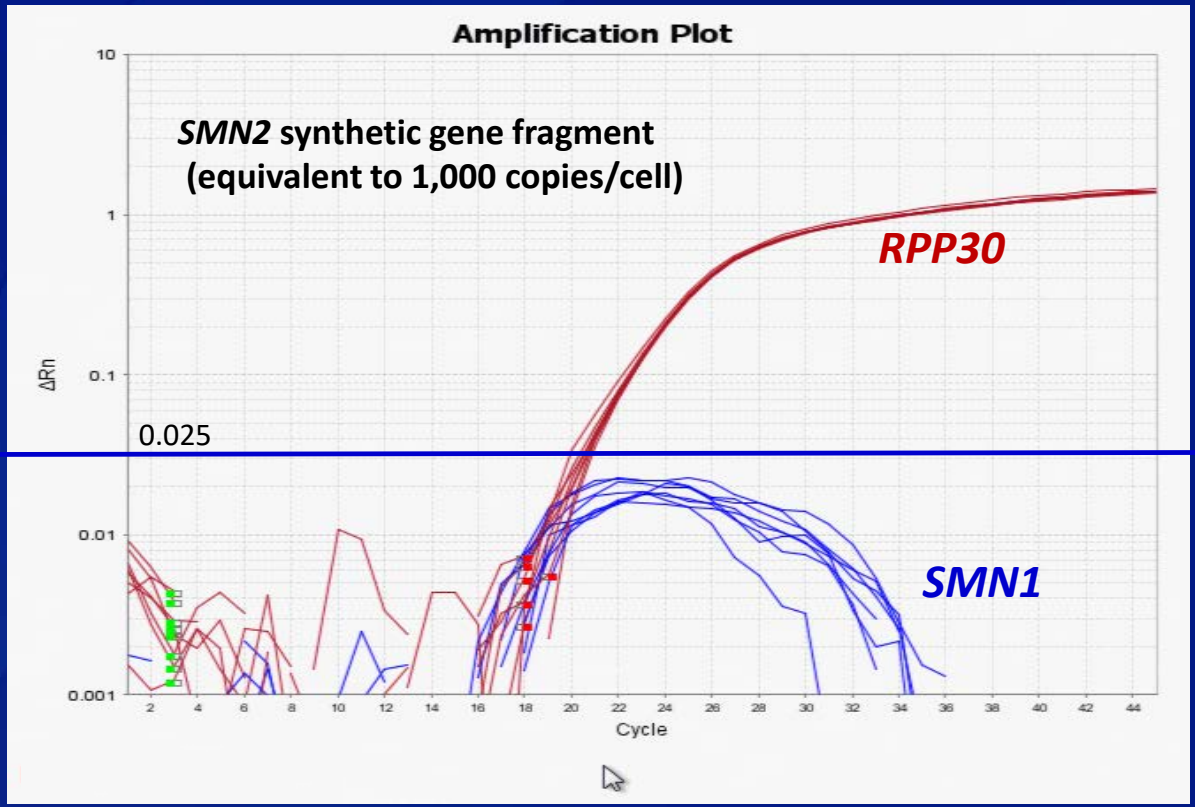
LNA probe was redesigned for maximum specificity

- Factors important in the design of LNA probe for mismatch discrimination:
  - short length (10-12 nucleotides)
  - Location of mismatch nucleotide in the center of probe
  - LNA substitution in triplet at site of mismatch

Probe with LNA modification of pyrimidine (C or T) at mismatch site within probe

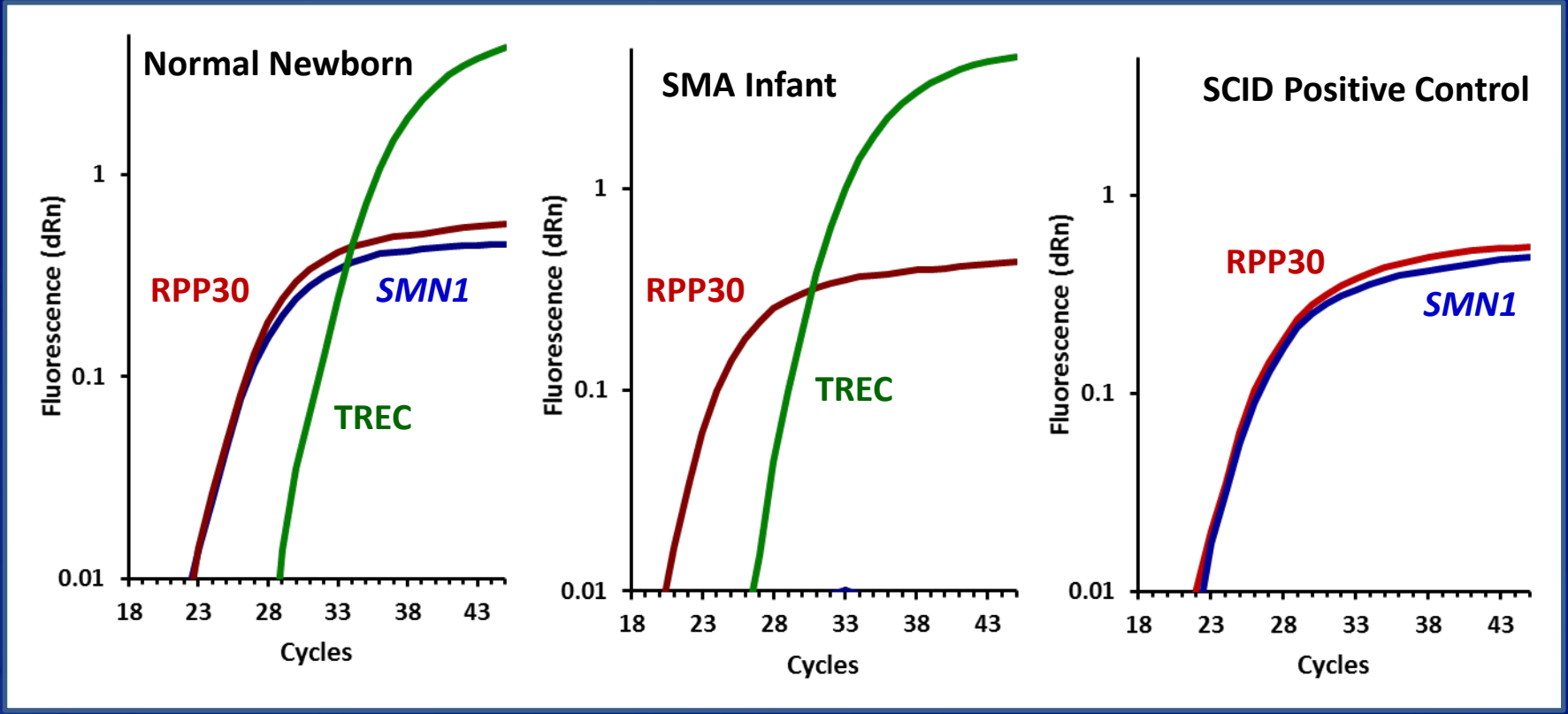
You, Y. et al., *Nucleic Acids Research*, (2006), 34(8)

The Assay ver. 2 utilizes an *SMN1*-specific LNA probe with forward strand sequence



- We do not observe any non-specific signal in *SMN1* null samples even when challenged with an excess of *SMN2* sequence

SMN1 can also be multiplexed into the current TREC assay (SMN1-TREC-RPP30)



SMA patients are correctly identified from dried blood spots when using the multiplex assay

	Assay Results		Clinical Category
Donor Number	Cq - *SMN1* Exon 7	*SMN1* Result	SMA Status
1	No Cq	Absent	Affected
2	No Cq	Absent	Affected
3	No Cq	Absent	Affected
4	No Cq	Absent	Affected
5	No Cq	Absent	Affected
6	No Cq	Absent	Affected
7	No Cq	Absent	Affected
8	No Cq	Absent	Affected
9	No Cq	Absent	Affected
10	No Cq	Absent	Affected
11	No Cq	Absent	Affected
12	22.6	Present	Unaffected/ Carrier
13	23.2	Present	Unaffected/ Carrier
14	24.0	Present	Unaffected/ Carrier
15	24.4	Present	Unaffected/ Carrier
16	24.4	Present	Unaffected/ Carrier
17	24.6	Present	Unaffected/ Carrier
18	24.7	Present	Unaffected/ Carrier
19	24.9	Present	Unaffected/ Carrier
20	25.0	Present	Unaffected/ Carrier
21	25.4	Present	Unaffected/ Carrier
22	25.4	Present	Unaffected/ Carrier
23	25.9	Present	Unaffected/ Carrier
24	26.5	Present	Unaffected/ Carrier
25	26.7	Present	Unaffected/ Carrier
26	28.4	Present	Unaffected/ Carrier

Technology Transfer to state newborn screening laboratories

Both versions of CDC SMA assay have been validated in state NBS labs, and is being used in state-wide screening

- Massachusetts (January 29, 2018)
- Utah (January 29, 2018)
- Minnesota (March 5, 2018)

- As of June, > 40,000 newborns have been screened
- Three SMA infants have been identified, confirmed and treated

Discussion

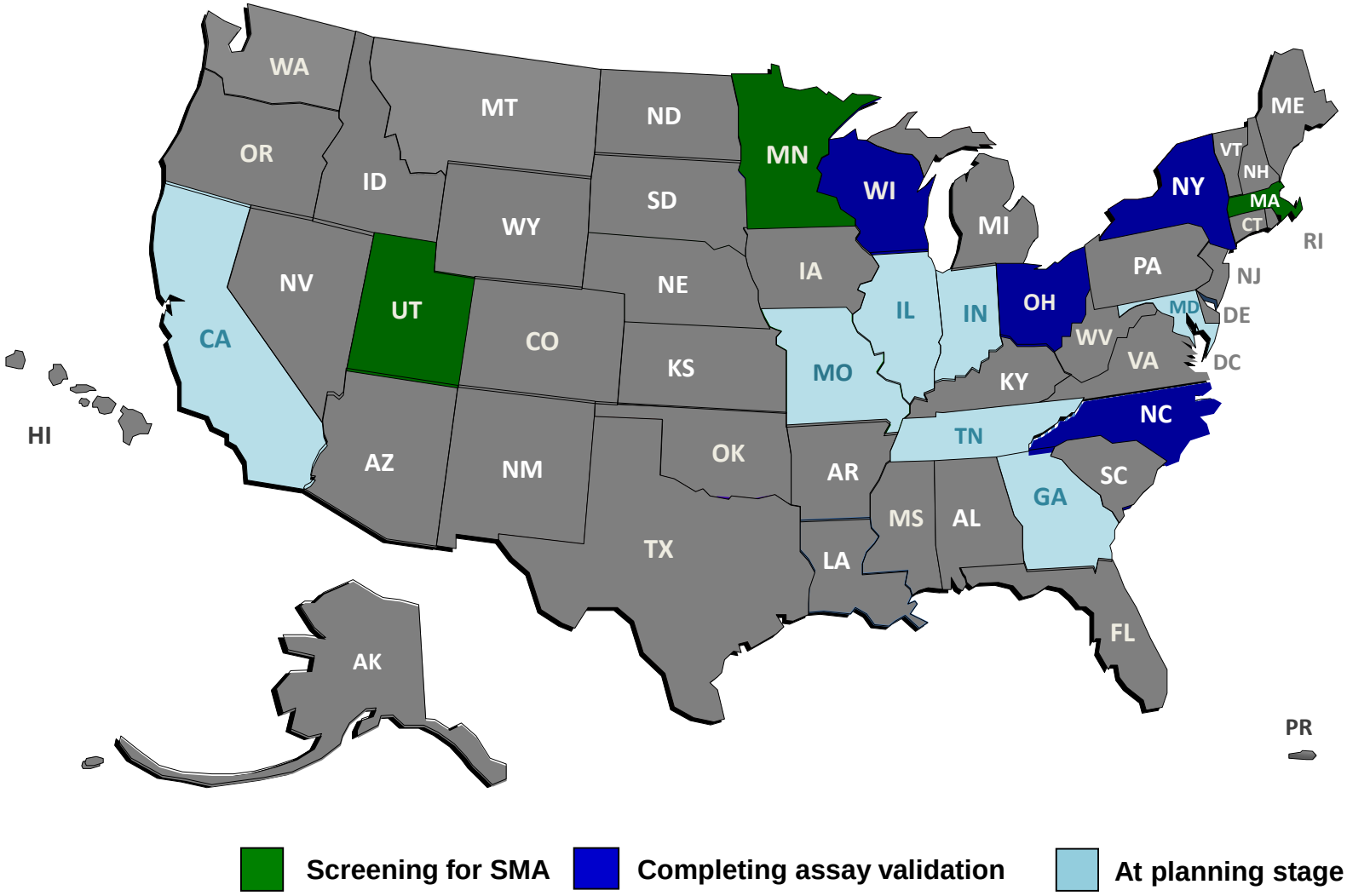
- *SMN1* assay is the first newborn screening 1st tier test based on genotype alone
- High specificity required to discriminate *SMN2* sequence to avoid false negative results
- Possible unknown non-pathogenic SNP, if present in the probe region can potentially lead to false positive
- Clinical diagnostic lab confirmation of screen positive cases, and determination of SMN2 copy numbers are important for medical management

CDC SMA NBS resources available to state labs

- If a state lab decides to try CDC assays, we provide reagents (enough for assay development), primers and probe sequences, QC materials and technical support
- Hands-on technical training at CDC, if requested
- SMA positive QC dried blood spot material: prepared from patient cell lines spiked into leukocyte - depleted blood

- CDC started SMA pilot proficiency testing program in June 6, 2018 (10 labs participating)

June 1, 2018 SMA Newborn Screening Implementation Status – US States and Territories



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Wuh-Liang Hwu

- **State NBS lab collaborators:**

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Wisconsin
Mei Baker, Sean Mochal

Massachusetts
Anne Comeau, Lan Ji

Utah
Andreas Rohrwasser
Katelyn Logerquist

Thank you for your attention!

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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.

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Spinal Muscular Atrophy Screening in New York State
APHL Webinar – June 28, 2018

Michele Caggana , Sc.D., FACMG
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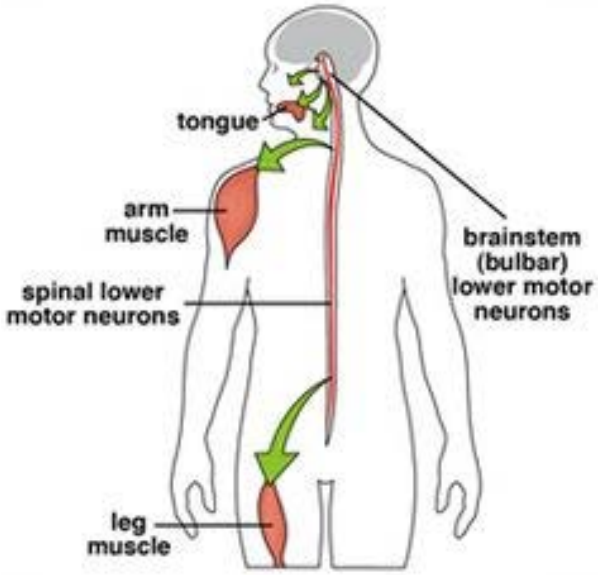
Wadsworth Center, NYS Department of Health

Disclosures

- **Biogen, Idec funded this study (screening, recruitment).**
- **Biogen had no role in data analysis, interpretation, or decisions regarding patient counseling or care.**

Spinal Muscular Atrophy (SMA)

- **Progressive degeneration & loss of spinal cord & brainstem motor neurons**
- **Muscle weakness, atrophy**
- **Difficulty breathing, poor weight gain, pneumonia, scoliosis, joint contractures**



Age at onset, symptoms, severity and survival vary

SMA

Most common ge
& toddler death
- **Incidence: 1 in**
- **Carriers: 1 in 5**

95%–98% homozyg
Survival of Motor N
exon 7

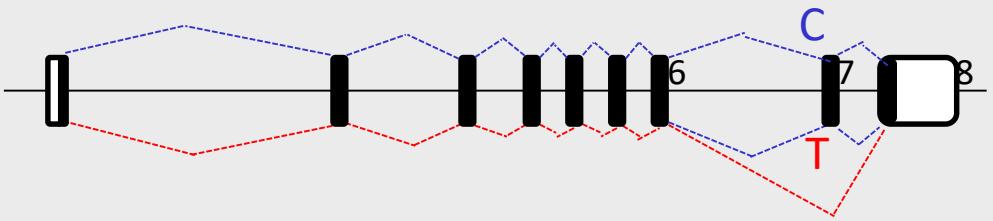
SMN1 (5q13)

exon 1 2a 2b 3 4 5 6 7 8

SMN2 = *SMN1* homologue

(differ by few nucleotides | both code for SMN)

SMN1 full-length SMN (100%)



SMN2

truncated, non-functional SMN (~85-95%)

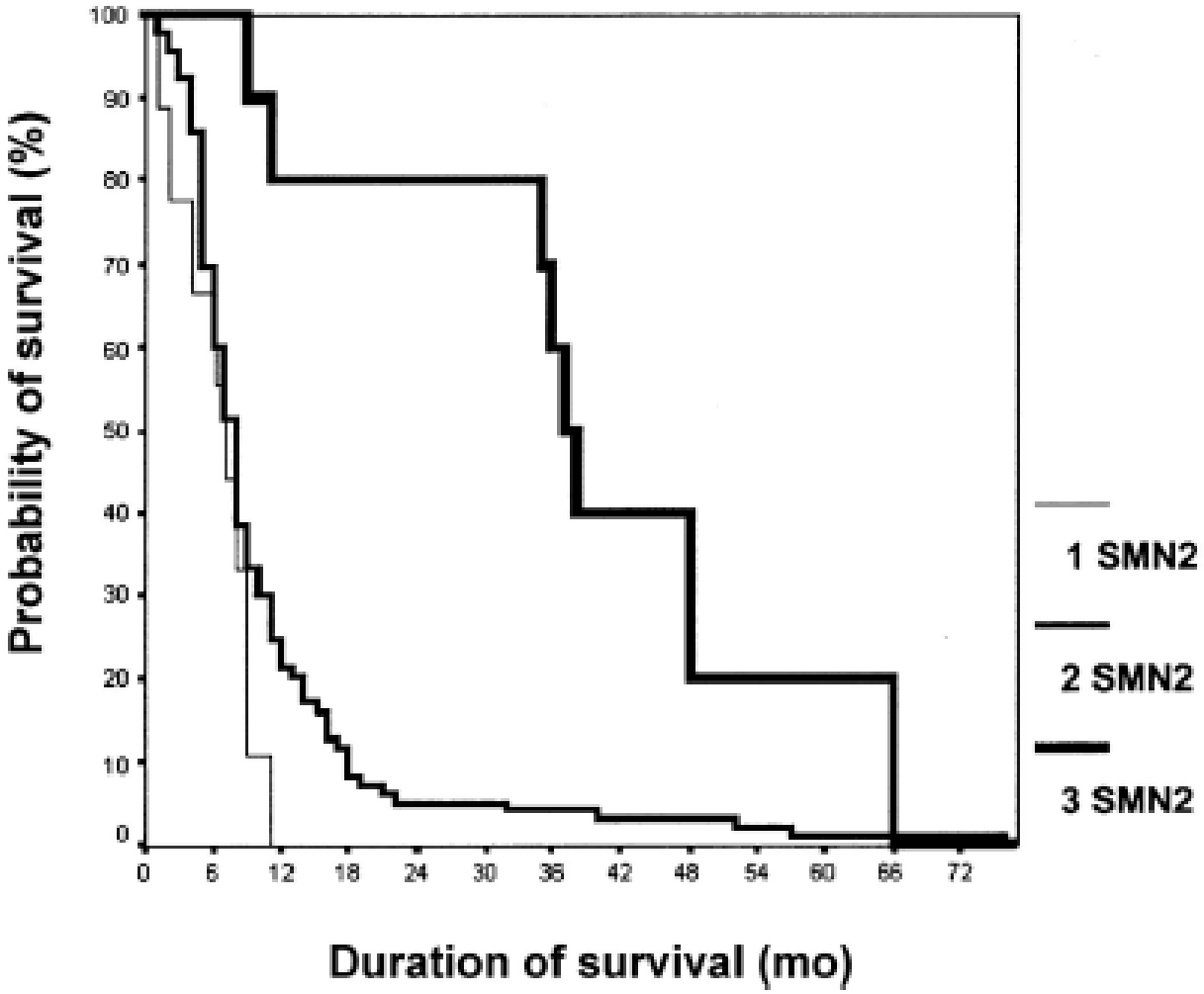
full-length SMN (~5-15%)

genomic copies of *SMN2* varies (0–5)
↑ *SMN2* ≈ less severe, later onset

Spinal Muscular Atrophy (SMA)

Age at onset, symptoms, severity and survival vary

SMA Type	A	*SMN2* copy #[1, 2]
Type I (Werdnig-Hoffmann)	<(	1 (7-13%) **2 (73-83%)** 3 (4-20%) 4 (---)
Type II	6	1 (---) 2 (11%) **3 (82%)** 4 (7%)
Type III (Kugelberg-Welander)	>	1 (---) 2 (0-4%) **3 (51-78%)** 4 (22-46%)
Type IV	2	1 2 3 4



[1]Mailman, 200

Path to SMA Newborn Screening

Should SMA be screened?

