

# SMA/SCID Control Panel: A novel panel of biologically-relevant, allele-based DBS mimic controls accurately monitoring SMA/SCID assay performance and SMN1/SMN2 Copy Number Variation

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## Introduction

Spinal Muscular Atrophy (SMA) and Severe Combined Immunodeficiency (SCID) represent critical targets on the Recommended Uniform Screening Panel, affecting 1/10,000 births and requiring urgent intervention. SMA results from SMN1 gene absence causing motor neuron degeneration, while SCID encompasses immunodeficiency disorders leading to fatal infections without treatment. Current diagnostic approaches rely on SMN1/SMN2 copy number analysis for SMA and TREC/KREC quantification from dried blood spots (DBS) for SCID. As testing methodologies evolve, a critical need for a robust source of comprehensive control materials to ensure accurate assay performance across both conditions is warranted. Our research addresses this gap by developing standardized controls, manufactured by a renewable process, that enhance reliability of these life-saving screening programs.

## Materials and Methods

A blood-mimic panel of synthetic DNA 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 INTROL® DBS SMA/SCID Control Panel G180.

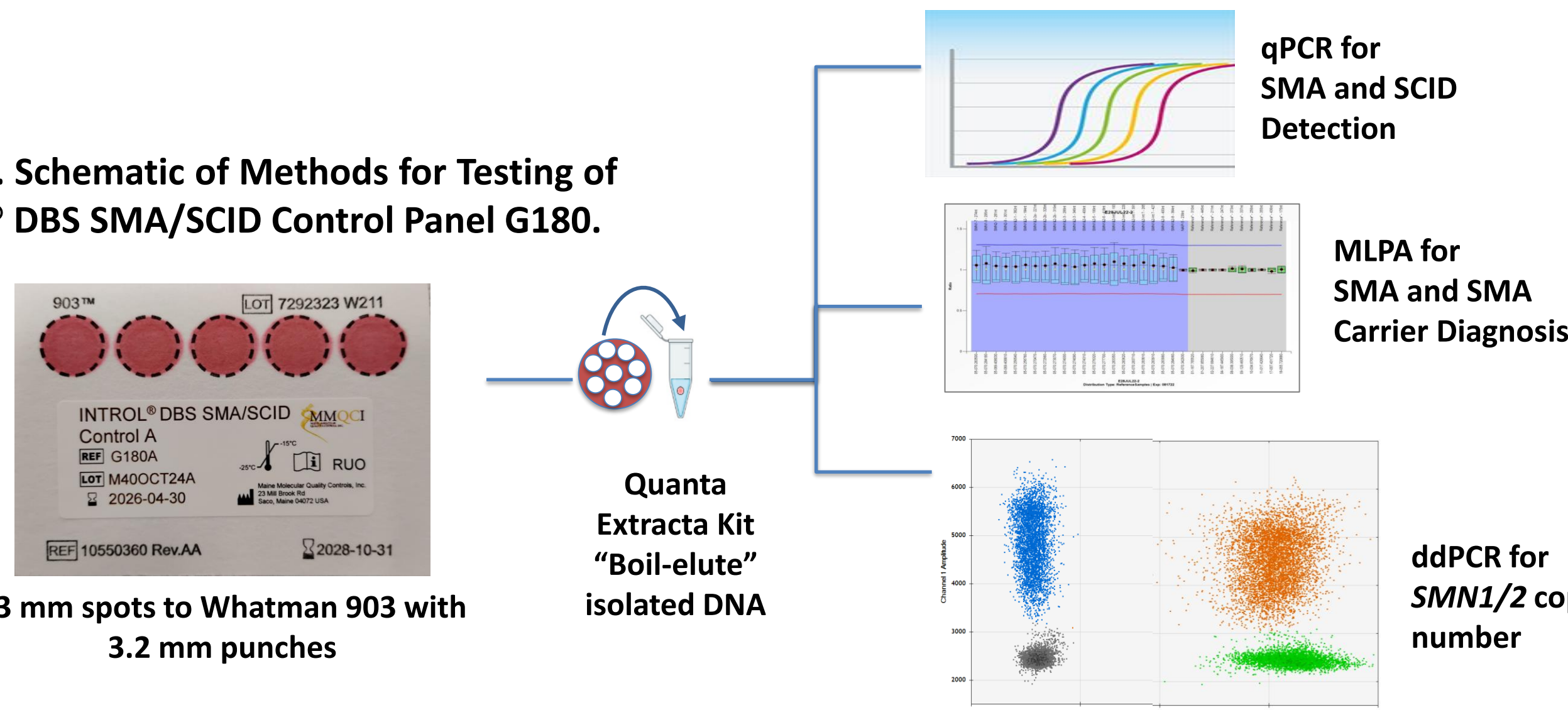
The control panel consists of 4 cards with different SMA genotypes and SCID levels. INTROL® DBS SMA/SCID Control A (WT) contains 2 copies of *SMN1*, 2 copies of *SMN2*, and a high-level copy number (Normal-high) of TREC and KREC. INTROL® DBS SMA Control B (SMA Carrier) contains 1 copy of *SMN1*, 3 copies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

