

# Spinal Muscular Atrophy/SCID Control Panel: A Control Panel of Biologically-Relevant, Allele-based Controls to Accurately Monitor SMA/SCID Assay Performance

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**G060**

## Introduction

Spinal Muscular Atrophy (SMA) and Severe Combined Immunodeficiency (SCID) are two diseases recommended as part of routine newborn screening due to severity of the disease and recently available therapies. SMA is an autosomal recessive disease affecting motor neurons caused by a homozygous mutation or loss of the *SMN1* (survival of motor neuron 1) gene, results in progressive muscular atrophy. Testing is primarily associated with determination of *SMN1* and *SMN2* (survival of motor neuron 2) copy numbers. Early diagnosis allows for critical intervention with new treatments to prevent further damage to nerve cells. SCID comprises a group of disorders caused by mutations in genes involved in the development and function of immune cells. SCID is recognized as a pediatric emergency since it leads to severe and recurrent infections and death in the first 2 years when untreated. SCID is primarily detected by quantifying T-cell receptor excision circles (TREC) and kappa-deleting element recombination circles (KREC) on newborn dried blood spots (DBS). As more assays and technologies are developed for newborn screening diagnostics, the need for a comprehensive control to accurately monitor both SMA and SCID assay performance is critical.

## Materials and Methods

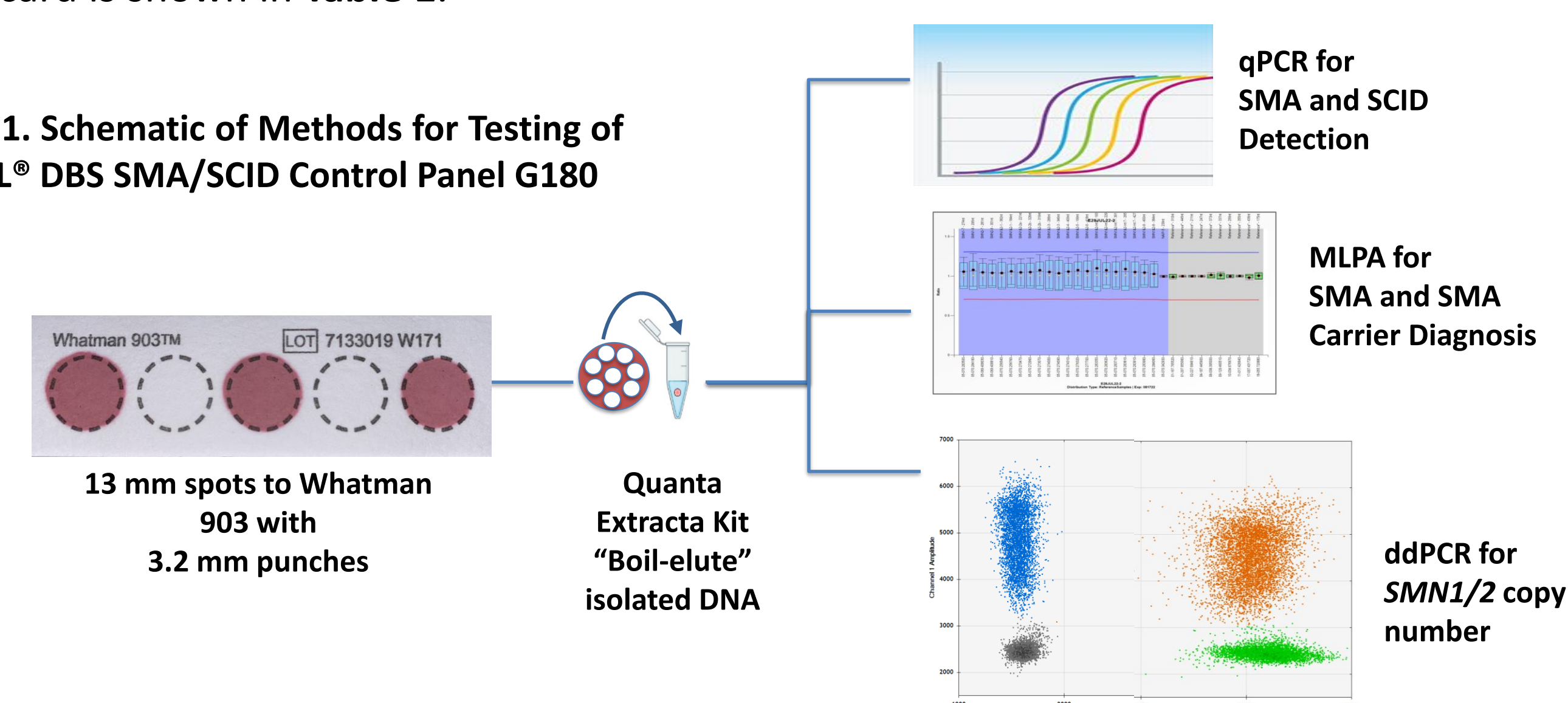
A panel of plasmids were created to include important SMA genetic markers within all exons plus intronic borders of *SMN1* and *SMN2* genes, TREC and KREC sequences, and gene segments of numerous reference genes. The plasmids were quantified by UV spectrophotometry, and suspended in a proprietary blood-like mimic and spotted on Whatman 903 Protein Saver cards to create the INTRLO<sup>®</sup> DBS SMA/SCID Control Panel G180.