- ☑ Important health problem
- ☑ Natural hx known
- ☑ Recognizable latent stage
- ☑ Biomarker & test (2000's)
- ☑ Acceptable Tx (2016)
- ☑ Demonstrated benefit of early detection, intervention & Tx (2016)

Screening criteria adapted from Wilson and Jungner (1968) Principles and practice of screening for disease

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photo: March of Dimes

Nomination of SMA for addition to RUSP (2017)

- **Evidence review by ACHDNC**
  - **NBS assay validated and implemented in traditional public health lab**
  - **Spinraza FDA-approved in 2016**
  - **Clinical trials (Spinraza, AVXS-101) published in 2017**

- **Recommendation to Secretary: Newborn Screening for SMA due to homozygous deletion of exon 7 in *SMN1* should be added to the RUSP as a core condition.**
  (February 8, 2018; 8-5 vote → June 9, 2018 was due)

Pilot Newborn Screening for SMA

Columbia University Medical Center, Columbia Presbyterian Hospitals, and NYS Newborn Screening Program

Major Goals

- Develop *SMN1* assay
- Demonstrate feasibility of high-throughput newborn SMA screening
- Offer screening, assess uptake and outcomes; including carrier status

Morgan Stanley Children's
Manhattan
4,400 births/yr

Allen Hospital
Upper Manhattan/Bronx
2,000 births/yr

Weill-Cornell Medical Center
Manhattan
5,800 births/yr

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Recruitment – Opt-in model

Hospitals - 3 NYC hospitals, 12,000 births/yr

Materials - video & brochure

Coordinators - describe study, answer questions, obtain consent on tablet (REDCap), mark Guthrie card

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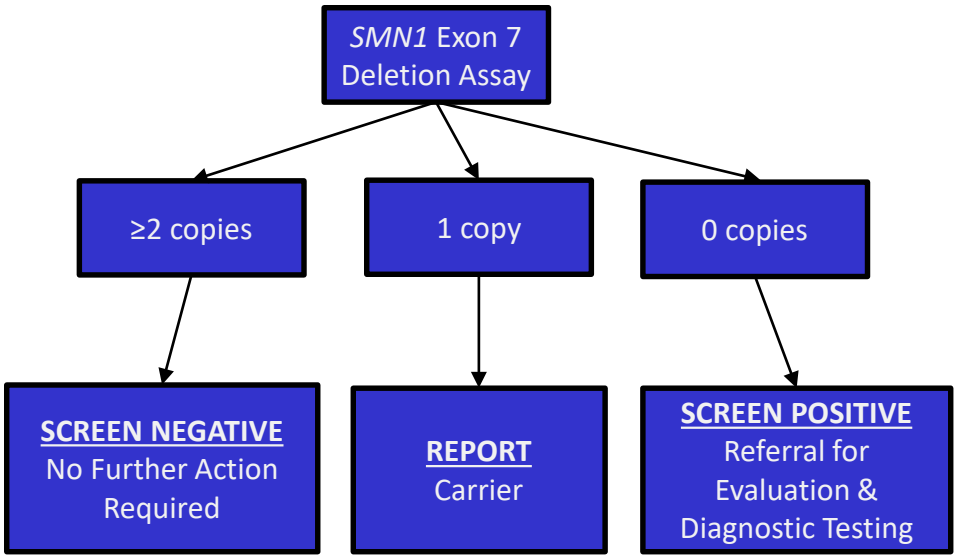
Screening – *SMN1* exon 7 deletion assay

- No biomarker; DNA-first test
- DNA extracted from dried blood spot
- TaqMan real-time qPCR assay
  - *SMN1* exon 7[1]
  - *RPPH1* (internal control gene)
- ABI 7900HT / QuantStudio 12K Flex
- ΔΔCt to calculate *SMN1* copy number

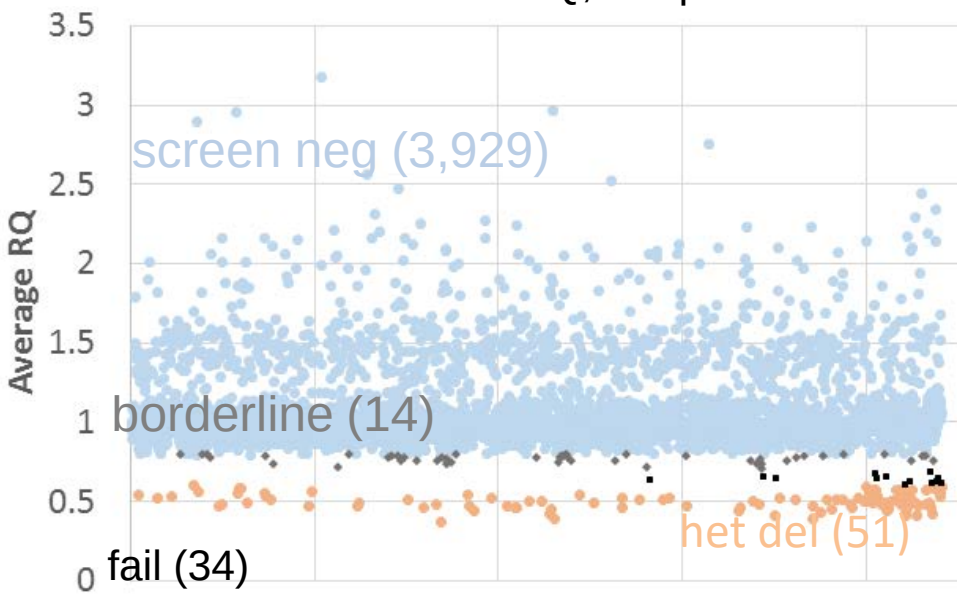


[1]Anhuf, Eggermann, Rudnik-Schöneborn, Zerres (2003) Human Mutation;22(1):74-8.

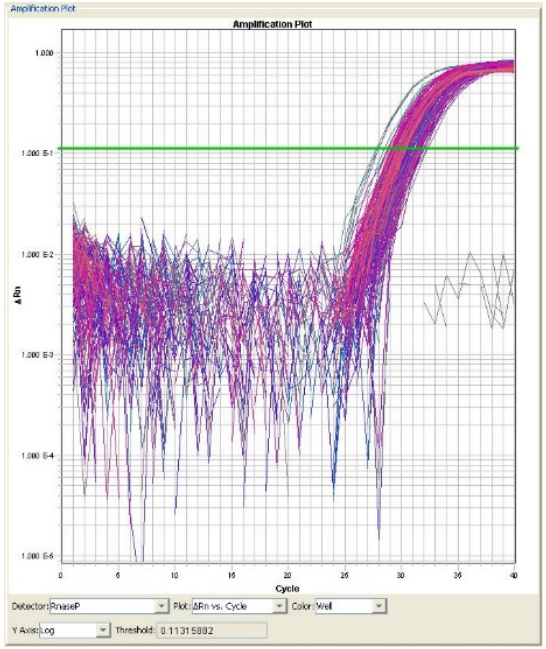
SMA Assay Validation

4,028 DBS

*each point=mean RQ, 3 replicates

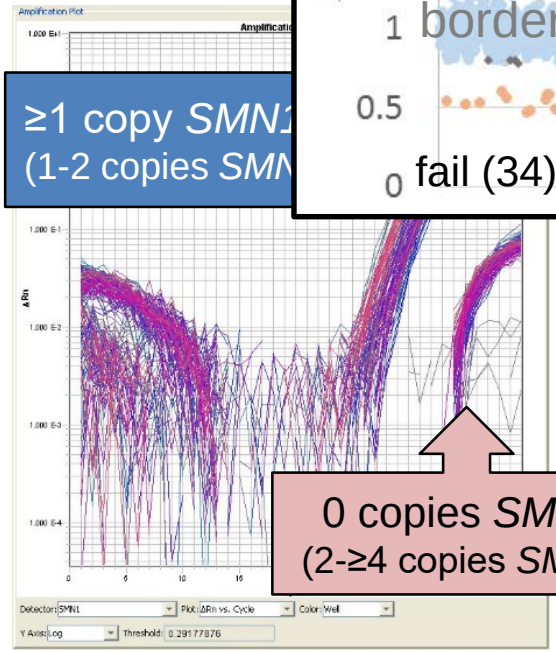


45 Positive Controls



RPPH1 amplification

≥1 copy *SMN1*
(1-2 copies *SMN*



SMN1 amplification

0 copies *SMN1*
(2-≥4 copies *SMN2*)

Probe
5’-FAM (SMN1)
5’-VIC (RPPH1)
3’ quencher – MGB-NFQ
ROX standard

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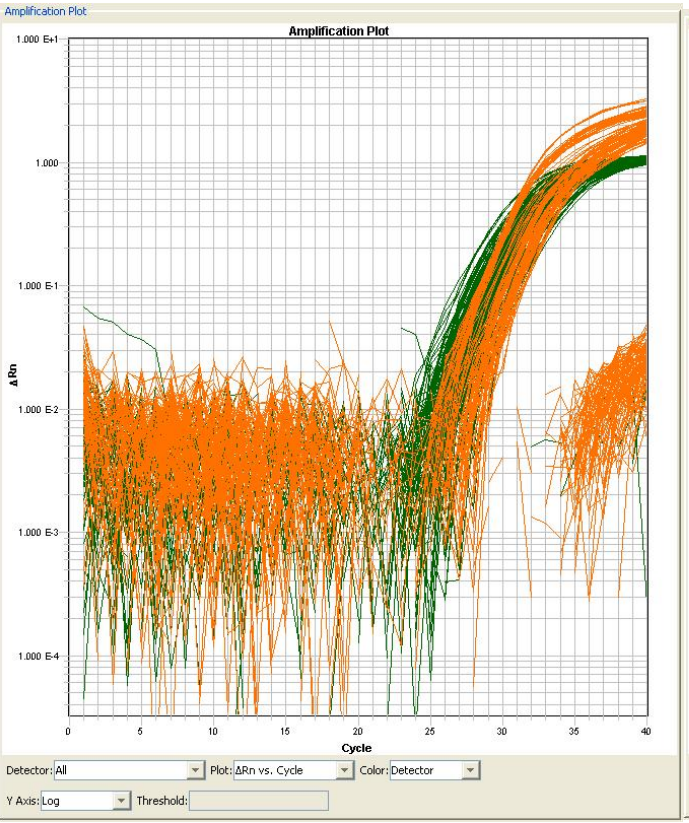
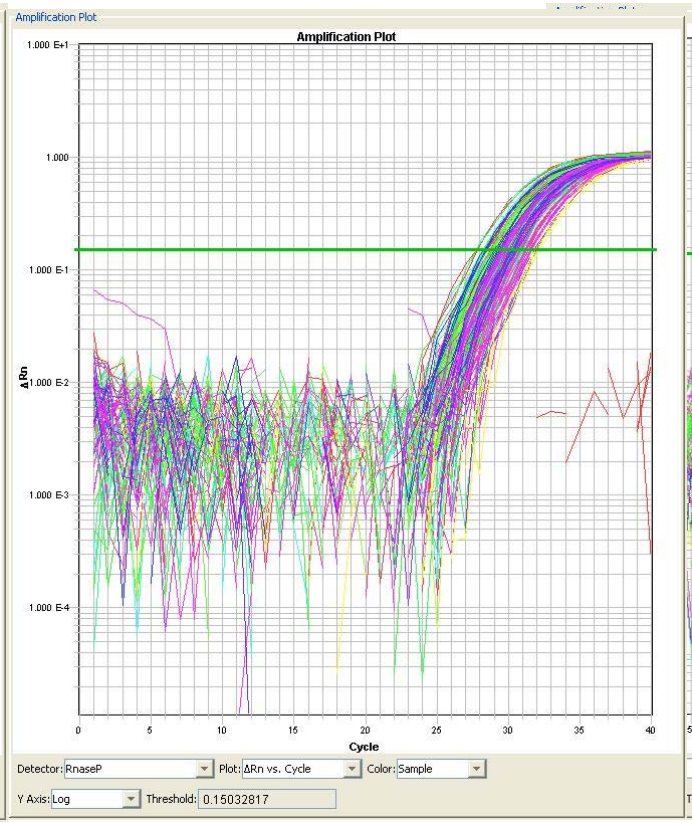
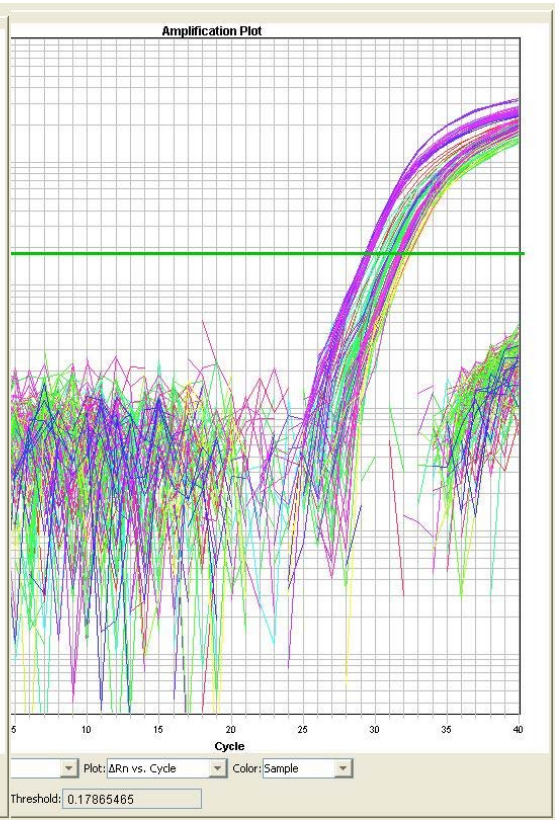
Biogen Samples







Both detectors **RPPH1** **SMN1**

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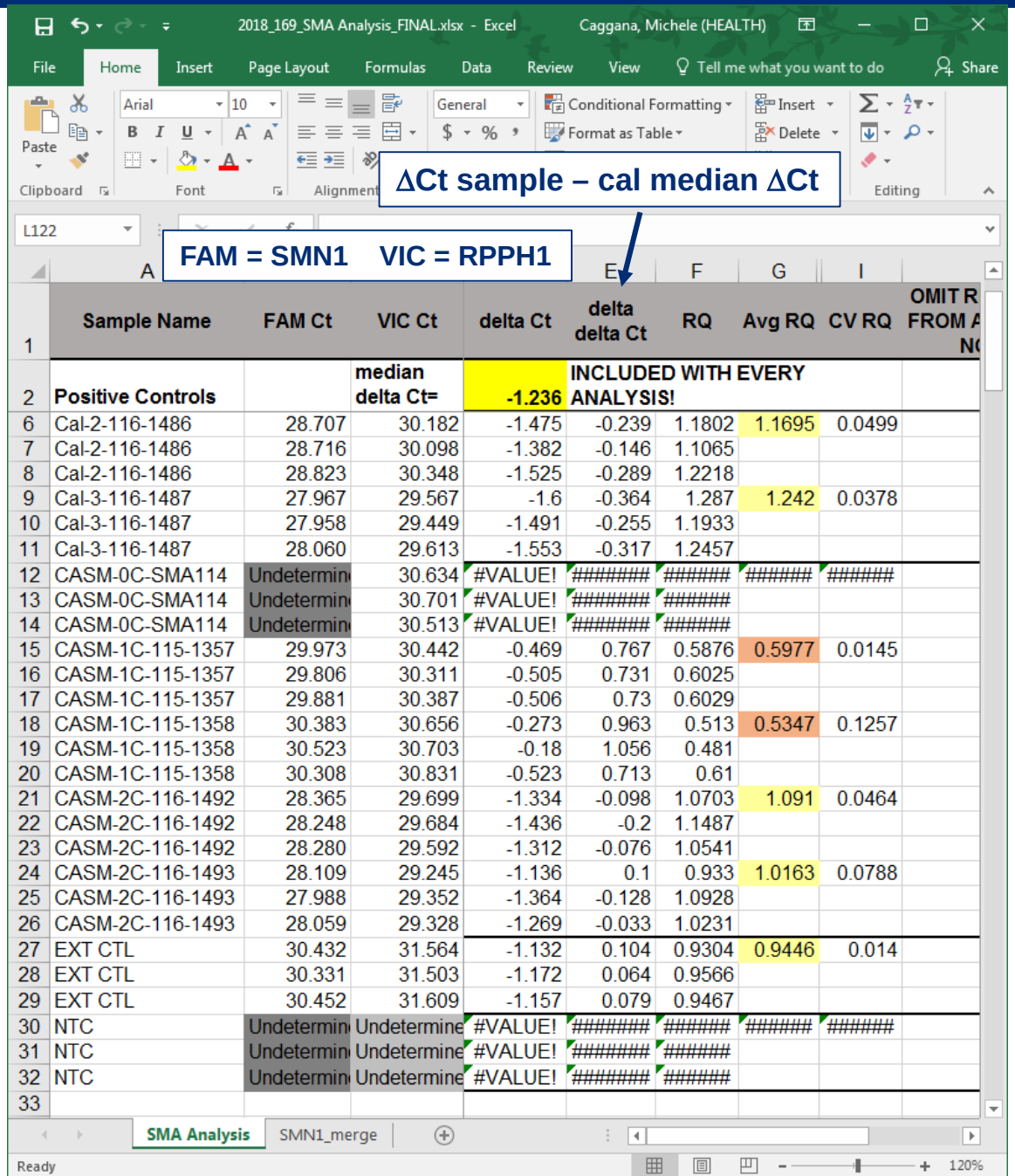

1	Sample Name	FAM Ct	VIC Ct	delta Ct	delta delta Ct	RQ	Avg RQ	CV RQ	OMIT R FROM A NC
2	Positive Controls		median delta Ct=	-1.236	INCLUDED WITH EVERY ANALYSIS!				
6	Cal-2-116-1486	28.707	30.182	-1.475	-0.239	1.1802	1.1695	0.0499	
7	Cal-2-116-1486	28.716	30.098	-1.382	-0.146	1.1065			
8	Cal-2-116-1486	28.823	30.348	-1.525	-0.289	1.2218			
9	Cal-3-116-1487	27.967	29.567	-1.6	-0.364	1.287	1.242	0.0378	
10	Cal-3-116-1487	27.958	29.449	-1.491	-0.255	1.1933			
11	Cal-3-116-1487	28.060	29.613	-1.553	-0.317	1.2457			
12	CASM-0C-SMA114	Undetermin	30.634	#VALUE!	########	#######	#######	#######	
13	CASM-0C-SMA114	Undetermin	30.701	#VALUE!	########	#######			
14	CASM-0C-SMA114	Undetermin	30.513	#VALUE!	########	#######			
15	CASM-1C-115-1357	29.973	30.442	-0.469	0.767	0.5876	0.5977	0.0145	
16	CASM-1C-115-1357	29.806	30.311	-0.505	0.731	0.6025			
17	CASM-1C-115-1357	29.881	30.387	-0.506	0.73	0.6029			
18	CASM-1C-115-1358	30.383	30.656	-0.273	0.963	0.513	0.5347	0.1257	
19	CASM-1C-115-1358	30.523	30.703	-0.18	1.056	0.481			
20	CASM-1C-115-1358	30.308	30.831	-0.523	0.713	0.61			
21	CASM-2C-116-1492	28.365	29.699	-1.334	-0.098	1.0703	1.091	0.0464	
22	CASM-2C-116-1492	28.248	29.684	-1.436	-0.2	1.1487			
23	CASM-2C-116-1492	28.280	29.592	-1.312	-0.076	1.0541			
24	CASM-2C-116-1493	28.109	29.245	-1.136	0.1	0.933	1.0163	0.0788	
25	CASM-2C-116-1493	27.988	29.352	-1.364	-0.128	1.0928			
26	CASM-2C-116-1493	28.059	29.328	-1.269	-0.033	1.0231			
27	EXT CTL	30.432	31.564	-1.132	0.104	0.9304	0.9446	0.014	
28	EXT CTL	30.331	31.503	-1.172	0.064	0.9566			
29	EXT CTL	30.452	31.609	-1.157	0.079	0.9467			
30	NTC	Undetermin	Undetermine	#VALUE!	########	#######	#######	#######	
31	NTC	Undetermin	Undetermine	#VALUE!	########	#######			
32	NTC	Undetermin	Undetermine	#VALUE!	########	#######			
33									