DBS cards were punched, and DNA was extracted using QuantaBio DBS Extracta Kit. Extracted samples were 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. At least three lots of the panel were tested using multiplex SMA/SCID qPCR, over multiple days and operators to determine the product stability at -20°C and RT.

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 INTROL® DBS SMA/SCID Control Panel G180.**



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

Control	SMN1, SMN2 Copies	SMA Status	SCID Status
INTROL® DBS SMA/SCID Control A	2 SMN1 2 SMN2	WT	TREC and KREC High
INTROL® DBS SMA/SCID Control B	1 SMN1 3 SMN2	SMA Carrier	SCID Positive (Zero TREC and KREC)
INTROL® DBS SMA/SCID Control C	3 SMN1 1 SMN2	SMA Silent Carrier	TREC and KREC Mid
INTROL® DBS SMA/SCID Control D	0 SMN1ex7 2 SMN2	SMA	TREC and KREC Low

**Table 2. The INTROL DBS SMA/SCID Control Panel G180 tested using the SMN1/SMN2 Copy Number Determination Kits (Bio-Rad)¹.** INTROL DBS SMA/SCID controls tested with SMN1/SMN2 ddPCR resulted in 100% accurate genotype calls across all the card types in manufactured lots. Testing for each lot was performed for at least N=10 punches, each punch was from a different card per lot.

Card Type	SMN1 Copies	SMN2 Copies	SMA Status	Lot	ddPCR			
					SMN1 Ave CNV	SMN1 STDV	SMN2 Ave CNV	SMN2 STDV
INTROL DBS SMA/SCID Control A (G180A)	2	2	WT	E28JUL22A	1.9	0.0889	1.9	0.0785
				B13JUN23A	1.9	0.0440	1.9	0.0754
				M47NOV23A	1.9	0.0566	1.9	0.0463
				M40OCT24A	1.9	0.0505	1.9	0.0596
INTROL DBS SMA/SCID Control B (G180B)	1	3	SMA carrier	M41OCT24A	2.0	0.0560	2.0	0.0453
				L04AUG22A	1.0	0.0356	3.1	0.1032
				E07JUN23A	0.9	0.0291	2.9	0.0439
				M49DEC23A	0.9	0.0561	2.9	0.1209
INTROL DBS SMA/SCID Control C (G180C)	3	1	SMA Silent Carrier g.27134T>G g.27706-27707delAT	M41APR25A	1.0	0.0254	3.1	0.0607
				T10AUG22A	3.0	0.1184	1.0	0.0393
				B12JUN23A	2.8	0.0660	0.9	0.0329
				M50DEC23A	3.0	0.1385	1.0	0.0541
INTROL DBS SMA/SCID Control D (G180D)	0	2	SMA	S10AUG22A	0.0	0.0007	2.1	0.0541
				E09JUN23A	0.0	0.0007	2.1	0.0933
				M51DEC23A	0.0	0.0013	2.0	0.0797
				M41JAN25A	0.0	0.0008	1.9	0.0383

**Table 3. INTROL DBS SMA/SCID Controls analyzed using a tri-plex qPCR for SMN1, TREC and RPP30 detection².** PCR amplification for detection of *SMN*, *TREC*, and *RPP30* resulted in accurate detection for presences of targets in all card types. Testing for each lot was performed for at least N=10 punches, each punch from a different card. Asterix indicates target concentration adjustment following customers' feedback.