The control panel consists of 4 cards with different SMA genotypes and SCID levels. INTRLO<sup>®</sup> DBS SMA/SCID Control A (WT) contains 2 copies of *SMN1*, 2 copies of *SMN2*, and a high-level copy number (Normal) of TREC and KREC. INTRLO<sup>®</sup> DBS SMA Control B (SMA Carrier) contains 1 copy of *SMN1*, 3 copies of *SMN2*, and no TREC or KREC DNA (SCID positive). INTRLO<sup>®</sup> DBS SMA/SCID Control C (SMA Silent Carrier) contains 3 copies of *SMN1*, 1 copy of *SMN2*, and a mid-level copy number (Normal) of TREC and KREC. INTRLO<sup>®</sup> DBS SMA/SCID Control D (SMA) contains 0 copies of *SMN1* exon 7, 2 copies of *SMN2*, and a low level copy number (Normal) of TREC and KREC.

Samples were extracted using QuantaBio DBS Extracta Kit and tested by qPCR, ddPCR and MLPA (Multiplex Ligation-Dependent Probe Amplification) to assess performance. Ten DBS extraction methods were evaluated using multiplex SMA/SCID qPCR and ddPCR *SMN1/SMN2* Copy Number Determination Kits. To represent a "patient" sample, whole blood was tested alongside the controls for comparison. Homogeneity was determined by testing variability across cards and within each spot. A single punch was extracted from 3 spots across 3 cards, and 7 punches were extracted within a spot across three spots on a single card. Each sample was tested using the SMA/SCID multiplex qPCR and a single factor ANOVA (JMP Version 16.1) was used to assess variability from punch to punch and across cards.

A schematic workflow for DBS card testing is shown in **Figure 1**; a table representing the expected results for each card is shown in **Table 1**.

**Figure 1. Schematic of Methods for Testing of INTRLO<sup>®</sup> DBS SMA/SCID Control Panel G180**



**Table 1. Expected Results for INTRLO DBS SMA/SCID Control Panel G180**

Control	<i>SMN1</i> , <i>SMN2</i> Copies	SMA Status	SCID Status
INTRLO <sup>®</sup> DBS SMA/SCID Control A	2 <i>SMN1</i> 2 <i>SMN2</i>	WT	Normal (TREC and KREC Normal-High)
INTRLO <sup>®</sup> DBS SMA/SCID Control B	1 <i>SMN1</i> 3 <i>SMN2</i>	SMA Carrier	SCID Positive (Zero TREC and KREC)
INTRLO <sup>®</sup> DBS SMA/SCID Control C	3 <i>SMN1</i> 1 <i>SMN2</i>	SMA Silent Carrier g.27134T>G g.27706-27707delAT	Normal (TREC and KREC Normal-Mid)
INTRLO <sup>®</sup> DBS SMA/SCID Control D	0 <i>SMN1</i> Ex7 2 <i>SMN2</i>	SMA	Normal (TREC and KREC Normal-Low)