SMA Assay Controls

All in triplicate

RQ = relative quantity = 2^(-ΔΔCt)

Known 2 *SMN1* copies As calibrators

0 copy *SMN1* control

1 copy *SMN1* control

2 copies *SMN1* control

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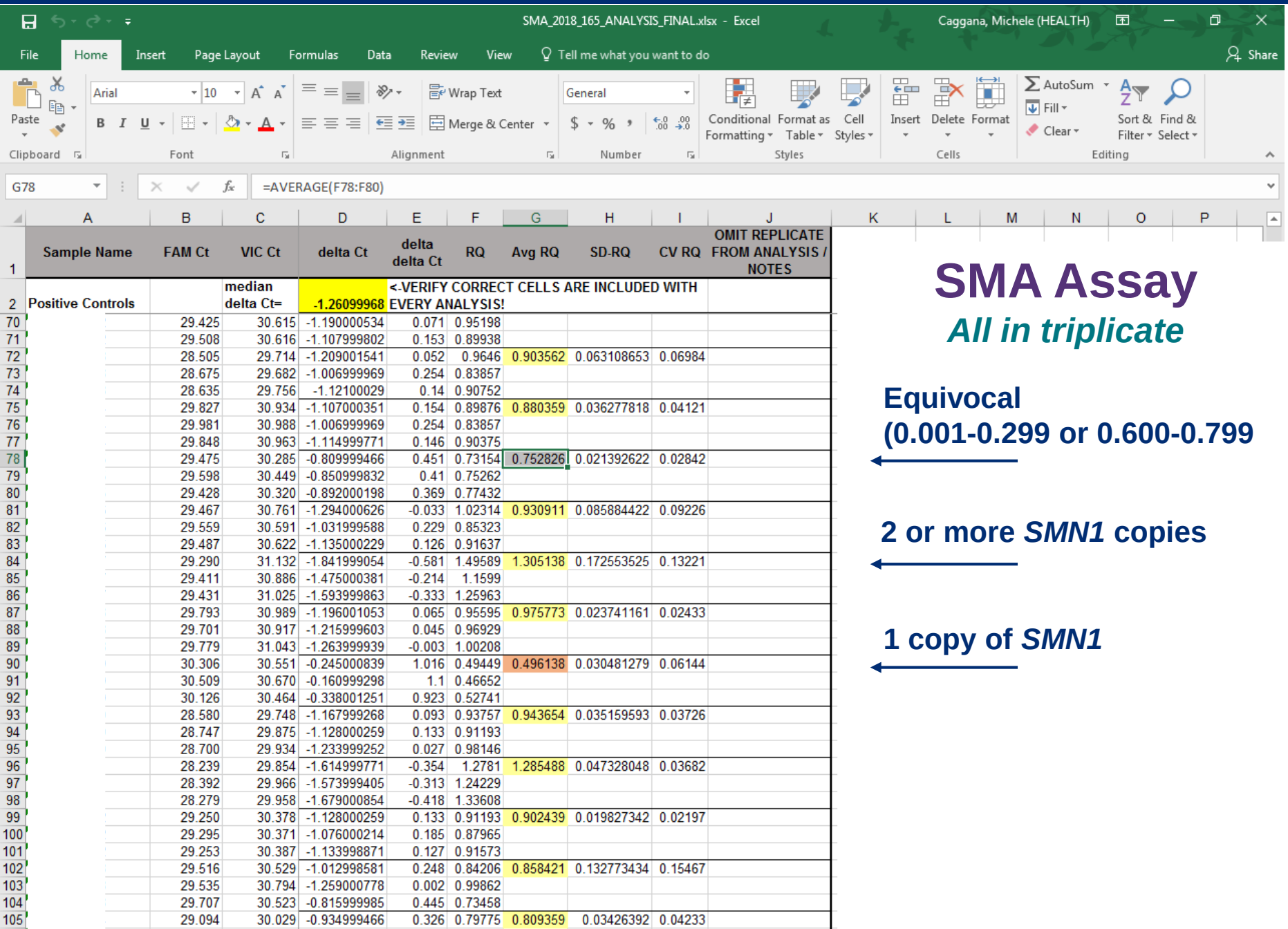


	A	B	C	D	E	F	G	H	I	J
1	Sample Name	FAM Ct	VIC Ct	delta Ct	delta delta Ct	RQ	Avg RQ	SD-RQ	CV RQ	OMIT REPLICATE FROM ANALYSIS / NOTES
2	Positive Controls		median delta Ct=	-1.26099968	<-VERIFY CORRECT CELLS ARE INCLUDED WITH EVERY ANALYSIS!					
70		29.425	30.615	-1.190000534	0.071	0.95198				
71		29.508	30.616	-1.107999802	0.153	0.89938				
72		28.505	29.714	-1.209001541	0.052	0.9646	0.903562	0.063108653	0.06984	
73		28.675	29.682	-1.006999969	0.254	0.83857				
74		28.635	29.756	-1.12100029	0.14	0.90752				
75		29.827	30.934	-1.107000351	0.154	0.89876	0.880359	0.036277818	0.04121	
76		29.981	30.988	-1.006999969	0.254	0.83857				
77		29.848	30.963	-1.114999771	0.146	0.90375				
78		29.475	30.285	-0.809999466	0.451	0.73154	0.752826	0.021392622	0.02842	
79		29.598	30.449	-0.850999832	0.41	0.75262				
80		29.428	30.320	-0.892000198	0.369	0.77432				
81		29.467	30.761	-1.294000626	-0.033	1.02314	0.930911	0.085884422	0.09226	
82		29.559	30.591	-1.031999588	0.229	0.85323				
83		29.487	30.622	-1.135000229	0.126	0.91637				
84		29.290	31.132	-1.841999054	-0.581	1.49589	1.305138	0.172553525	0.13221	
85		29.411	30.886	-1.475000381	-0.214	1.1599				
86		29.431	31.025	-1.593999863	-0.333	1.25963				
87		29.793	30.989	-1.196001053	0.065	0.95595	0.975773	0.023741161	0.02433	
88		29.701	30.917	-1.215999603	0.045	0.96929				
89		29.779	31.043	-1.263999939	-0.003	1.00208				
90		30.306	30.551	-0.245000839	1.016	0.49449	0.496138	0.030481279	0.06144	
91		30.509	30.670	-0.160999298	1.1	0.46652				
92		30.126	30.464	-0.338001251	0.923	0.52741				
93		28.580	29.748	-1.167999268	0.093	0.93757	0.943654	0.035159593	0.03726	
94		28.747	29.875	-1.128000259	0.133	0.91193				
95		28.700	29.934	-1.233999252	0.027	0.98146				
96		28.239	29.854	-1.614999771	-0.354	1.2781	1.285488	0.047328048	0.03682	
97		28.392	29.966	-1.573999405	-0.313	1.24229				
98		28.279	29.958	-1.679000854	-0.418	1.33608				
99		29.250	30.378	-1.128000259	0.133	0.91193	0.902439	0.019827342	0.02197	
100		29.295	30.371	-1.076000214	0.185	0.87965				
101		29.253	30.387	-1.133998871	0.127	0.91573				
102		29.516	30.529	-1.012998581	0.248	0.84206	0.858421	0.132773434	0.15467	
103		29.535	30.794	-1.259000778	0.002	0.99862				
104		29.707	30.523	-0.815999985	0.445	0.73458				
105		29.094	30.029	-0.934999466	0.326	0.79775	0.809359	0.03426392	0.04233	

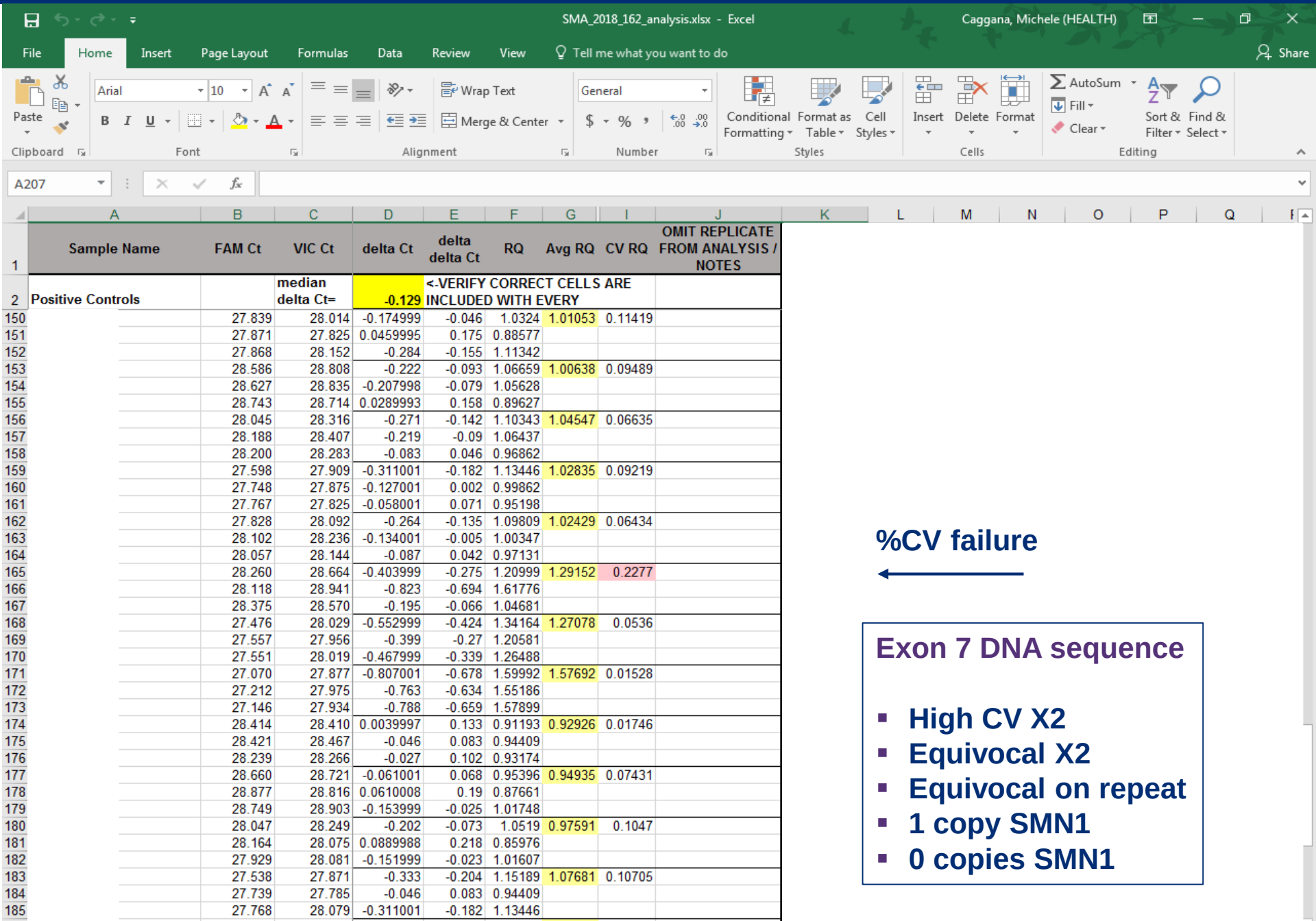
SMA Assay

All in triplicate

Equivocal (0.001-0.299 or 0.600-0.799

2 or more *SMN1* copies

1 copy of *SMN1*



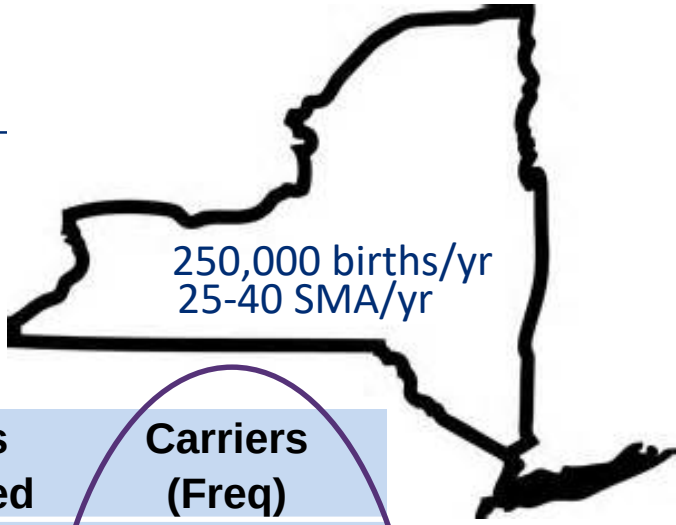
	Sample Name	FAM Ct	VIC Ct	delta Ct	delta delta Ct	RQ	Avg RQ	CV RQ	OMIT REPLICATE FROM ANALYSIS / NOTES
1									
2	Positive Controls		median delta Ct=	-0.129	<-VERIFY CORRECT CELLS ARE INCLUDED WITH EVERY				
150		27.839	28.014	-0.174999	-0.046	1.0324	1.01053	0.11419	
151		27.871	27.825	0.0459995	0.175	0.88577			
152		27.868	28.152	-0.284	-0.155	1.11342			
153		28.586	28.808	-0.222	-0.093	1.06659	1.00638	0.09489	
154		28.627	28.835	-0.207998	-0.079	1.05628			
155		28.743	28.714	0.0289993	0.158	0.89627			
156		28.045	28.316	-0.271	-0.142	1.10343	1.04547	0.06635	
157		28.188	28.407	-0.219	-0.09	1.06437			
158		28.200	28.283	-0.083	0.046	0.96862			
159		27.598	27.909	-0.311001	-0.182	1.13446	1.02835	0.09219	
160		27.748	27.875	-0.127001	0.002	0.99862			
161		27.767	27.825	-0.058001	0.071	0.95198			
162		27.828	28.092	-0.264	-0.135	1.09809	1.02429	0.06434	
163		28.102	28.236	-0.134001	-0.005	1.00347			
164		28.057	28.144	-0.087	0.042	0.97131			
165		28.260	28.664	-0.403999	-0.275	1.20999	1.29152	0.2277	
166		28.118	28.941	-0.823	-0.694	1.61776			
167		28.375	28.570	-0.195	-0.066	1.04681			
168		27.476	28.029	-0.552999	-0.424	1.34164	1.27078	0.0536	
169		27.557	27.956	-0.399	-0.27	1.20581			
170		27.551	28.019	-0.467999	-0.339	1.26488			
171		27.070	27.877	-0.807001	-0.678	1.59992	1.57692	0.01528	
172		27.212	27.975	-0.763	-0.634	1.55186			
173		27.146	27.934	-0.788	-0.659	1.57899			
174		28.414	28.410	0.0039997	0.133	0.91193	0.92926	0.01746	
175		28.421	28.467	-0.046	0.083	0.94409			
176		28.239	28.266	-0.027	0.102	0.93174			
177		28.660	28.721	-0.061001	0.068	0.95396	0.94935	0.07431	
178		28.877	28.816	0.0610008	0.19	0.87661			
179		28.749	28.903	-0.153999	-0.025	1.01748			
180		28.047	28.249	-0.202	-0.073	1.0519	0.97591	0.1047	
181		28.164	28.075	0.0889988	0.218	0.85976			
182		27.929	28.081	-0.151999	-0.023	1.01607			
183		27.538	27.871	-0.333	-0.204	1.15189	1.07681	0.10705	
184		27.739	27.785	-0.046	0.083	0.94409			
185		27.768	28.079	-0.311001	-0.182	1.13446			

%CV failure

Exon 7 DNA sequence

- **High CV X2**
- **Equivocal X2**
- **Equivocal on repeat**
- **1 copy SMN1**
- **0 copies SMN1**

Results



January 15, 2016 – June 15, 2018
Infants screened: 14,089 (200 carriers)
Opt in rate: 91-93%

Hospital	Recruitment period	Infants Screened	Carriers (Freq)
Morgan Stanley Children's Hospital	1/14/2016 – 5/9/2018*	5,840	74 (1 in 79)
Weill-Cornell Medical Center	7/13/2016 – 5/9/2018	4,851	95 (1 in 51)
Allen Hospital	1/26/2016 – 5/9/2018	2,523	20 (1 in 126)
Total		**13,214**	**189 (1 in 70)**

False positives: 0% (0/13,214)
False negatives: 0% (0/13,214)

Retest rate ~1%; mostly around carrier calls; live = no CV fails

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Follow-up – Carriers

14.1% (16/113) agreed to genetics referral
- **73.3% (11/15) made appt**
- **72.7% (8/11) maintained appt**

photo: Mass general

Most parents expressed concern; after speaking with counselor, expressed understanding of "carrier" status versus "affected“

42.9% (81/189) knew they were carriers
- **less concerned, better understanding**

Results

Affected infant identified by NBS

Genotype:

***SMN1*: homozygous Δ exon 7**

***SMN2*: 2 copies**

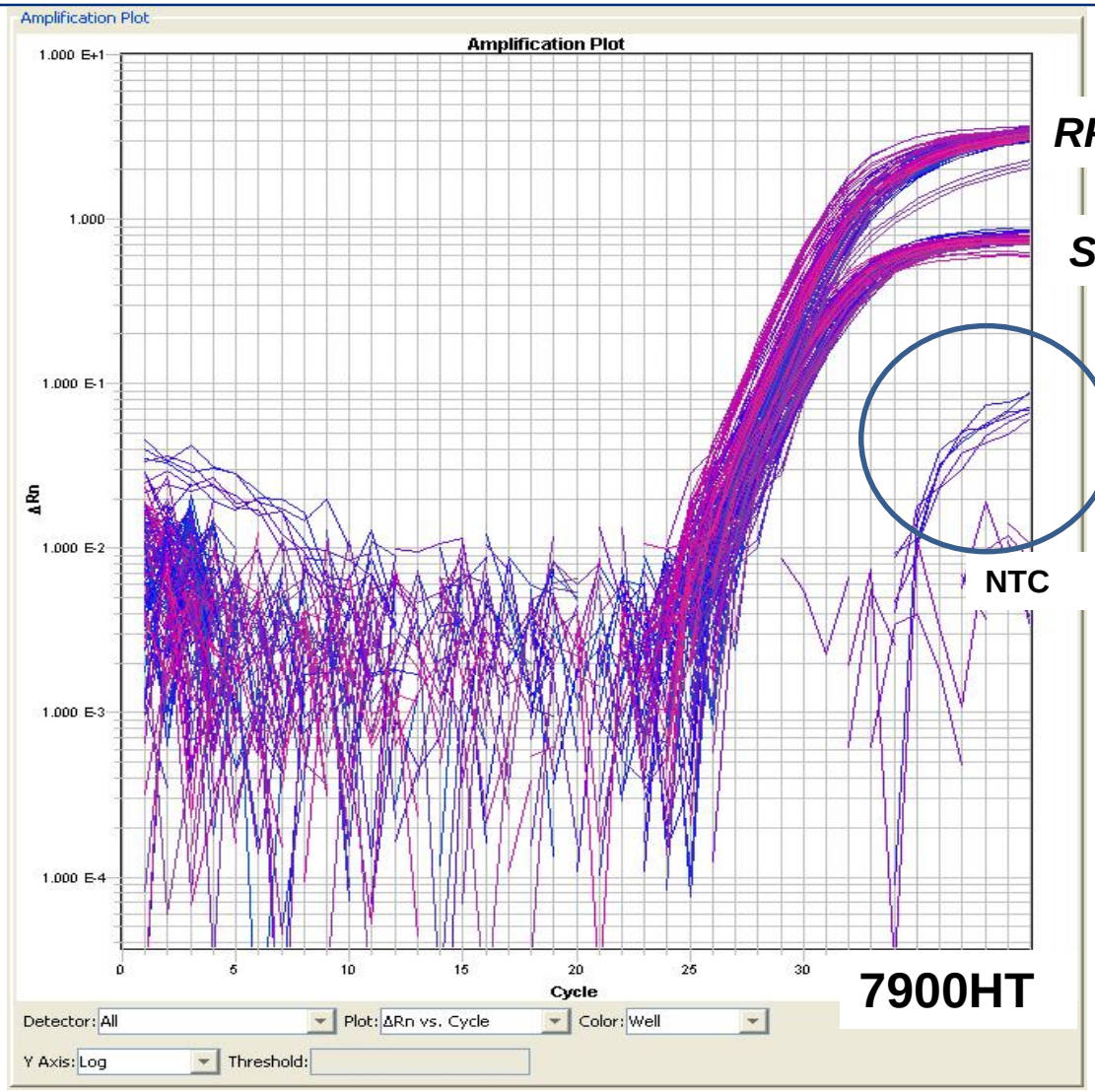
Predicts SMA type 1

SMA Type 1 Natural History

- **Onset: <6 months**
- **Survival: ≤2 years**
- **Major motor milestones reached: None; never sit unassisted.**
- **Sx: Profound hypotonia and flaccidity, no head control, poor suck & swallow; respiratory and nutritional problems**