Card Type	SMN1 Copies	SMN2 Copies	SMA Status	SCID Status	Lot	qPCR					
						SMN1 Ave Ct	SMN1 STDV	RPP30 Ave Ct	RPP30 STDV	TREC Ave Ct	TREC STDV
INTROL DBS SMA/SCID Control A (G180A)	2	2	WT	High-level (TREC and KREC Normal-High)	E28JUL22A	23.2	0.4671	23.8	0.1783	25.7*	0.1776
					B13JUN23A	22.8	0.2880	22.6	0.2913	27.5	1.3006
					M47NOV23A	22.9	0.3388	22.5	0.3773	27.9	0.9308
					M40OCT24A	22.7	0.3261	22.2	0.1592	27.4	0.2086
INTROL DBS SMA/SCID Control B (G180B)	1	3	SMA carrier	SCID Positive (Zero TREC and KREC)	M41OCT24A	22.6	0.1583	22.4	0.1681	27.6	0.1481
					L04AUG22A	24.0	0.7002	23.1	0.3515	0.0	0.0000
					E07JUN23A	23.9	0.3880	23.1	0.2534	0.0	0.0000
					M49DEC23A	23.9	0.8493	23.6	0.8264	0.0	0.0000
INTROL DBS SMA/SCID Control C (G180C)	3	1	SMA Silent Carrier g.27134T>G g.27706-27707delAT	Mid-level (TREC and KREC Normal)	M41APR25A	24.8	0.2461	23.8	0.3112	0.0	0.0000
					T10AUG22A	24.5	0.5431	23.7	0.5260	32.0	0.5193
					B12JUN23A	23.6	0.2932	22.4	0.2791	30.9	0.2579
					M50DEC23A	23.7	0.5106	22.6	0.2052	31.0	0.2843
INTROL DBS SMA/SCID Control D (G180D)	0	2	SMA	Low-level (TREC and KREC Low)	S10AUG22A	0.0	0.0000	24.2	0.2260	36.9*	0.4885
					E09JUN23A	0.0	0.0000	23.1	0.6233	34.9	0.6242
					M51DEC23A	0.0	0.0000	23.3	0.4071	36.8	0.6400
					M41JAN25A	0.0	0.0000	23.1	0.1252	35.1	0.2951