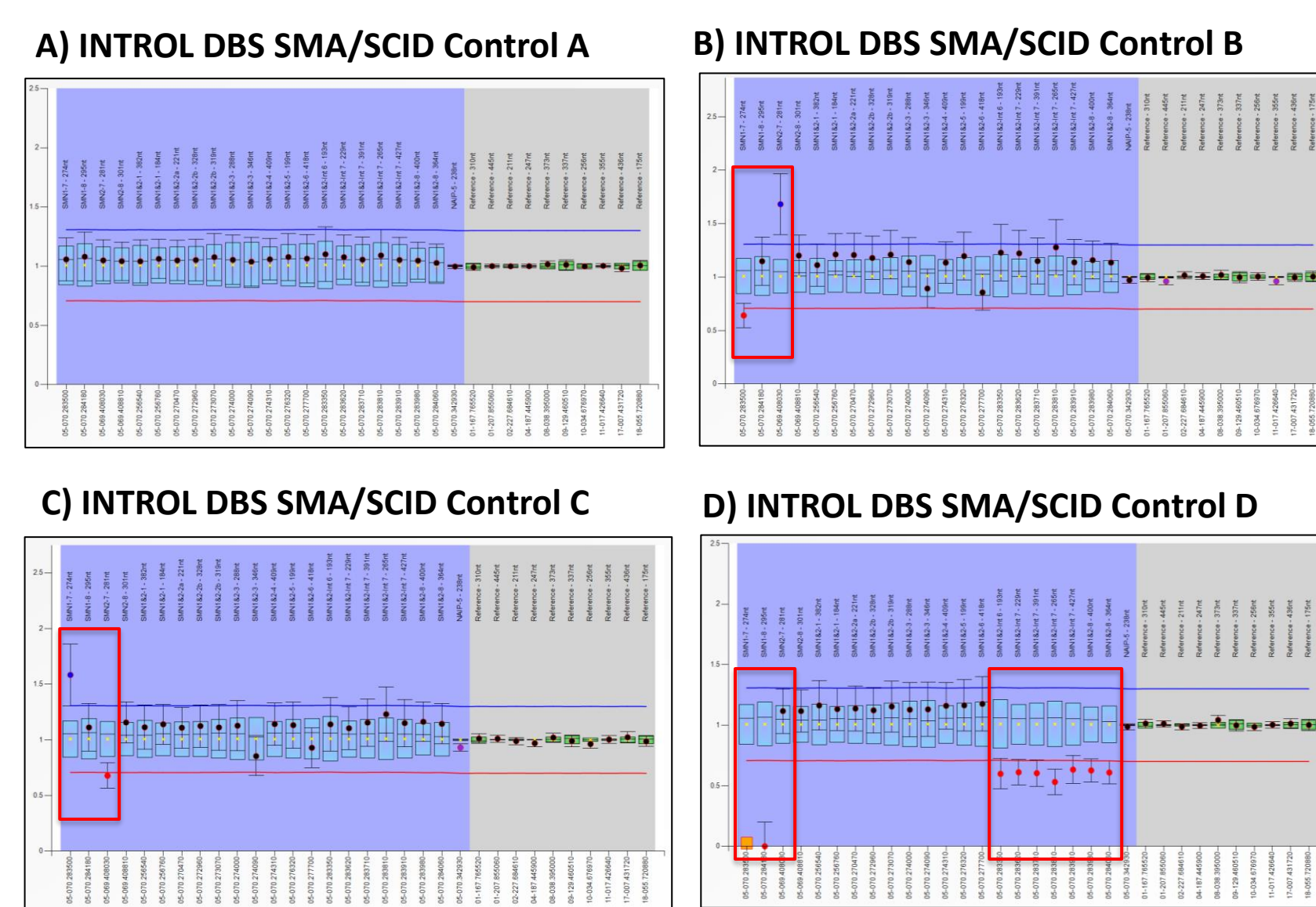
## INTRLO DBS SMA/SCID Control Panel G180 Evaluated by ddPCR, qPCR and MLPA

**Table 2. The INTRLO DBS SMA/SCID Control Panel G180 tested using the *SMN1/SMN2* Copy Number Determination Kits (Bio-Rad)<sup>1</sup>. INTRLO DBS SMA/SCID controls tested with *SMN1/SMN2* ddPCR resulted in 100% accurate genotype calls across all the card types. N=2; Each replicate is from a separate extraction/spot on one card.**