@ 29 months – tolerates medication, meeting milestones on time, walking, running, talking

Results



SMA Assay
Detected homozygous deletion

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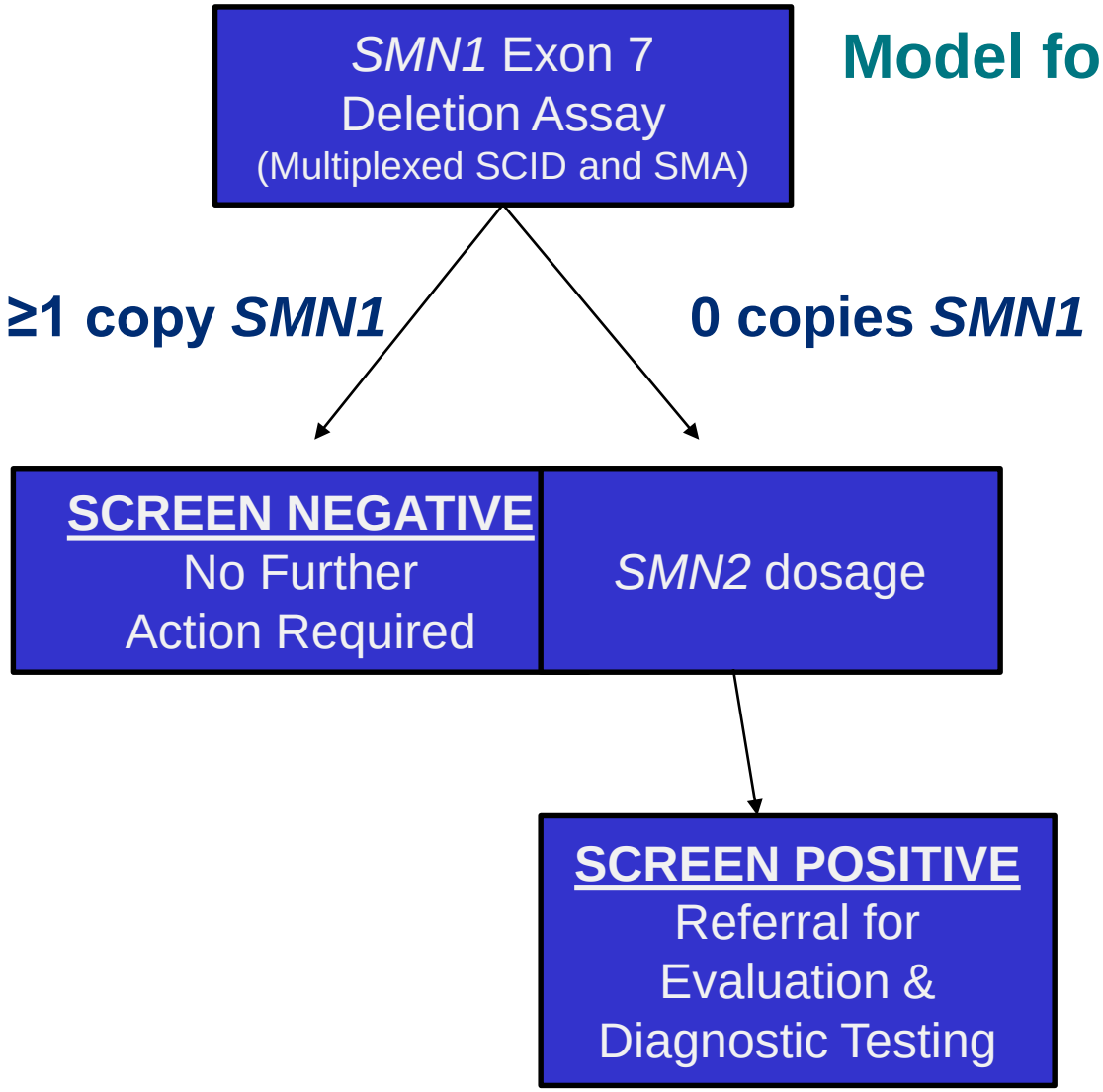
Conclusions

- SMA newborn screening is feasible
  - Sensitive, specific, robust, high-throughput
  - No false positives/negatives
- NYS families want testing (93%)
- Carrier rate = 1 in 70
- 1 infant predicted to have type 1 infantile SMA (1 in 13,214)
  - treated with nusinersen (Spinraza)
  - asymptomatic at 29 months

Population-wide Screening in NYS

- **Regulatory amendment** ***(bill pending currently)***
- **Specialty Care Centers** ***(certifying)***
  - **Genetics, neuromuscular specialists (n = 11)**
- **No carrier reporting**
- **Multiplex with severe combined immunodeficiency (SCID) qPCR assay; singlicate**
  - **$0.10/baby for SMA FOR TEST**
- ***SMN2*** **dosage (digital droplet PCR), about $25 per baby**

Screening – *SMN1* exon 7 deletion assay



Model for universal screening

Will use Ct cut-off rather than ΔΔCt to calculate *SMN1* copy number

Probes
5'-VIC (SMN1)
5'-ABY (RPPH1)
5'-FAM (TREC)
3' quencher – MGB-NFQ
(SMN1 + TREC)
3' quencher – QSY
(RPPH1)

Purple haze standard

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Universal SMA Screening – New York Plan

Multiplex with SCID TREC assay

Carriers

- Not reported

Late onset SMA

- *SMN2* copy number
- When to treat
- How will detection impact the incidence of SMA?

Non-deletion mutations

- Will not be detected; report language important
- 2 – 5%

Treatment

- Long-term effects? Renal toxicity?
- Availability, cost and compliance?
- Insurance Coverage

Acknowledgement

Thank you to Denise Kay, Ph.D. for slides

Acknowledgements

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- Michele Caggana, ScD, FACMG
- Colleen Stevens, PhD
- Ritu Jain, PhD
- Sandra Levin, BS
- Patrick Wilson, BS
- NYS Newborn Screening Program

Recruitment

- Jennifer Kraszewski, MS
- Bianca Haser, BS
- Veronica Ortiz, MHS
- Anthony Albertorio, BA
- Emilia Naranjo
- Talia Weitz
- Katiana Rufino
- Jacqueline Gomez, RN
- Angela Pena
- Columbia Presbyterian Hospitals

Clinical

- Wendy Chung, MD, PhD
- Carrie Koval, MS, CGC
- Julia Wynn, MS
- Lilian Cohen, MD
- Sarah Andrew, BA
- Sally Dunaway Young, PT, DPT
- Nicole LaMarca, DNP, MSN, CPNP
- Darryl De Vivo, MD
- Columbia University Medical Center

Funding

- Biogen, Idec

Controls

- Pediatric Neuromuscular Research Clinic (PNRC)
- Biogen, Idec


Screening for Spinal Muscular Atrophy
Early Data from Massachusetts Newborn Screening

APHL SMA Webinar Series Part Two:
Overview of Available Screening Methods

Anne Marie Comeau, Ph.D
Deputy Director, New England Newborn Screening Program
Professor of Pediatrics, UMass Medical School

New England Newborn Screening Program

University of Massachusetts Medical School
UMASS
umassmed.edu

DISCLOSURE

The University of Massachusetts holds intellectual property that is used in

1 of 17

pipeline therapies that are listed by Cure SMA.

Spinal Muscular Atrophy (SMA)

- Most common lethal autosomal recessive disorder in infants.
- Progressive muscle weakness resulting from degeneration of an anterior horn neurons
- FDA-approved therapy
- Recommended for RUSP by SACHDNC
- Estimated Incidence : 1 in 6,000 to 20,000
- 1 in 40 people are heterozygote carriers

Assay Development for SMA NBS

Francis K Lee and Kristina Mercer

Newborn Screening and Molecular Biology Branch,
Centers for Disease Control and Prevention

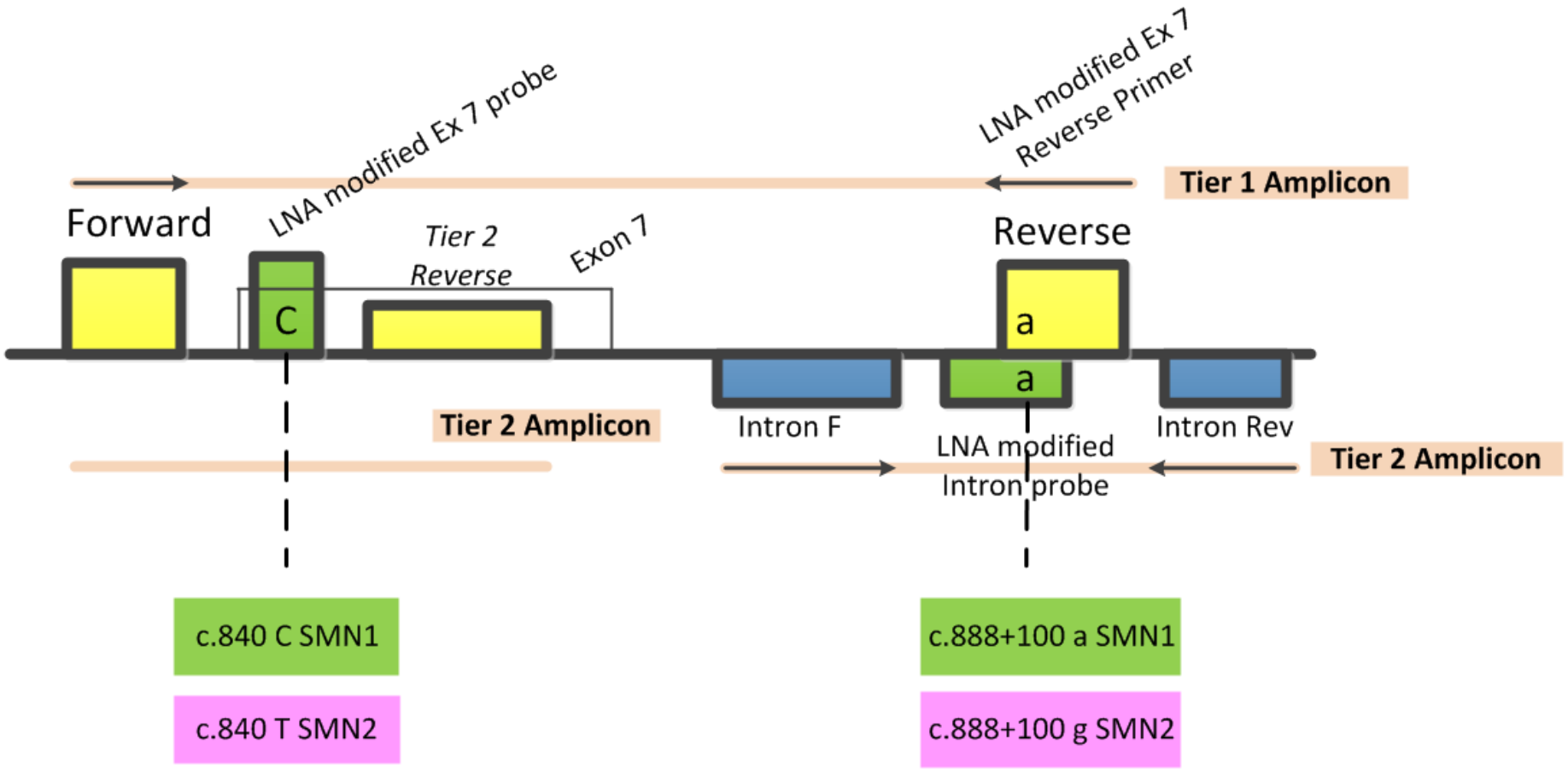
Lan Ji and Jennifer Navas

New England Newborn Screening Program
UMMS

Assay Development for SMA NBS

Two factors key to development:

- SMA is related to the absence of a fully functional gene that produces a Survival of Motor Neuron (SMN) protein, *SMN1*
- 95% SMA patients show homozygous loss of *SMN1* exon 7



Validation

Pre-characterized samples from Corielle n=7

Pre-characterized samples from CDC n= 2

Pre-characterized samples from Biogen
n= 22 SMA patients
n= 44 obligate carriers (parents)

100% pass

The Massachusetts SMA NBS Workgroup

Representatives from Newborn Screening, Neurology, Genetics

Baystate Children's Hospital

Mary Alice Abbott, MD

Children's Hospital Boston

Basil Darras, MD

UMassMemorial Medical Center
A Member of UMass Memorial Health Care

Beverly N. Hay, MD

MassGeneral Hospital for Children

Kathryn J. Swoboda, MD

New England Newborn Screening Program

Anne Marie Comeau, PhD
Jaime E. Hale, MS
Inderneel Sahai, MD
Roger B. Eaton, PhD

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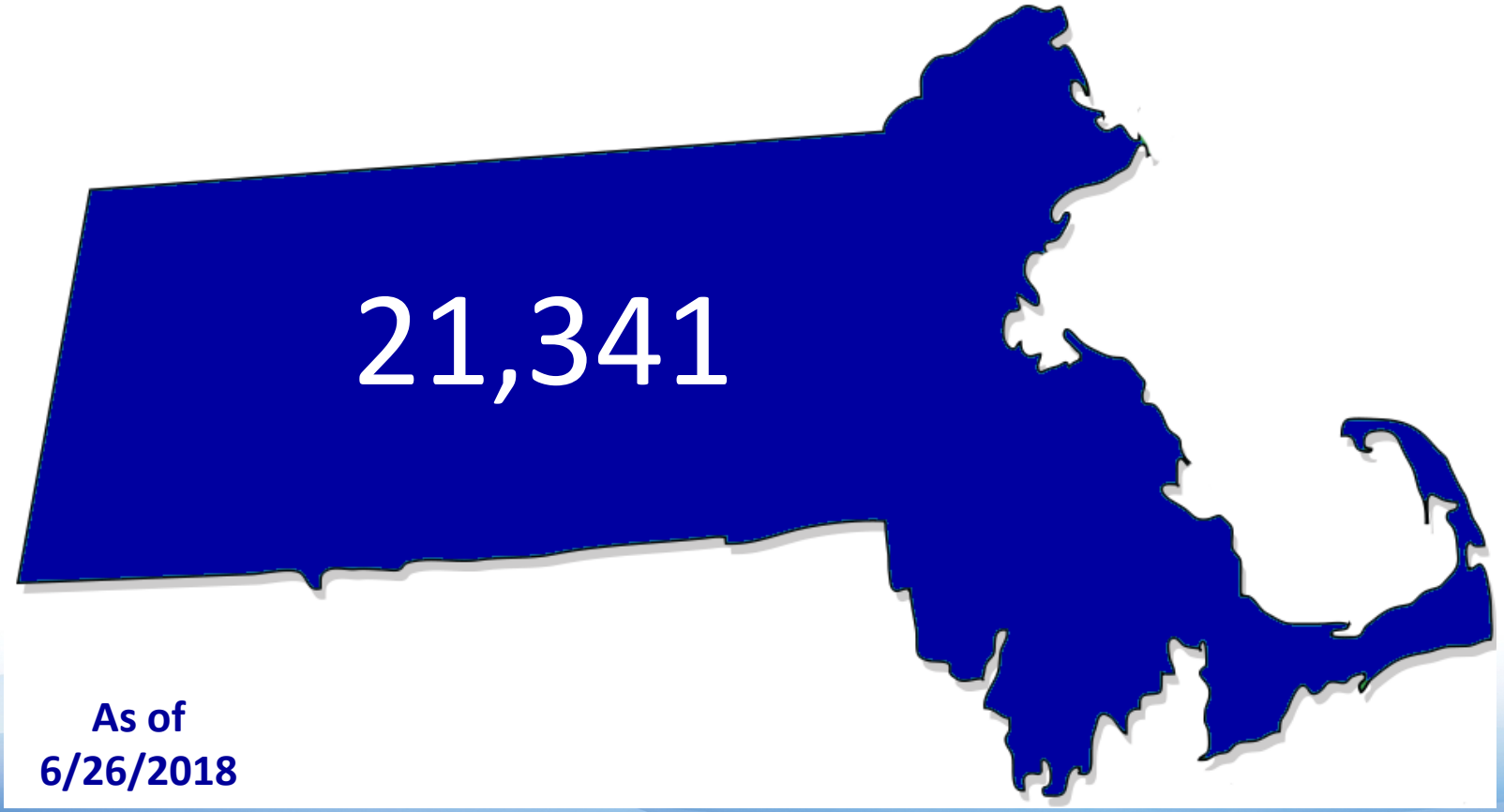
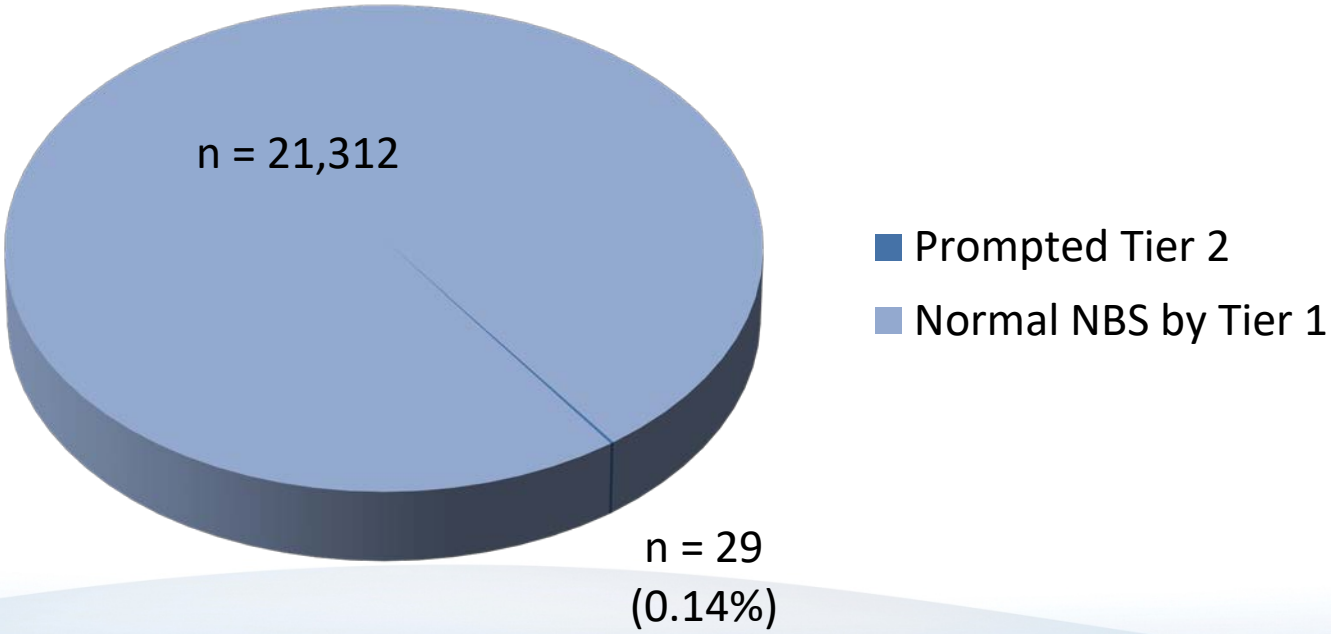
Number of Babies Screened for SMA