Card Type	SALSA MLPA Probemix P021 SMA															
	INTROL DBS SMA/SCID Control A				INTROL DBS SMA/SCID Control B				INTROL DBS SMA/SCID Control C				INTROL DBS SMA/SCID Control D			
SMN1 Copies	2				1				3				0			
SMN2 Copies	2				3				1				2			
SMA Status	WT		SMA carrier		SMA silent carrier		SMA		WT		SMA carrier		SMA silent carrier		SMA	
	Ave ratio	StDev	Copy number	Ave ratio	StDev	Copy number	Ave ratio	StDev	Copy number	Ave ratio	StDev	Copy number	Ave ratio	StDev	Copy number	Ave ratio
SMN1-7	1.01	0.1012	2.01	0.66	0.0208	1.31	1.62	0.0513	3.25	0.03	0.0058	0.05	0.00	0.0000	0.00	0.00
SMN1-8	1.01	0.1102	2.01	1.14	0.0115	2.29	1.15	0.0361	2.30	0.00	0.0000	0.00	0.00	0.0000	0.00	0.00
SMN2-7	1.01	0.0929	2.01	1.69	0.0265	3.38	0.67	0.0306	1.34	1.12	0.0058	2.25	0.00	0.0000	0.00	0.00
SMN2-8	1.00	0.0907	2.01	1.20	0.0000	2.40	1.16	0.0058	2.31	1.13	0.0173	2.26	0.00	0.0000	0.00	0.00
SMN1&2-1	1.01	0.1041	2.01	1.12	0.0173	2.24	1.11	0.0058	2.23	1.13	0.0252	2.27	0.00	0.0000	0.00	0.00
SMN1&2-1	1.01	0.0896	2.01	1.22	0.0153	2.45	1.17	0.0361	2.34	1.13	0.0451	2.27	0.00	0.0000	0.00	0.00
SMN1&2-2a	1.01	0.0929	2.01	1.21	0.0153	2.43	1.17	0.0551	2.35	1.15	0.0231	2.31	0.00	0.0000	0.00	0.00
SMN1&2-2b	1.01	0.0929	2.01	1.19	0.0173	2.38	1.16	0.0404	2.31	1.14	0.0208	2.29	0.00	0.0000	0.00	0.00
SMN1&2-2b	1.01	0.1044	2.02	1.21	0.0153	2.41	1.15	0.0361	2.30	1.15	0.0000	2.30	0.00	0.0000	0.00	0.00
SMN1&2-3	1.01	0.1153	2.02	1.17	0.0265	2.34	1.15	0.0289	2.29	1.14	0.0100	2.28	0.00	0.0000	0.00	0.00
SMN1&2-3	1.01	0.1179	2.02	0.90	0.0058	1.79	0.87	0.0252	1.75	1.13	0.0200	2.26	0.00	0.0000	0.00	0.00
SMN1&2-4	1.01	0.0954	2.02	1.16	0.0231	2.31	1.15	0.0321	2.31	1.15	0.0231	2.29	0.00	0.0000	0.00	0.00
SMN1&2-5	1.01	0.1044	2.02	1.23	0.0361	2.46	1.16	0.0300	2.32	1.16	0.0400	2.32	0.00	0.0000	0.00	0.00
SMN1&2-6	1.01	0.1102	2.01	0.90	0.0379	1.81	1.00	0.0404	2.00	1.19	0.0208	2.37	0.00	0.0000	0.00	0.00
SMN1&2-Int 6	1.01	0.1229	2.02	1.25	0.0200	2.50	1.20	0.0709	2.41	0.61	0.0173	1.22	0.00	0.0000	0.00	0.00
SMN1&2-Int 7	1.01	0.1021	2.01	1.23	0.0115	2.45	1.16	0.0557	2.32	0.62	0.0208	1.23	0.00	0.0000	0.00	0.00
SMN1&2-Int 7	1.01	0.1044	2.02	1.16	0.0173	2.32	1.15	0.0252	2.31	0.59	0.0173	1.18	0.00	0.0000	0.00	0.00
SMN1&2-Int 7	1.01	0.1136	2.02	1.33	0.0557	2.66	1.34	0.1332	2.69	0.53	0.0058	1.07	0.00	0.0000	0.00	0.00
SMN1&2-Int 7	1.01	0.1021	2.01	1.16	0.0208	2.31	1.16	0.0361	2.32	0.62	0.0115	1.23	0.00	0.0000	0.00	0.00
SMN1&2-8	1.01	0.0839	2.01	1.17	0.0231	2.35	1.17	0.0305	2.34	1.16	0.0400	1.22	0.00	0.0000	0.00	0.00
SMN1&2-8	1.01	0.0917	2.02	1.17	0.0300	2.34	1.13	0.0058	2.27	0.61	0.0058	1.21	0.00	0.0000	0.00	0.00

**Table 4. INTROL DBS SMA/SCID Controls evaluated by SALSA MLPA Probemix P021 SMA assay³.** Correct ratio reported across 32 MLPA probes, spanning exons 1-8 of *SMN1* and *SMN2* across all 4 card types. The relative signal height was normalized against the reference samples with a normal copy number of 2. A final ratio of 1.0 = copy number of 2, a ratio of 0.5 = copy number of 1, and a ratio of 1.5 = a copy number of 3. Ratio below 1 is depicted in red; ratio above 1 is depicted in blue.

## Acknowledgements

¹ddPCR performed using SMN1/SMN2 Copy Number Determination Kits (Bio-Rad, Product Code 1863500/1863503).

²SMA/SCID qPCR assay taken from Taylor JL, Lee PK, Yastaparajah GK, Stappert JF, Liu M, Garulli JP, Sin G, Dobrowolski SF, Hannon WH, Vogt RF. Newborn blood spot screening test using multiplexed 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: PMC7906865.

³MLPA performed using MRC Holland's SALSA MLPA Probemix P021-B1 SMA, P060-B2 SMA Carrier, and P460-A1 SMA (Silent) Carrier (MRC Holland, Product Code P021-100R, P060-100R, P460-100R).

## Results

**Table 7. Evaluation of 10 DBS extraction methods and homogeneity of recovery.** A) INTROL 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. B) Punch-to-punch variability and card-to-card variability tests demonstrates homogeneity of the synthetic control product. Eight punches within a single DBS control show homogenous distribution with no significant variability based on SMA/SCID qPCR assay. C) Evaluation across 3 cards, 1 punch from each 3 spots on tested cards demonstrated no significant changes in SMA/SCID qPCR results.

Sample	Extraction	SMN1-RPP30-TREC qPCR										SMN1/SMN2 ddPCR		
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