Card Type	<i>SMN1</i> Copies	<i>SMN2</i> Copies	SMA Status	ddPCR					
				<i>SMN1</i> CNV	<i>SMN1</i> Ave CNV	<i>SMN1</i> STDV	<i>SMN2</i> CNV	<i>SMN2</i> Ave CNV	<i>SMN2</i> STDV
INTRLO DBS SMA/SCID Control A	2	2	WT	1.97 1.94	1.96	0.021	1.91 1.92	1.92	0.007
INTRLO DBS SMA/SCID Control B	1	3	SMA carrier	1.04 1.06	1.05	0.015	3.29 3.27	3.28	0.014
INTRLO DBS SMA/SCID Control C	3	1	SMA Silent Carrier g.27134T>G g.27706-27707delAT	3.09 2.99	3.04	0.071	1.06 1.05	1.05	0.006
INTRLO DBS SMA/SCID Control D	0	2	SMA	0.00 0.00	0.00	0.000	2.03 2.13	2.08	0.071

**Table 3. INTRLO DBS SMA/SCID Controls analyzed using a tri-plex qPCR for *SMN1*, TREC and *RPP30* detection<sup>2</sup>. PCR amplification for detection of *SMN*, TREC, and *RPP30* resulted in accurate detection for presences of *SMN1* gene, TREC and the *RPP30* for all card types. N=3; Each replicate is from a separate extraction/spot on one card.**

Card Type	<i>SMN1</i> Copies	<i>SMN2</i> Copies	SMA Status	qPCR								
				<i>SMN1</i> Ct	<i>SMN1</i> Ave Ct	<i>SMN1</i> STDV	<i>RPP30</i> Ct	<i>RPP30</i> Ave Ct	<i>RPP30</i> STDV	TREC Ct	TREC Ave Ct	TREC STDV
INTRLO DBS SMA/SCID Control A	2	2	WT	21.90 21.87 21.63	21.80	0.15	22.47 22.31 22.14	22.31	0.17	24.52 24.34 24.15	24.34	0.19
INTRLO DBS SMA/SCID Control B	1	3	SMA carrier	22.98 22.95 22.98	22.97	0.02	22.84 22.75 22.74	22.78	0.06	-	-	-
INTRLO DBS SMA/SCID Control C	3	1	SMA Silent Carrier g.27134T>G g.27706-27707delAT	23.98 24.12 24.15	24.08	0.09	23.28 23.46 23.48	23.41	0.11	31.65 31.97 31.94	31.85	0.18
INTRLO DBS SMA/SCID Control D	0	2	SMA	- - -	-	-	23.45 23.33 23.45	23.41	0.07	35.61 35.76 35.92	35.76	0.16



**Figure 2. INTRLO DBS SMA/SCID Controls evaluated by SALSA MLPA Probenix P021 SMA assay<sup>3</sup>. A) Control A containing WT *SMN1* and *SMN2*, B) Control B containing 1 copy of exon 7 *SMN1* and 3 copies of exon 7 *SMN2*, C) Control C containing 3 copies of exon 7 *SMN1* and 1 copy of exon 7 *SMN2* and D) Control D containing 0 copies of exon 7-8 *SMN1* and 2 copies of exon 7 *SMN2*.**