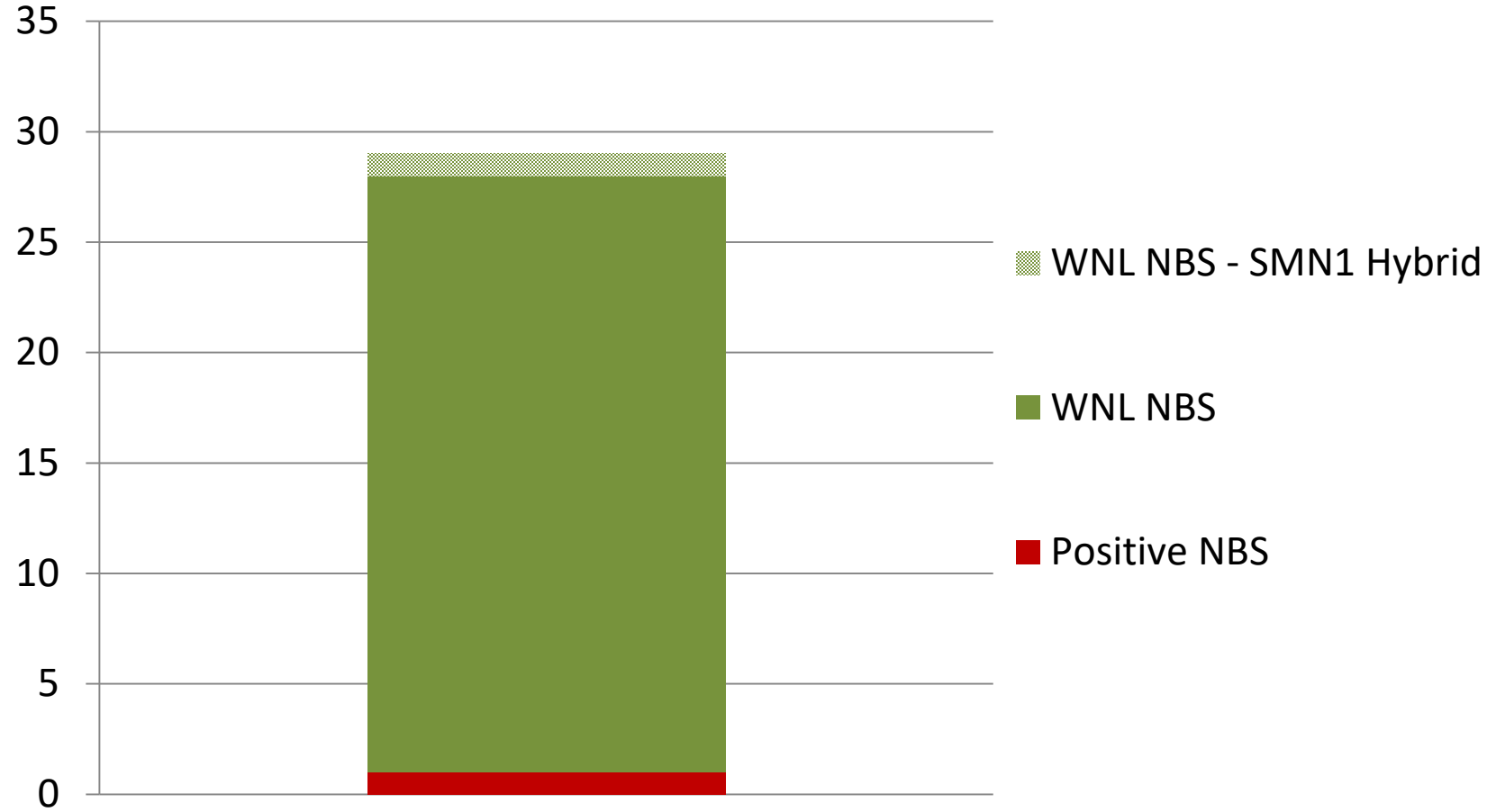


As of 6/26/2018

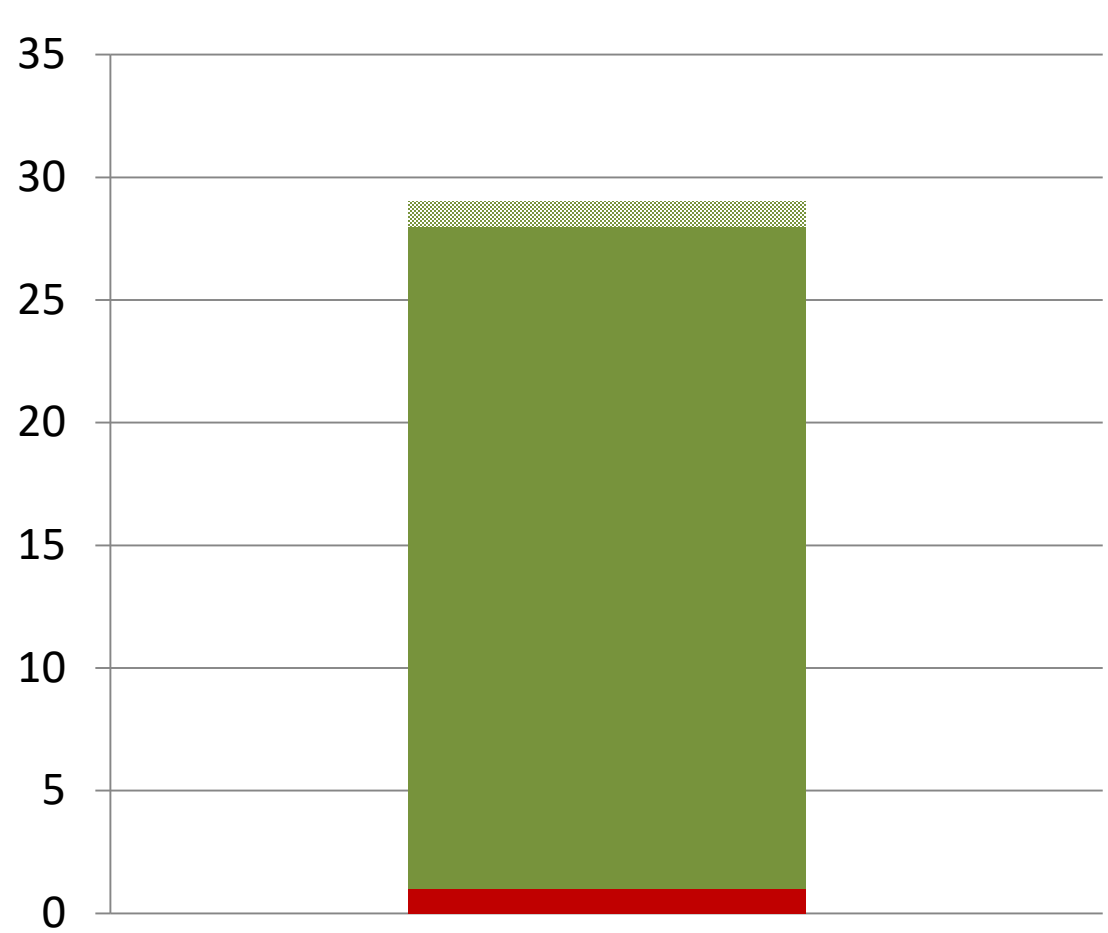
Number of infants with a specimen prompting Tier 2



Infants with a specimen prompting Tier 2
n = 29



Infants with a specimen prompting Tier 2 n = 29



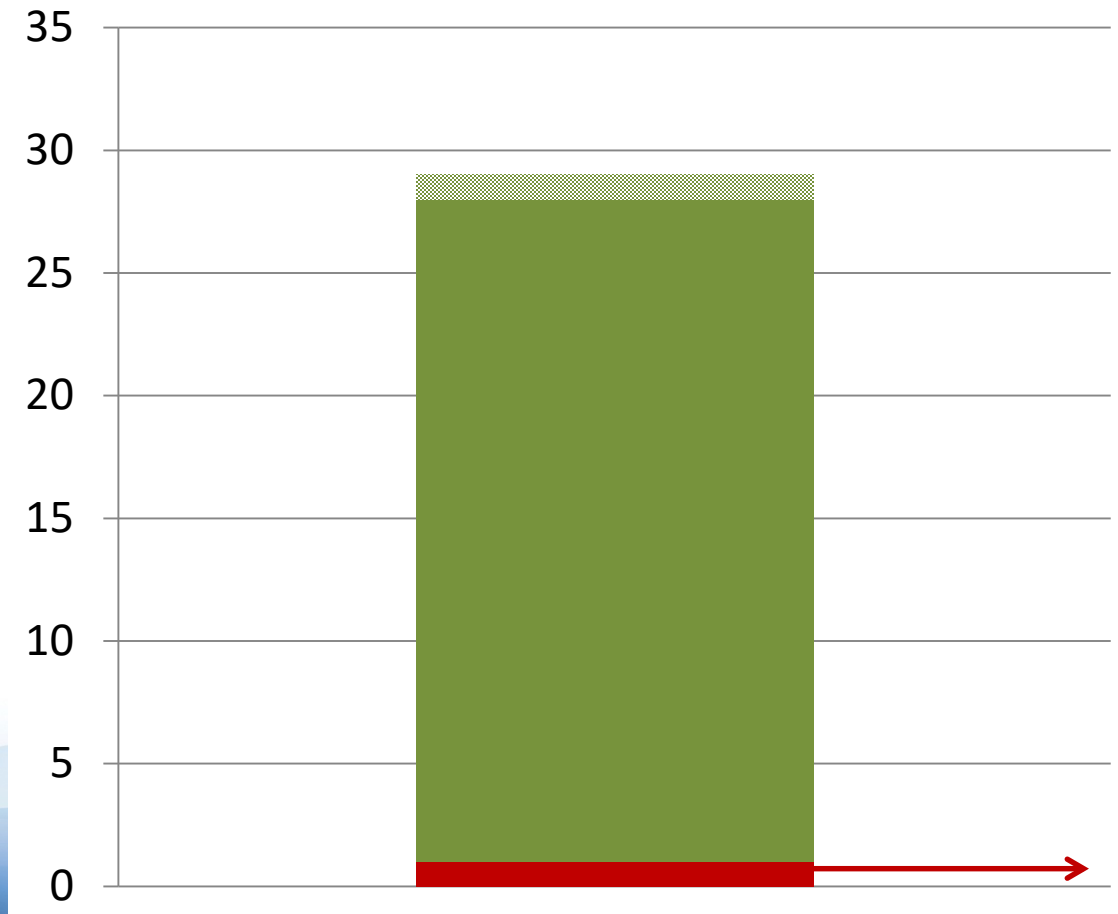
72% prompting Tier 2 have been NICU specimens

WNL NBS - SMN1 Hybrid

WNL NBS

Positive NBS

Infants with a specimen prompting Tier 2 n = 29



72% prompting Tier 2 have been NICU specimens

WNL NBS - SMN1 Hybrid

WNL NBS

Positive NBS

False positive; specimen apparently contained an inhibitor

Implementation of SMA/TREC LDT Assay

Katelyn Logerquist, MLS(ASCP)^{CM}

David E. Jones, PhD

Andy Rohrwasser, PhD

SMA Webinar

June 28, 2018

UTAH DEPARTMENT OF HEALTH

NEWBORN SCREENING

SMA/TREC Assay Method

- PCR-Based Triplex Assay (described by Dr. Lee)
  - *SMN1* – Deletion of exon 7 of *SMN1* gene (SMA)
  - TREC – T-cell receptor excision circles (SCID)
  - *RPP30* – Internal control
- Extraction
  - Automated – TECAN Freedom EVO
  - PBS/Tween 20 wash/Qiagen Solution 2 wash and elution
  - 96 well format to 384 well format
- Real-Time PCR
  - Roche LightCycler 480 II
  - 384 well block

Extraction

1. 3.2 mm punch
2. Wash 1: 80ul PBS/Tween 20, 8 mins, shaking 700rpm (RT, Inheco)
3. Wash 2: 80ul Qiagen Solution 2, 8 mins, RT, shaking 700rpm
4. Elution: 140ul Qiagen solution 2, 30 mins, 70C, shaking 700rpm
5. Transfer 3.5 ul into 384 well, PCR volume 12 ul


TECAN.
TECAN.
MASTER MIX
96pcs
TECAN.

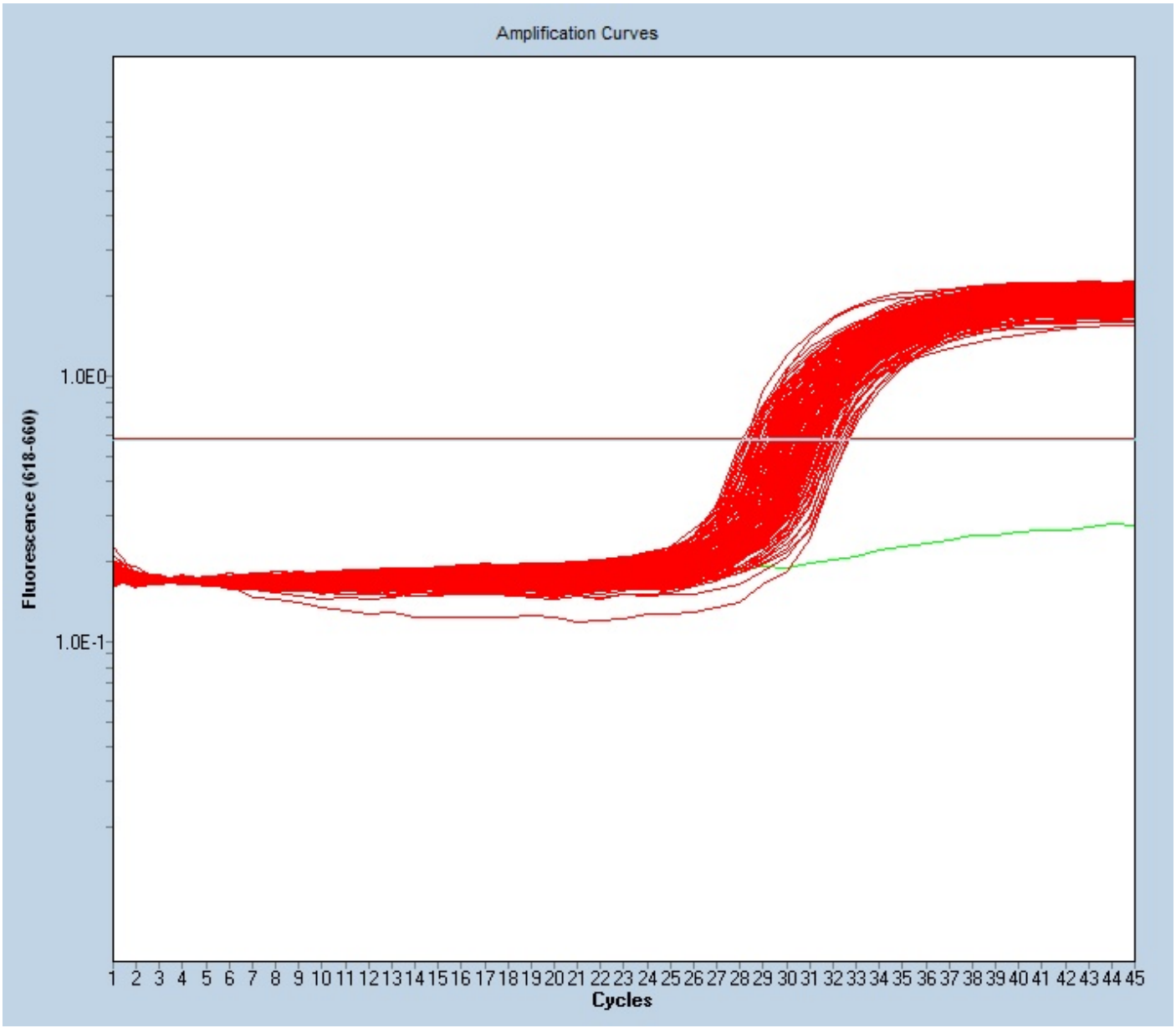

TECAN.
TECAN.
3
2
3
4


SOLUTION 2
MASTER MIX
with filter
96pcs
natural 50µl
TECAN
1
3
4
2

SMA/TREC Assay Results

- Normal Control
  - Pooled known normal specimens
- Abnormal Control
  - Negative control
    - *SMN1*
    - TREC

SMN1


Amplification Curves
Fluorescence (618-660)
1.0E0
1.0E-1
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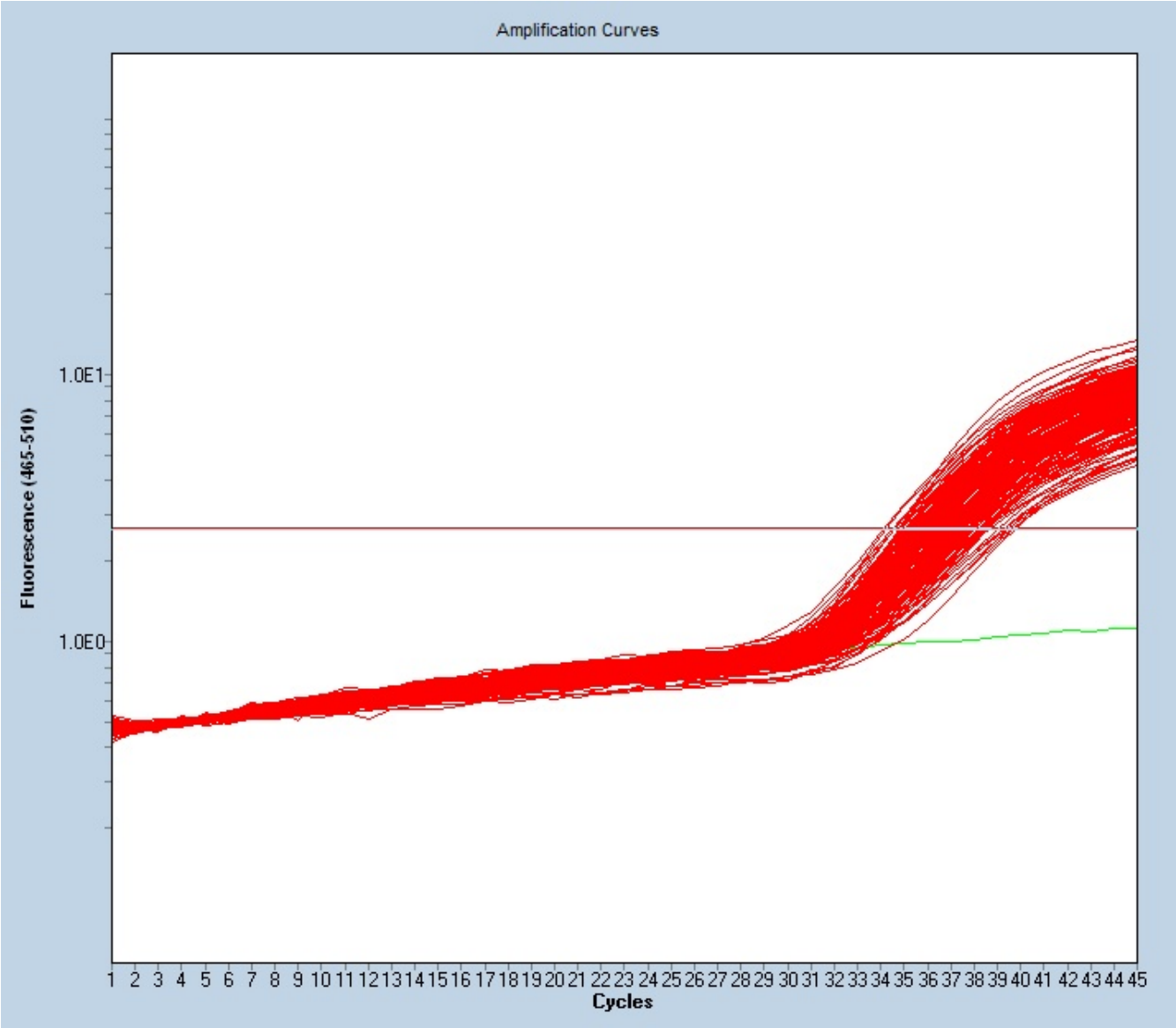
Cycles

TREC


Amplification Curves
Fluorescence (465-510)
1.0E1
1.0E0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Cycles

Validation of SMA/TREC Assay

- Reproducibility Study
- Limited Case Control Study (BLINDED!)
- Population Analysis (5000 (SMA), 3000 (SCID))

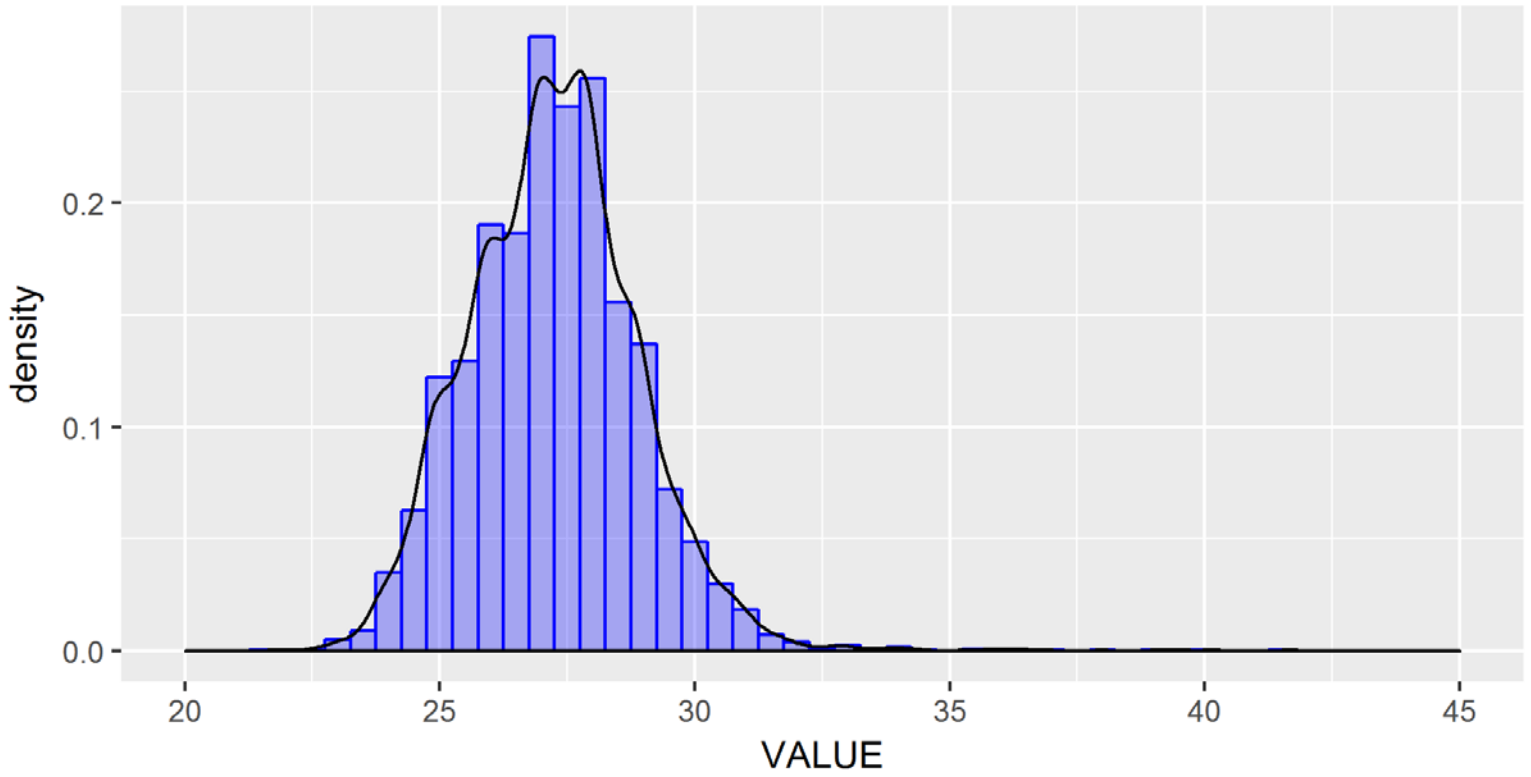
SMA Abnormals

Patient	Origin	*SMN1* Cp	*RPP30* Cp	LDT Determination	Dx
1	Biogen	No Amp	27.64	Abnormal	SMA
2	Biogen	No Amp	26.41	Abnormal	SMA
3	Biogen	No Amp	27.61	Abnormal	SMA
4	Biogen	No Amp	28.91	Abnormal	SMA
5	Biogen	No Amp	28.45	Abnormal	SMA
6	Biogen	No Amp	28.67	Abnormal	SMA
7	Biogen	No Amp	29.82	Abnormal	SMA
8	Biogen	No Amp	29.67	Abnormal	SMA
9	Biogen	No Amp	27.91	Abnormal	SMA
10	Biogen	No Amp	28.85	Abnormal	SMA
11	Biogen	No Amp	29.55	Abnormal	SMA
12	Biogen	No Amp	28.12	Abnormal	SMA
13	Biogen	No Amp	29.92	Abnormal	SMA
14	Biogen	No Amp	28.89	Abnormal	SMA
15	Biogen	No Amp	27.28	Abnormal	SMA
16	CDC	No Amp	26.14	Abnormal	SMA
17	CDC	No Amp	27.85	Abnormal	SMA
18	Utah	No Amp	28.59	Abnormal	SMA
19	Utah	No Amp	29.08	Abnormal	SMA
20	Utah	No Amp	28.64	Abnormal	SMA
21	Utah	No Amp	28.55	Abnormal	SMA
22	Utah	No Amp	29.41	Abnormal	SMA
23	Utah	No Amp	29.82	Abnormal	SMA
24	Utah	25.58	26.21	Normal	Normal

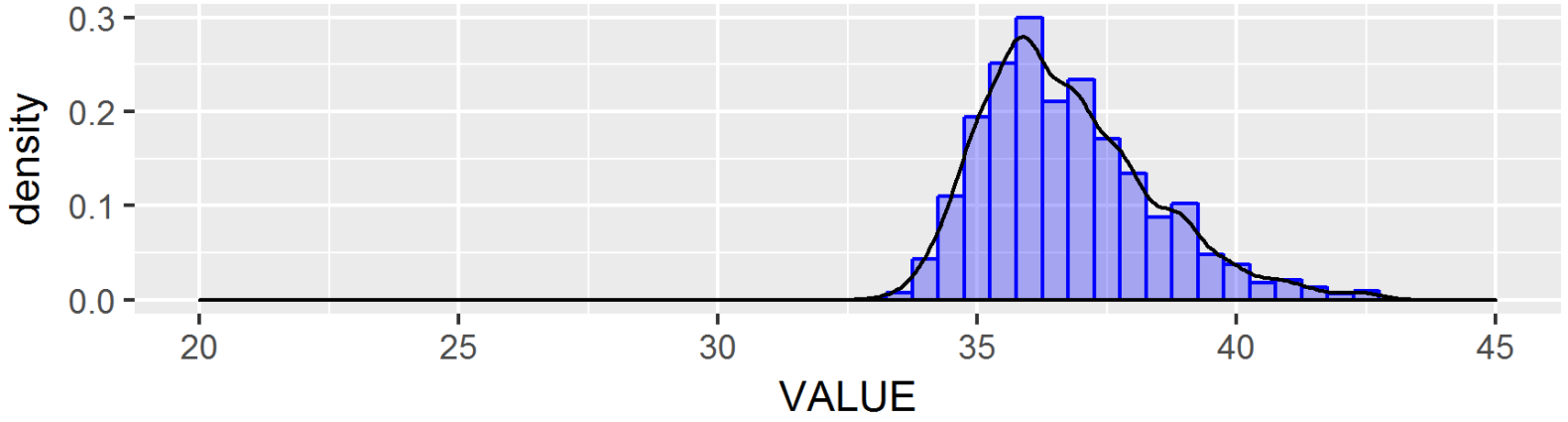
SCID Abnormals

Patient	TREC Cp	Z-Score	*RPP30* Cp	LDT Determination	Dx
1	No Amp	No Amp	28.97	Abnormal	Classic SCID
2	No Amp	No Amp	26.98	Abnormal	Classic SCID
3	No Amp	No Amp	30.34	Abnormal	SCID ADA
4	No Amp	No Amp	29.94	Abnormal	SCID ADA
5	No Amp	No Amp	29.94	Abnormal	DiGeorge Syndrome
6	No Amp	No Amp	30.21	Abnormal	DiGeorge Syndrome
7	No Amp	No Amp	33.13	Abnormal	Secondary T-cell Lymphopenia
8	No Amp	No Amp	31.37	Abnormal	Secondary T-cell Lymphopenia
9	No Amp	No Amp	28.86	Abnormal	Secondary T-cell Lymphopenia
10	No Amp	No Amp	26.54	Abnormal	Idiopathic T-cell lymphopenia asymptomatic
11	No Amp	No Amp	30.58	Abnormal	Variant T-cell lymphopenia
12	No Amp	No Amp	27.16	Abnormal	Microdeletion syndrome
13	40.8	2.30	29.35	Normal	Secondary T-cell Lymphopenia
14	41.39	2.66	31.61	Normal	Secondary T-cell Lymphopenia
15	39.23	1.36	30.71	Normal	Normal

SMN1 Population Analysis



TREC Population Analysis



Z-Score

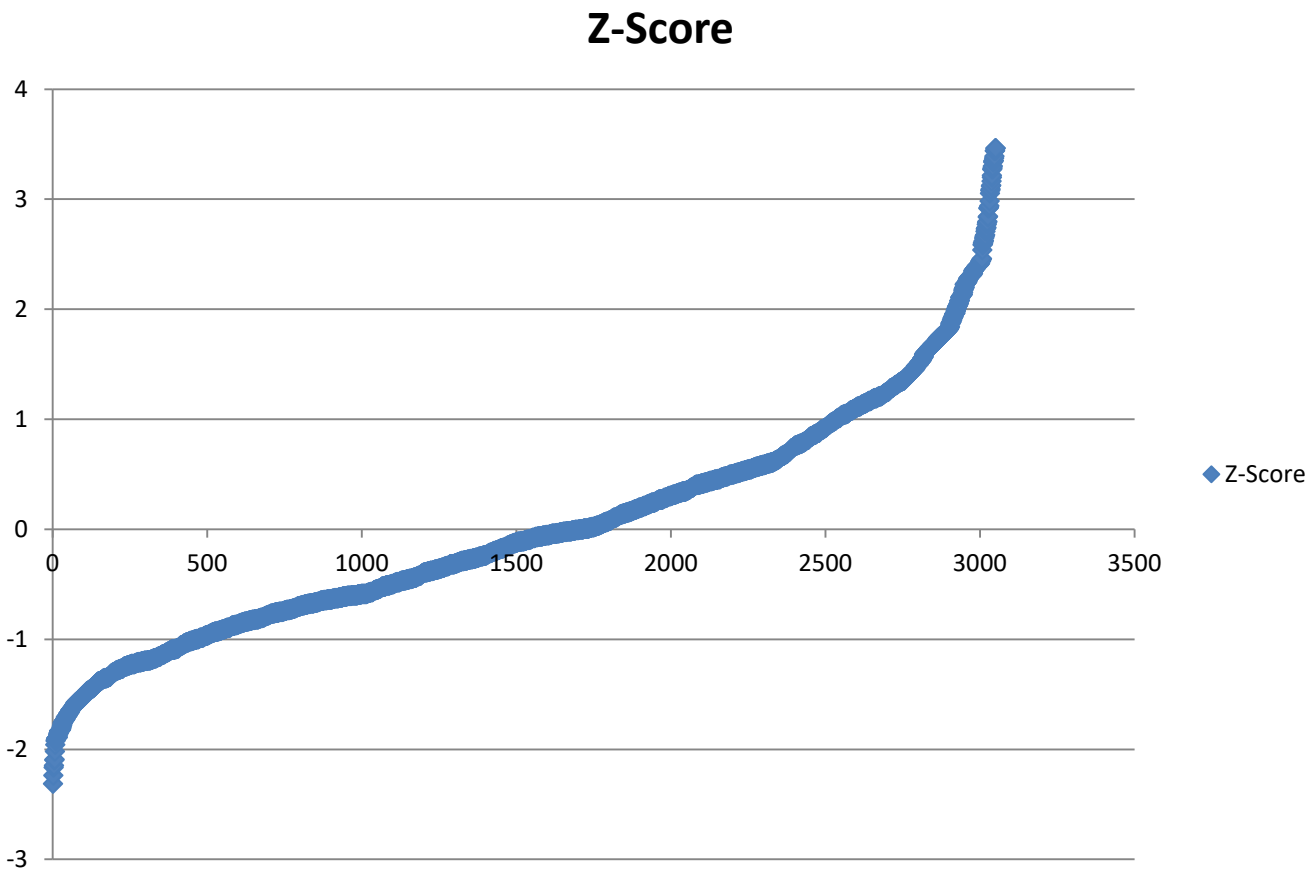
Population Z - score

$$z = \frac{x - \mu}{\sigma}$$

Individual measurement:
How many standard deviations below or above the population mean?

Requires sufficiently large population study (knowledge of population mean and population standard deviation).

TREC Population Analysis

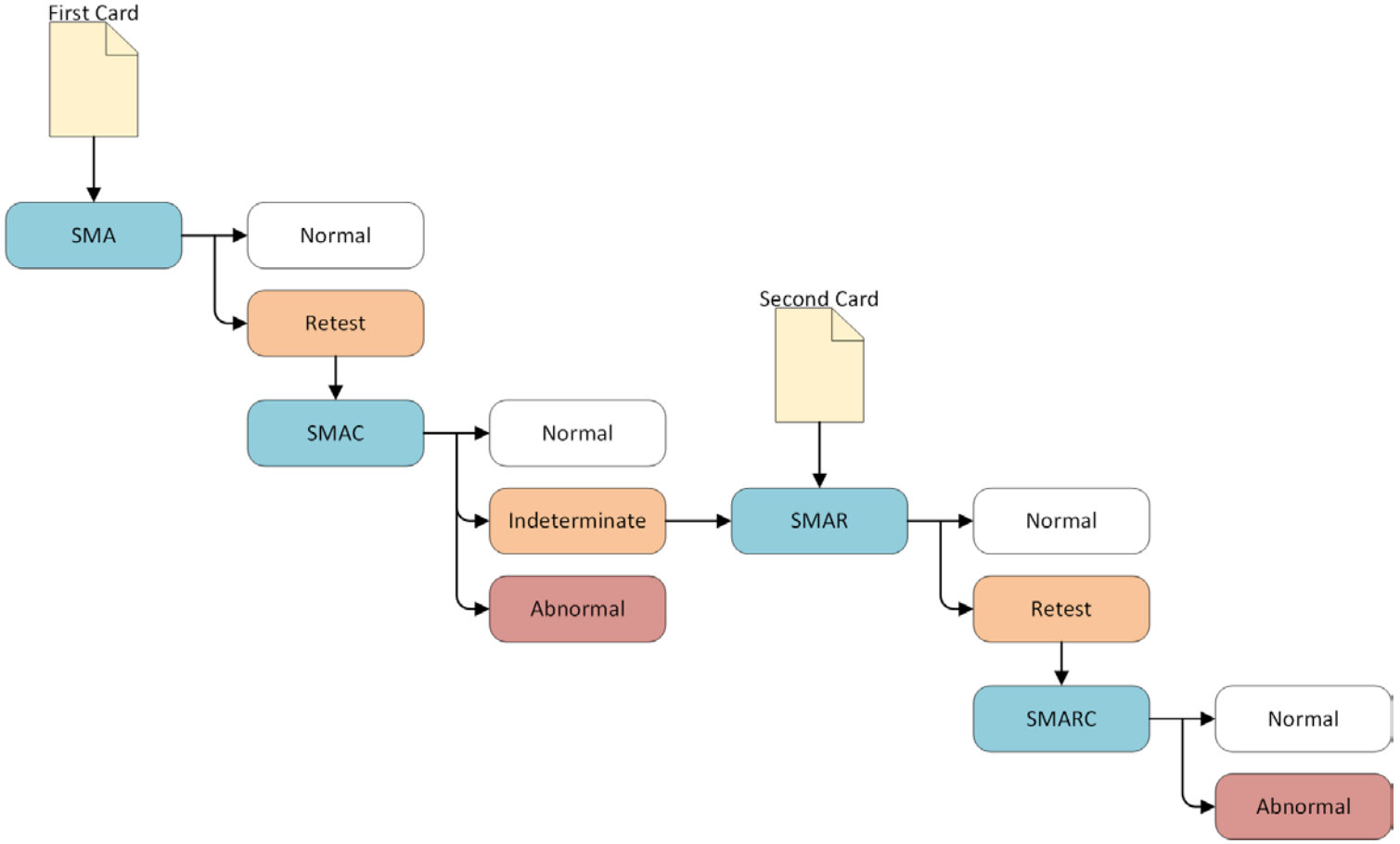


SMA/TREC Assay Cut-Offs

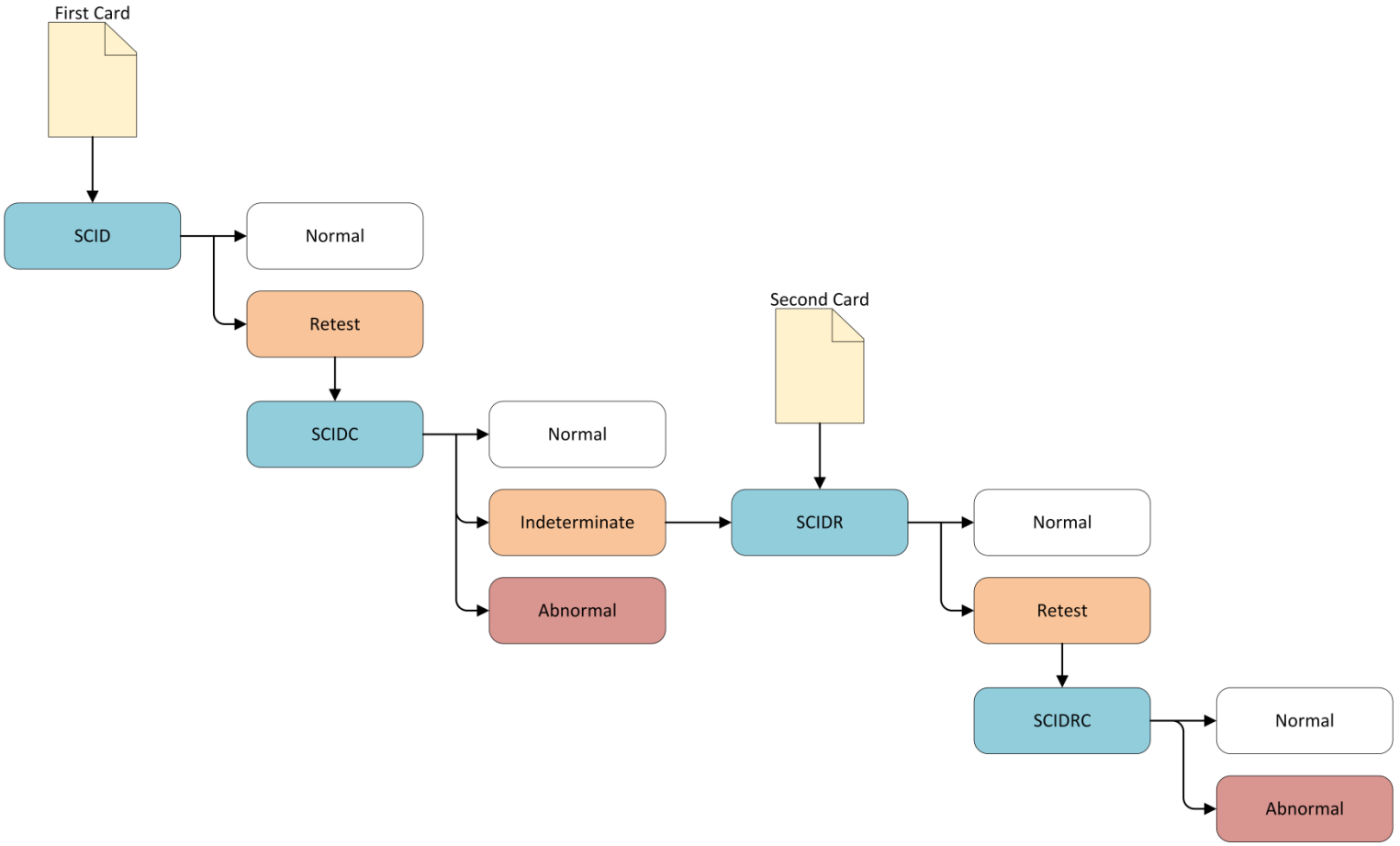
Analyte	Mean ± SD	2 SD	99th Percentile	3SD	99.5th Percentile
SMN1	29.15 ± 1.35	31.85	32.91	33.20	33.81
TREC	36.98 ± 1.66	40.31	41.54	41.97	42.18
RPP30	29.71 ± 1.39	32.49	32.99	33.88	34.14

*The cut-off for TREC is a Z-score of 2.8 (corresponds with a Cp ≈ 41.65).