Card Type	<i>SMN1</i> Copies	<i>SMN2</i> Copies	SMA Status	SALSA MLPA Probenix P060 SMA Carrier											
				INTRLO DBS SMA/SCID Control A	INTRLO DBS SMA/SCID Control B	INTRLO DBS SMA/SCID Control C	INTRLO DBS SMA/SCID Control D	WT	SMA carrier	SMA silent carrier	SMA	1	2	3	4
SMN1-7	1.06	0.89	1.07	0.68	0.64	0.65	1.41	1.58	1.68	2%	3%	3%	0	0	0
SMN1-8	1.08	0.88	1.06	1.15	1.15	1.13	1.16	1.11	1.18	0	0	0	0	0	0
SMN2-7	1.05	0.90	1.07	1.72	1.68	1.69	0.72	0.68	0.66	1.12	1.12	1.13	1.12	1.13	1.13
SMN2-8	1.04	0.90	1.07	1.20	1.20	1.20	1.16	1.16	1.15	1.12	1.12	1.12	1.12	1.12	1.12
SMN1&2-1	1.04	0.89	1.09	1.11	1.11	1.14	1.11	1.11	1.12	1.16	1.13	1.11	1.11	1.11	1.11
SMN1&2-2	1.06	0.90	1.05	1.24	1.21	1.22	1.16	1.14	1.21	1.13	1.09	1.18	1.18	1.18	1.18
SMN1&2-3	1.05	0.90	1.07	1.20	1.21	1.23	1.21	1.11	1.20	1.14	1.14	1.14	1.14	1.14	1.14
SMN1&2-4	1.05	0.90	1.07	1.21	1.18	1.18	1.15	1.12	1.20	1.12	1.15	1.15	1.15	1.15	1.15
SMN1&2-5	1.08	0.89	1.06	1.22	1.21	1.19	1.16	1.11	1.18	1.15	1.15	1.15	1.15	1.15	1.15
SMN1&2-6	1.06	0.88	1.08	0.92	0.86	0.93	0.95	0.93	1.12	1.18	1.21	1.17	1.17	1.17	1.17
SMN1&2-int6	1.1	0.87	1.06	1.25	1.23	1.27	1.19	1.14	1.28	0.6	0.6	0.63	0.63	0.63	0.63
SMN1&2-int7	1.08	0.89	1.05	1.22	1.22	1.24	1.17	1.10	1.21	0.63	0.6	0.64	0.64	0.64	0.64
SMN1&2-int8	1.06	0.89	1.08	1.18	1.15	1.15	1.13	1.15	1.18	0.6	0.6	0.57	0.57	0.57	0.57
SMN1&2-int9	1.09	0.88	1.06	1.39	1.28	1.32	1.31	1.23	1.49	0.53	0.54	0.53	0.53	0.53	0.53
SMN1&2-int10	1.05	0.89	1.08	1.18	1.14	1.15	1.13	1.15	1.20	0.63	0.61	0.61	0.61	0.61	0.61
SMN1&2-8	1.05	0.91	1.06	1.20	1.16	1.16	1.15	1.16	1.20	0.61	0.61	0.59	0.59	0.59	0.59
SMN1&2-8 (MUT)	1.03	0.91	1.09	1.20	1.14	1.17	1.13	1.14	1.13	0.61	0.6	0.61	0.61	0.61	0.61

**Table 5. INTRLO DBS SMA/SCID Controls evaluated by SALSA MLPA Probenix P060 SMA Carrier assay<sup>3</sup>. Correct copy number variations reported for exons 7 and exon 8 of *SMN1* and *SMN2* across all 4 card types.**

## Acknowledgements

<sup>1</sup>ddPCR performed using *SMN1/SMN2* Copy Number Determination Kits (Bio-Rad, Product Code 1863500/1863503).  
<sup>2</sup>SMA/SCID qPCR assay taken from Taylor JL, Lee FK, Yazdanzadeh GK, Stropoli JF, Liu M, Garulli JP, Sun C, Dobrowolski SF, Hannon WH, Vogt RE. Newborn blood spot screening test using a multiplex real-time PCR to simultaneously screen for spinal muscular atrophy and severe combined immunodeficiency. Clin Chem. 2015 Feb;61(2):412-9. doi: 10.1373/clinchem.2014.231019. Epub 2014 Dec 11. PMID: 25502182; PMCID: PMC7908665.  
<sup>3</sup>MLPA performed using MRC Holland's SALSA MLPA Probenix P021-B1 SMA, P060-B2 SMA Carrier, and P460-A1 SMA (Silent) Carrier (MRC Holland, Product Code P021-100R, P060-100R, P460-100R).

## Results

### Evaluation of Multiple Extraction Methods

**Table 7. Evaluation of 10 DBS extraction methods. INTRLO DBS SMA/SCID Control A extracted with 10 different DBS extraction methods resulted in varying recovery based on extraction efficiencies. All were reproducible with %CV < 10% tested by SMA/SCID Multiplex qPCR and eight were reproducible with %CV of <5% when tested by *SMN1/SMN2* ddPCR.**