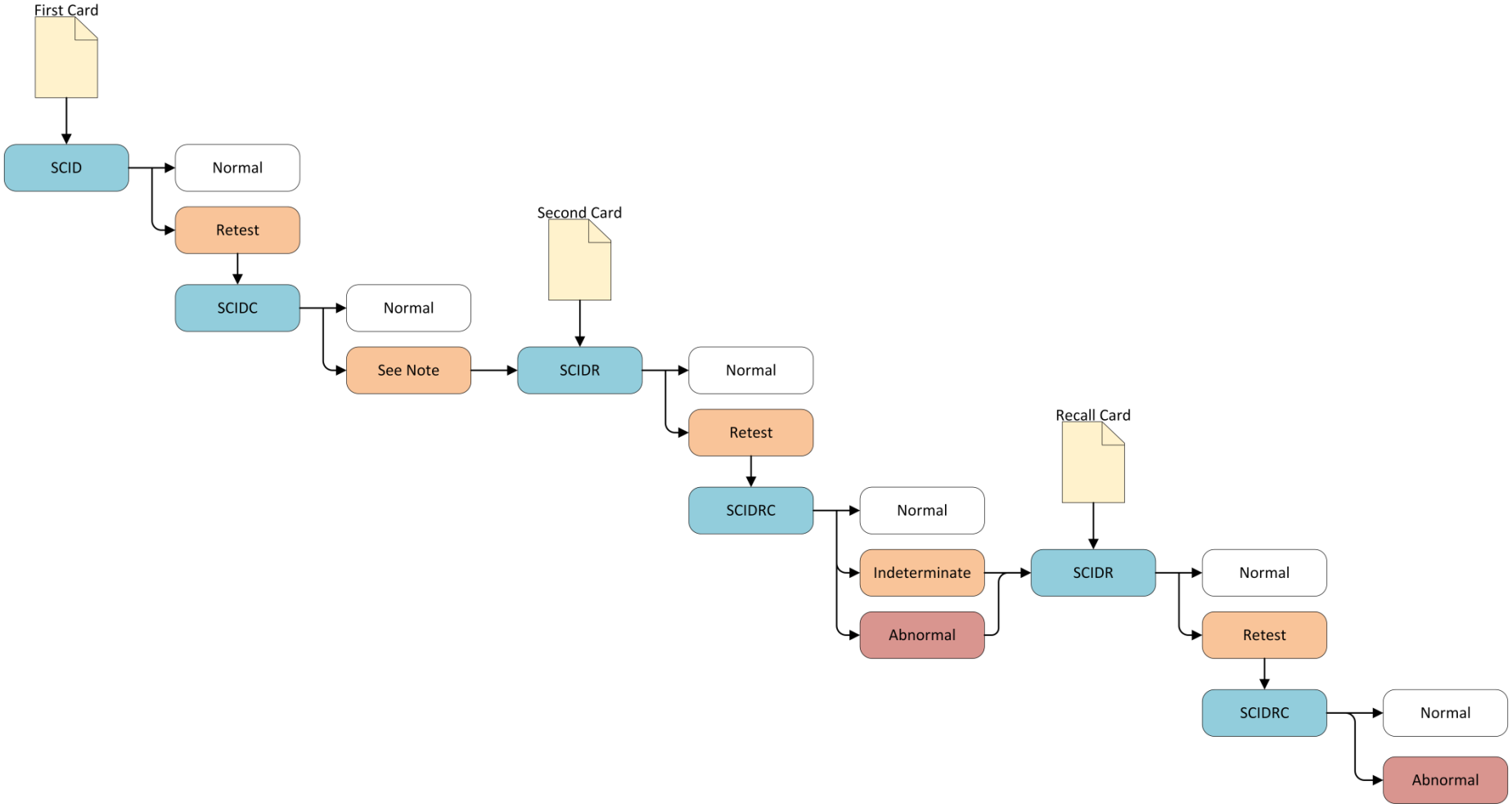
SMA Workflow



Term SCID Workflow



Premie SCID Workflow



SMA Production Data

Category	Old Method Count (n)	New Method Count (n)	Total (n)
Total Screened	10,989	5,548	16,537
Repeat First Screen	204	43	247
Second Specimens Screened	12	9	21
Total Abnormal	1 + 1	0	1 + 1
True SMA Case	1	0	1

*Summary of patients screened from January 29, 2018 – May 31, 2018

*About 5% repeat requirement for first NBS

Abnormal Case 1

- Positive screen reported
- Assessed in clinic no symptoms present
- Confirmatory testing confirmed diagnosis of SMA (0 *SMN1* and 3 *SMN2*)
- Patient with family history and predicted SMA Type 2 phenotype

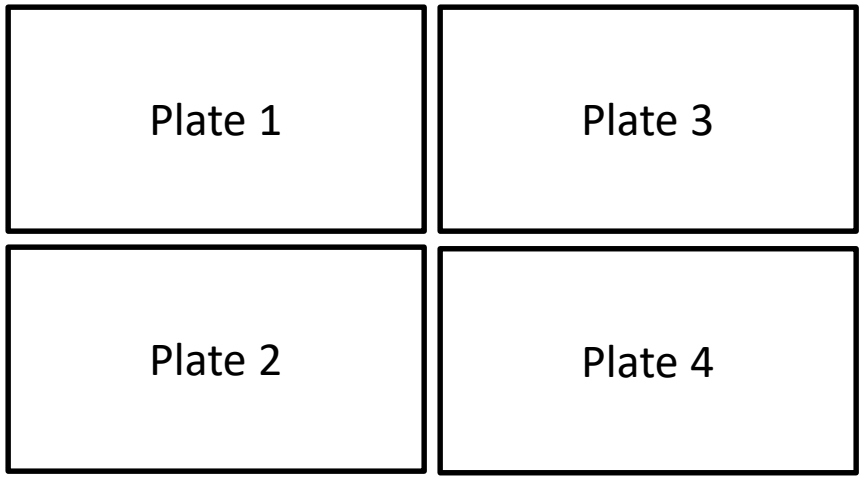
Abnormal Case 2

- **<u>Internal decision to send for diagnostic testing (early testing stage) instead of resorting to repeat screen/recall specimen</u>**
- Assessed in clinic with no symptoms present
- Confirmatory testing showed 2 copies *SMN1* and 1 copy *SMN2* (confirmed in 2 independent laboratories)
- *SMN1* repeated on second NBS and was normal

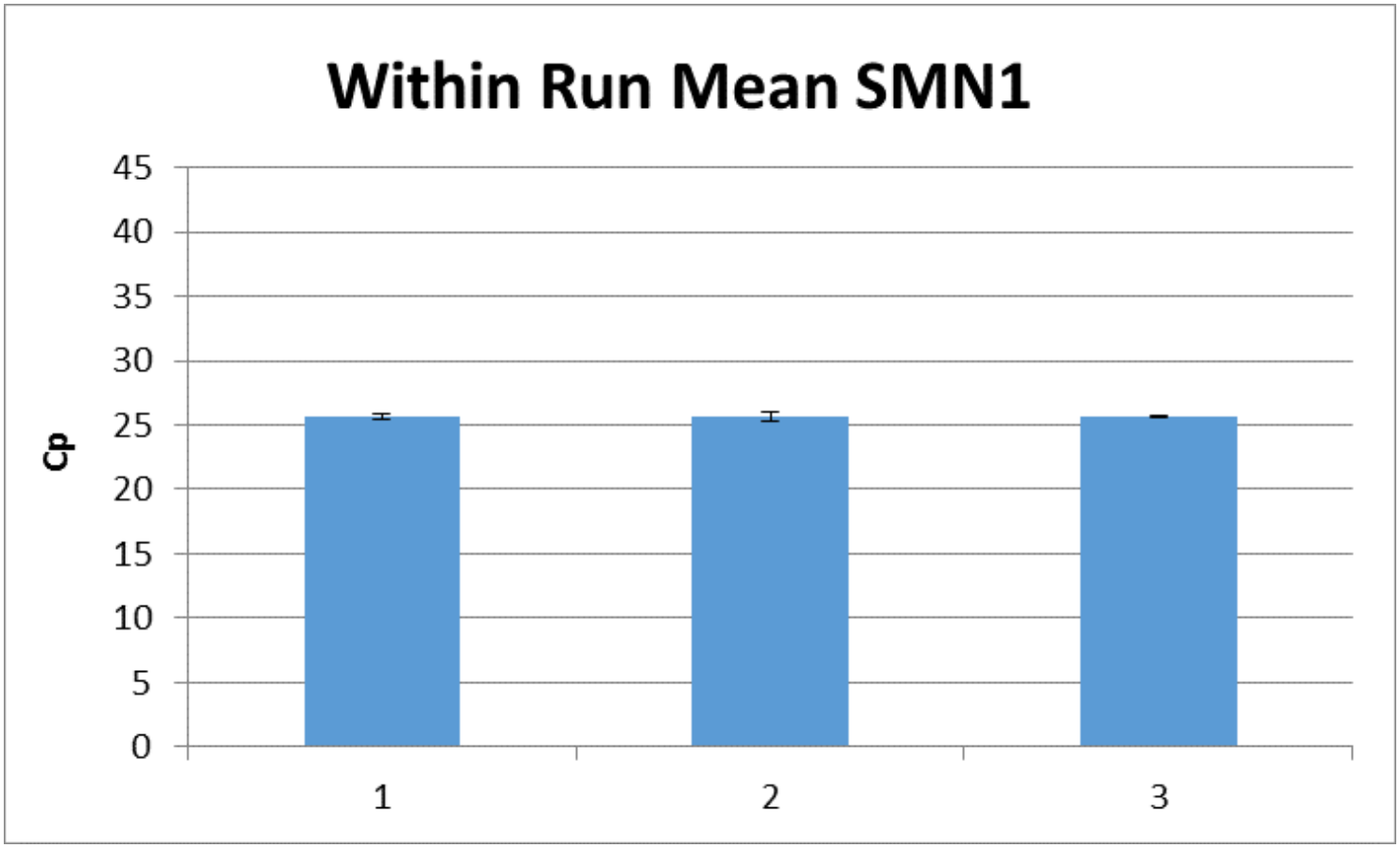
Summary

- True cases show no amplification of *SMN1*
- In production assay works for *SMN1* and TREC
- Concordant performance with EnLite
- 384 well format allows economies of scale
- Passed initial PT

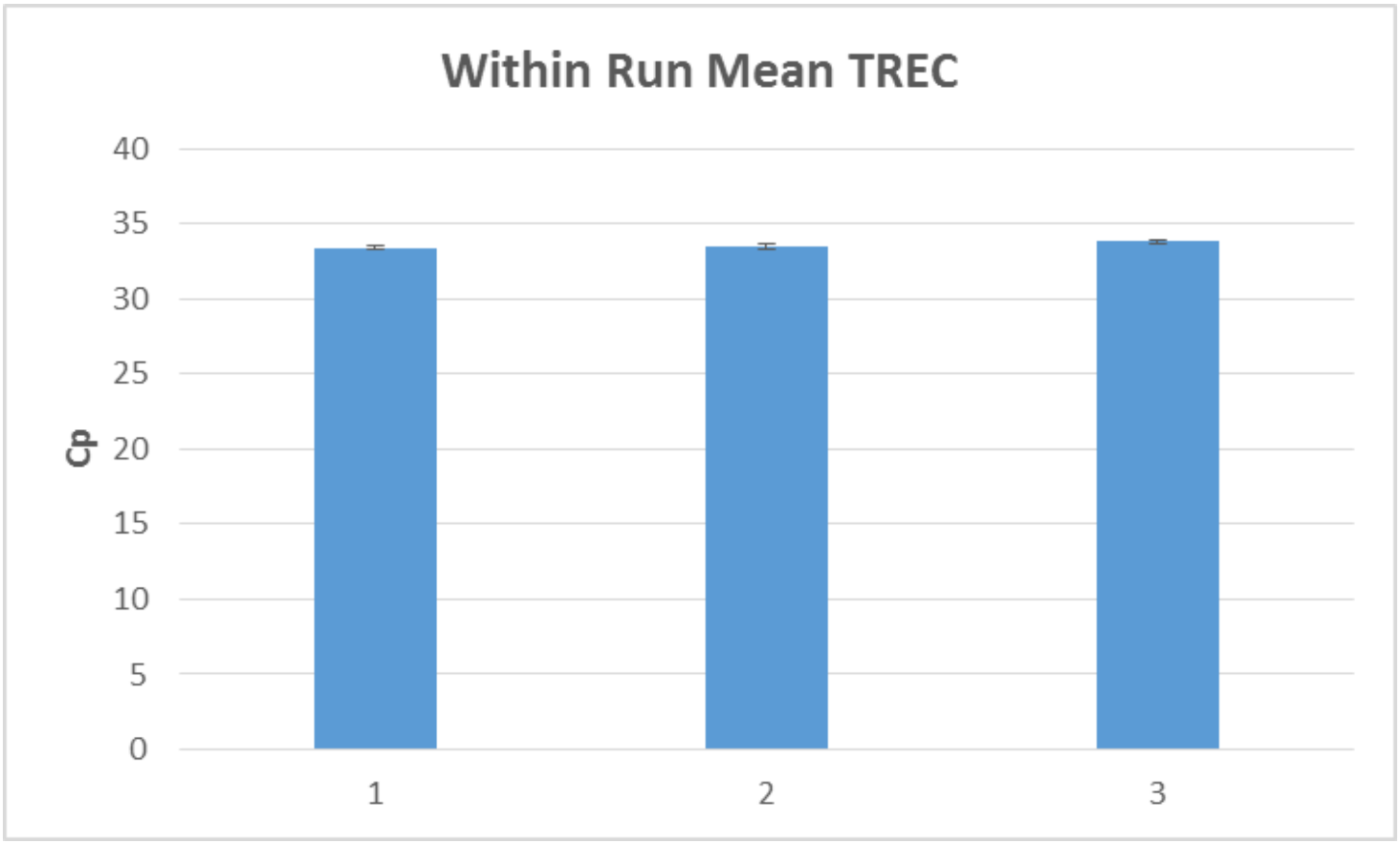
96 to 384 conversion



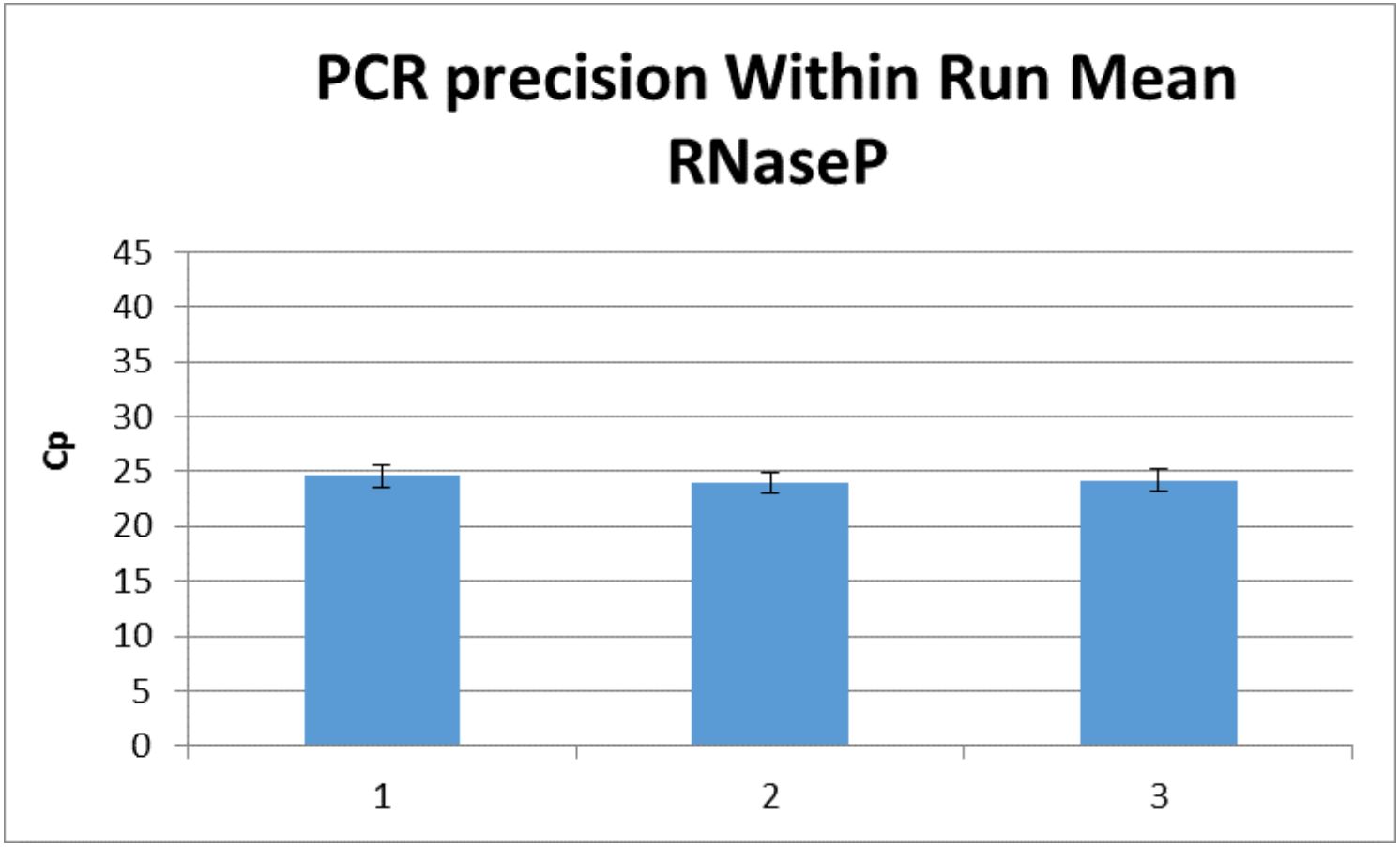
SMN1 Reproducibility



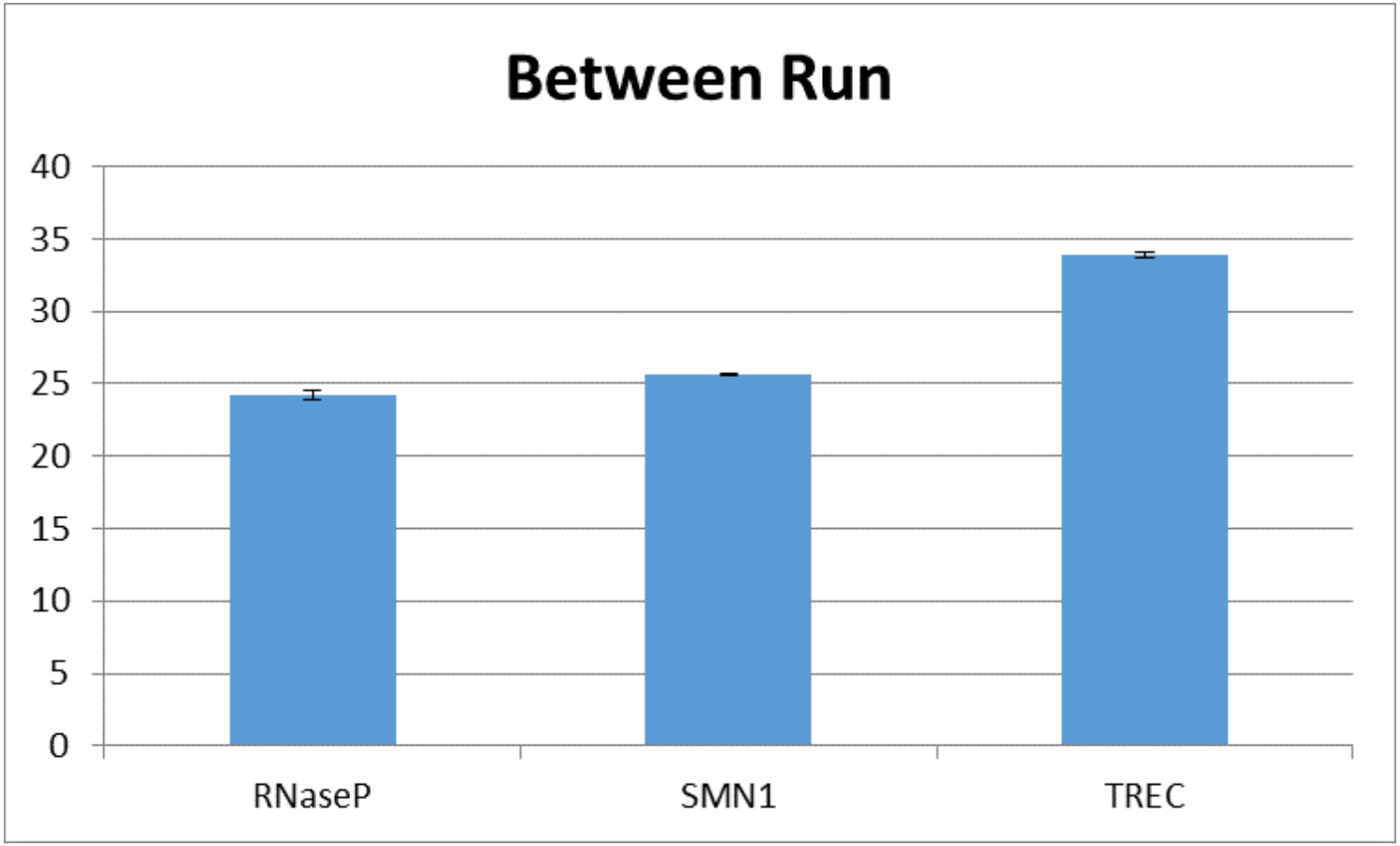
TREC Reproducibility



RPP30 Reproducibility



Reproducibility



SMN2 Copy number Assessment in NBS for SMA

Mei Baker, MD, FACMG

Co-Director, Newborn Screening Laboratory at WSLH
Wynne Mateffy Professor, Department of Pediatrics
University of Wisconsin School of Medicine and Public Health