Sample	Extraction	<i>SMN1-RPP30-TREC</i> qPCR											
		<i>SMN1</i>			<i>RPP30</i>			TREC					
		Avg Cp	STDV	%CV	cps/mL	Avg Cp	STDV	%CV	cps/mL	Avg Cp	STDV	%CV	cps/mL
INTRLO DBS SMA/SCID Control A	Qiagen QiaAmp DNA micro column	27.13	0.16	0.60	2.93E+06	27.87	0.20	0.72	5.14E+06	29.38	0.38	1.29	3.47E+06
INTRLO DBS SMA/SCID Control A	Qiagen DNA purification and Elution	21.99	0.31	1.41	8.88E+07	22.39	0.38	1.70	1.70E+08	24.19	0.37	1.53	1.06E+08
INTRLO DBS SMA/SCID Control A	Qiagen Elution	21.85	0.13	0.62	9.63E+07	22.13	0.08	0.36	1.97E+08	24.00	0.07	0.29	1.17E+08
INTRLO DBS SMA/SCID Control A	One-step (DNA3)	21.58	0.20	0.92	1.29E+08	21.97	0.10	0.46	2.44E+08	23.85	0.14	0.59	1.45E+08
INTRLO DBS SMA/SCID Control A	One-step (DNA3 one wash)	21.73	0.07	0.32	1.16E+08	22.10	0.08	0.36	2.25E+08	24.01	0.06	0.25	1.30E+08
INTRLO DBS SMA/SCID Control A	TritonX-MgCl2 (Method 1)	27.84	1.27	4.54	5.33E+07	26.34	0.67	2.54	2.59E+07	29.59	1.33	4.49	6.82E+06
INTRLO DBS SMA/SCID Control A	TritonX-MgCl2 (Method 2)	25.60	1.35	5.27	1.23E+07	24.79	0.98	3.95	4.91E+07	27.31	1.35	4.94	2.03E+07
INTRLO DBS SMA/SCID Control A	Quanta Extracta	21.86	0.07	0.32	1.09E+08	22.26	0.05	0.22	2.07E+08	24.19	0.03	0.12	1.18E+08
INTRLO DBS SMA/SCID Control A	PerkinElmer	22.98	0.22	0.95	7.32E+07	23.13	0.19	0.82	1.68E+08	25.23	0.21	0.83	8.38E+07
INTRLO DBS SMA/SCID Control A	MRC Holland SALSA	22.11	0.45	2.04	9.45E+07	22.48	0.39	1.73	1.84E+08	24.30	0.34	1.40	1.12E+08
Fresh Patient sample	Quanta Extracta	25.70	0.13	0.50	7.53E+06	25.10	0.04	0.16	2.97E+07	37.75	0.59	1.56	3.94E+04
Patient sample (w/EDTA)	Quanta Extracta	24.45	0.49	2.00	1.77E+07	24.66	0.43	1.74	4.02E+07	38.58	2.02	5.24	1.61E+04

Sample	Extraction	<i>SMN1/SMN2</i> ddPCR									
		Target	Ave. CNV	STDV	%CV	Target	Ave. CNV	STDV	%CV		
INTRLO DBS SMA/SCID Control A	Qiagen QiaAmp DNA micro column	<i>SMN1</i>	2.06	0.06	2.7	<i>SMN2</i>	2.27	0.08	3.7		
INTRLO DBS SMA/SCID Control A	Qiagen DNA purification and Elution	<i>SMN1</i>	1.91	0.01	0.7	<i>SMN2</i>	1.86	0.03	1.5		
INTRLO DBS SMA/SCID Control A	Qiagen Elution	<i>SMN1</i>	1.90	0.00	0.3	<i>SMN2</i>	1.86	0.01	0.8		
INTRLO DBS SMA/SCID Control A	One-step (DNA3)	<i>SMN1</i>	1.90	0.01	0.8	<i>SMN2</i>	1.87	0.02	1.3		
INTRLO DBS SMA/SCID Control A	One-step (DNA3 one wash)	<i>SMN1</i>	1.90	0.01	0.6	<i>SMN2</i>	1.94	0.07	3.8		
INTRLO DBS SMA/SCID Control A	Quanta Extracta	<i>SMN1</i>	1.94	0.03	1.7	<i>SMN2</i>	1.96	0.02	1.1		
INTRLO DBS SMA/SCID Control A	PerkinElmer	<i>SMN1</i>	1.89	0.02	1.1	<i>SMN2</i>	1.84	0.06	3.1		
INTRLO DBS SMA/SCID Control A	MRC Holland SALSA	<i>SMN1</i>	1.72	0.17	1.0	<i>SMN2</i>	1.80	0.15	8.3		
Fresh Patient sample	Quanta Extracta	<i>SMN1</i>	2.00	0.00	0.0	<i>SMN2</i>	1.02	0.01	0.7		
Patient sample (w/EDTA)	Quanta Extracta	<i>SMN1</i>	1.95	0.04	2.2	<i>SMN2</i>	1.95	0.08	4.0		

### Synthetic Dried Blood Spot Homogeneity Testing

**Figure 3. Homogeneity of recovery across a single synthetic DBS SMA/SCID control. Punch-to-punch variability and card-to-card variability tests demonstrates homogeneity of the synthetic control**