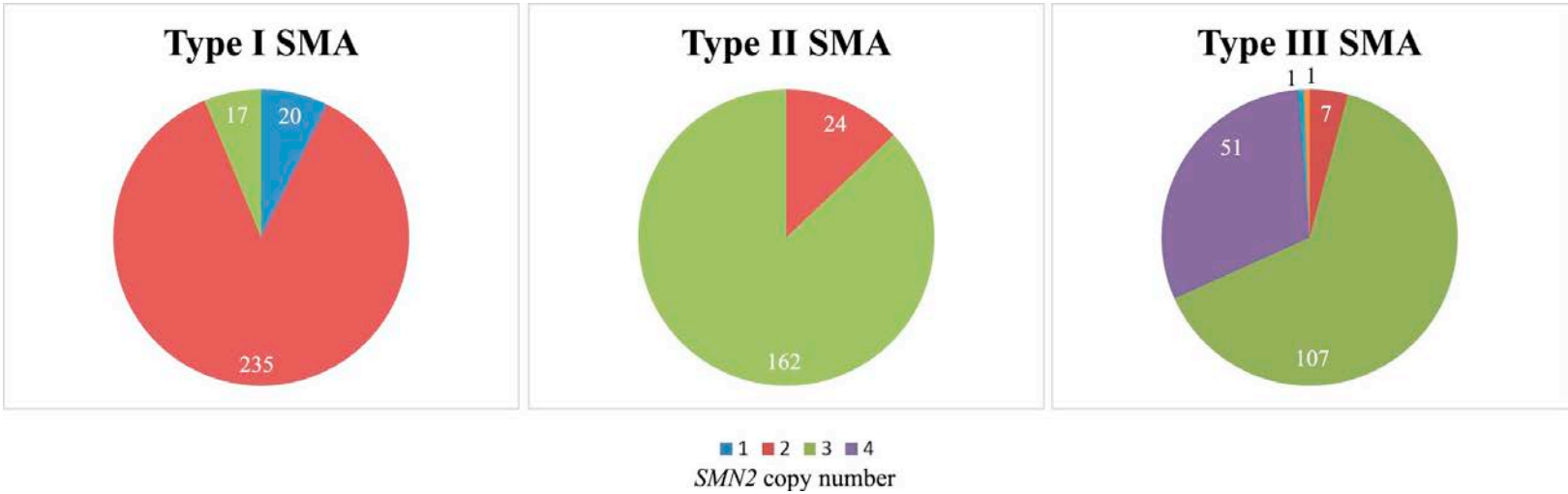
APHL webinar series on spinal muscular atrophy (SMA)

June 28, 2018

SMA Types and Clinical Classification

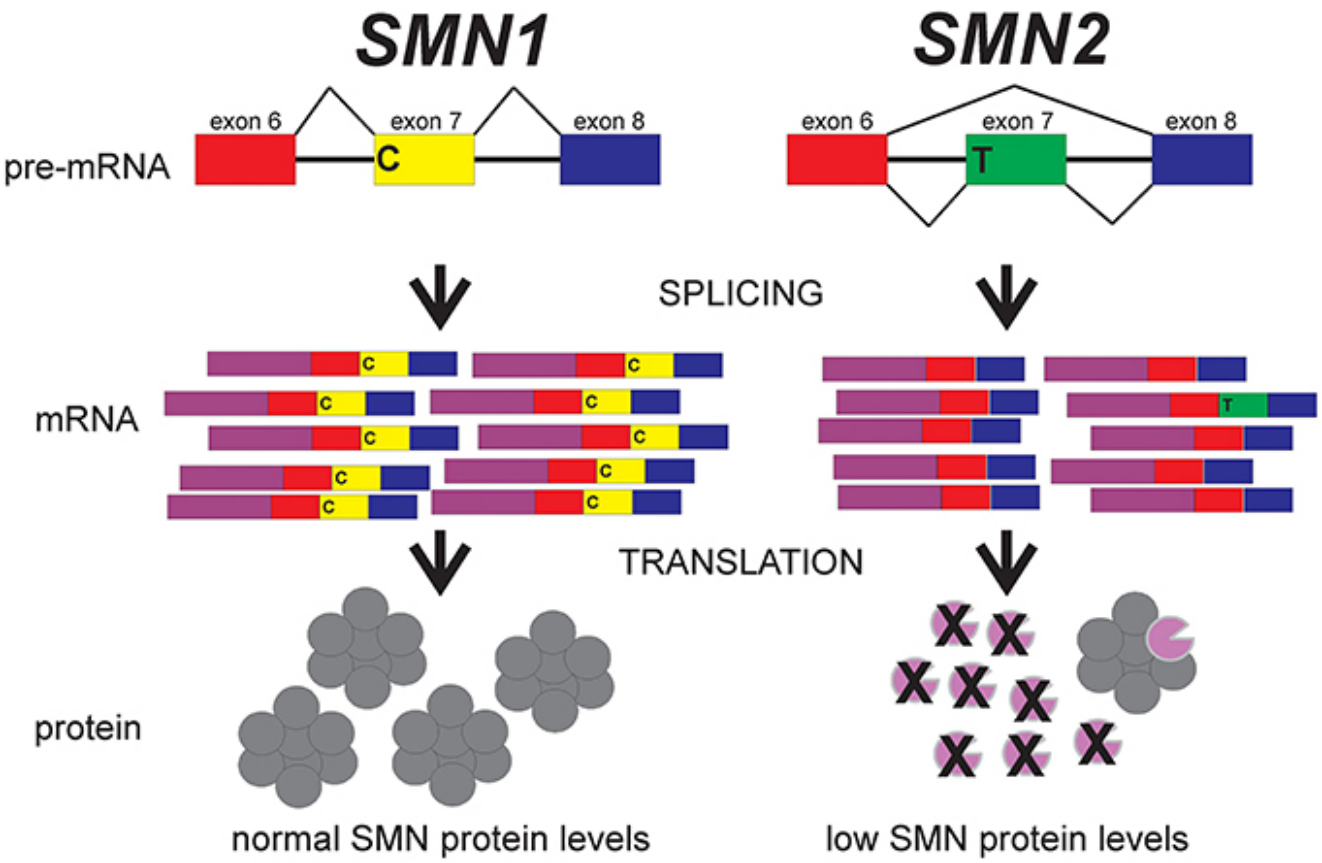
SMA Type	Age of Onset	Motor Ability	Life Expectancy	SMN2 Copy Number
SMA Type I	< 6 months	Cannot sit	< 2years	2 copies
SMA Type II	< 18 months	Sit independently, cannot stand Breathing difficulty	2^{nd} - 3^{rd} decade	3-4 copies
SMA Type III	> 18 months	Stand and walk independently	Normal life expectancy	3-4 copies
SMA Type IV	Adolescent or adult onset	Retain walking, muscle pain	Normal life expectancy	4-8 copies

SMA Type and SMN2 Copies



M. Calucho et al, Neuromuscular Disorders (2018)

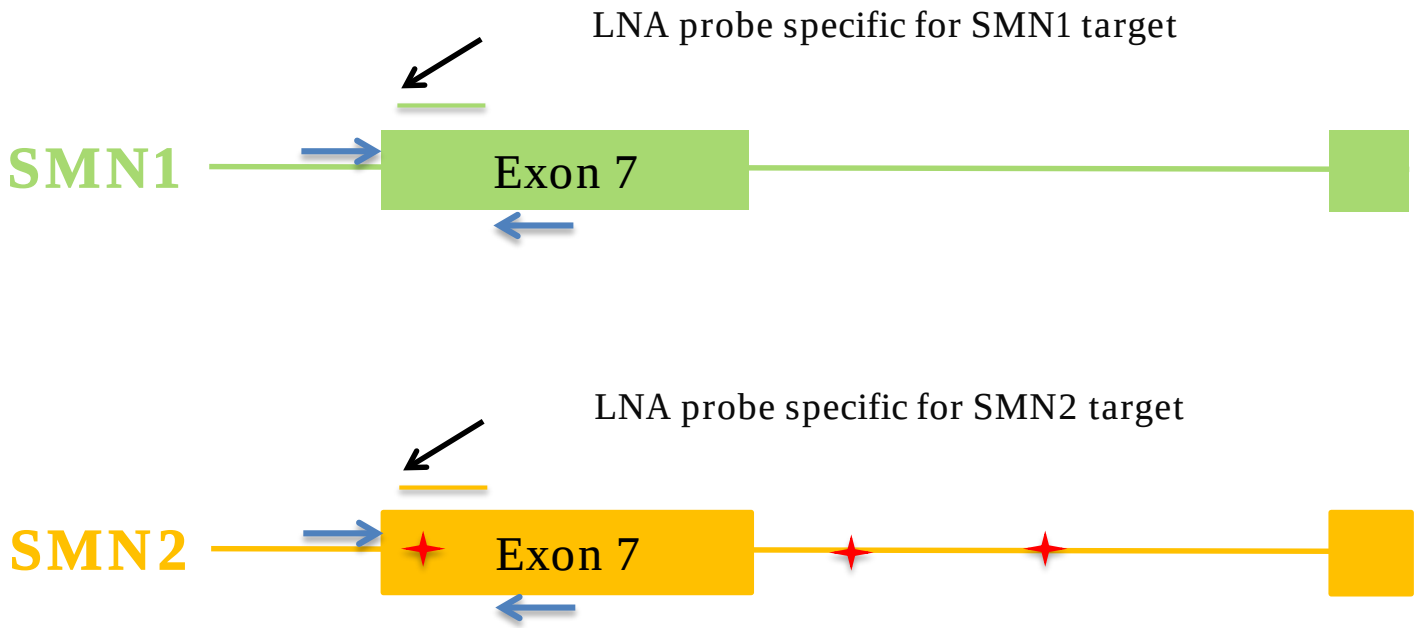
SMN1 and SMN2 in SMA



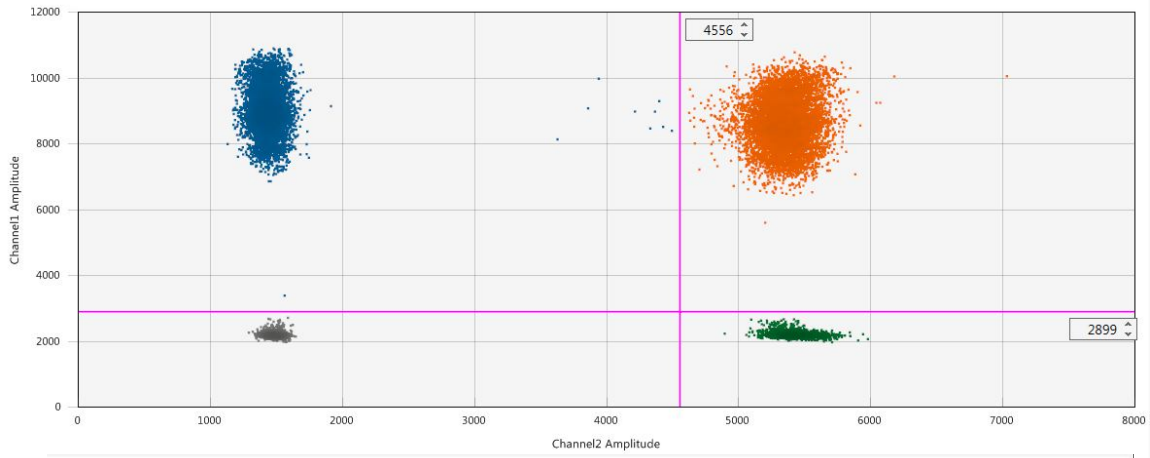
M. Butchbach et al, Frontiers in Molecular Biosciences (2016)

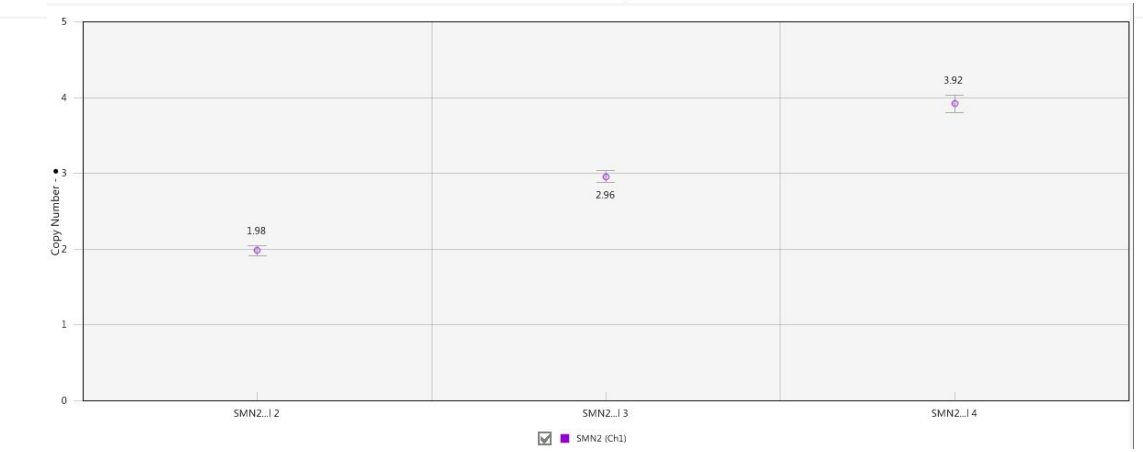
Real-time PCR Assay

Targeting Single Base Variant in Exon 7



SMN2 Copy Number Assessment by Droplet Digital PCR



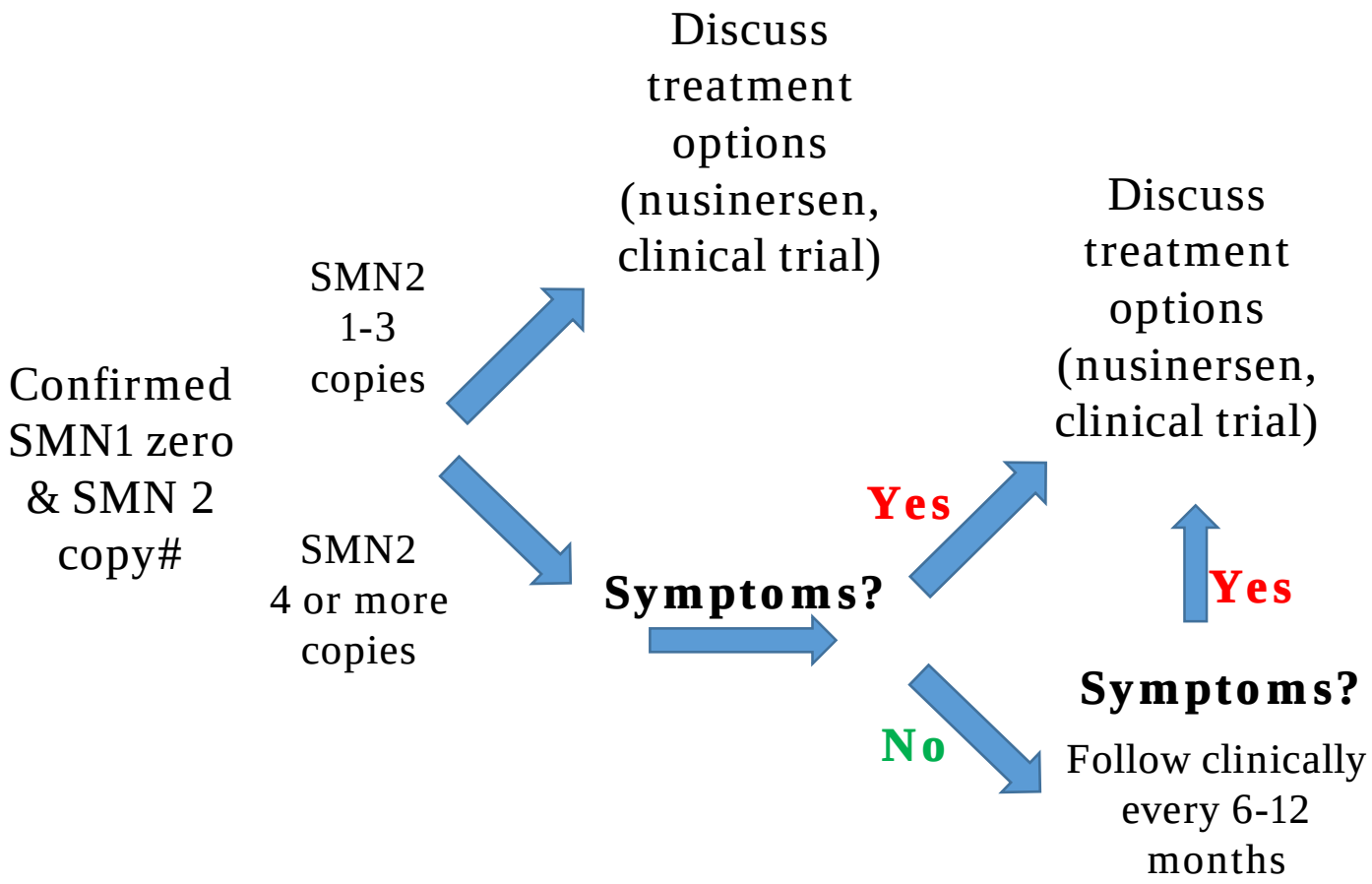


SMN2 Copy Numbers in SMN1 Zero Samples

ID		SMN2 Copy Numbers		
	Clinical Diagnosis	Provided	Real-time PCR Assay	Droplet Digital PCR Assay
WI SMA 1	SMA Type II	**3**	**4**	**3**
WI SMA 2	SMA Type I	2	2	2
WI SMA 3	SMA Type II	**4**	**4**	**3**
WI SMA 4	SMA Type I	Not Provided	2	2
WI SMA 5	SMA Type I	Not Provided	2	2
WI SMA 6	SMA Type I	2	2	2
WI SMA 7	SMA Type II	**Not Provided**	**>4**	**3**

Wisconsin
SMA Screening Protocol

Wisconsin
SMA Follow-up Protocol



SMA Screening Assay Summary

- It is technically feasible to incorporate SMA screening test into the current ongoing SCID screening test

  MULTIPLEX

- It is feasible to avoid SMA carrier identification by only detecting **"SMN1 ZERO"**

- Screening sensitivity of the proposed method is about 95%

- It is beneficial to include SMN2 copy number assessment in NBS for SMA protocol

Acknowledgments

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  Dept. of Pediatrics, UWSMPH

- **Sean Mochal, BS**
- **Mandie Loehe, BS**
- **Bethany Zeitler, BS**
  Newborn Screening Laboratory at WSLH

Questions?

- Please press *7 to unmute, or type your question in the chat box.

Archived Webinar Series

The SMA webinar series has been archived and recorded. It will be posted on APHL.org within the next week.

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