

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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Poster#56

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**.

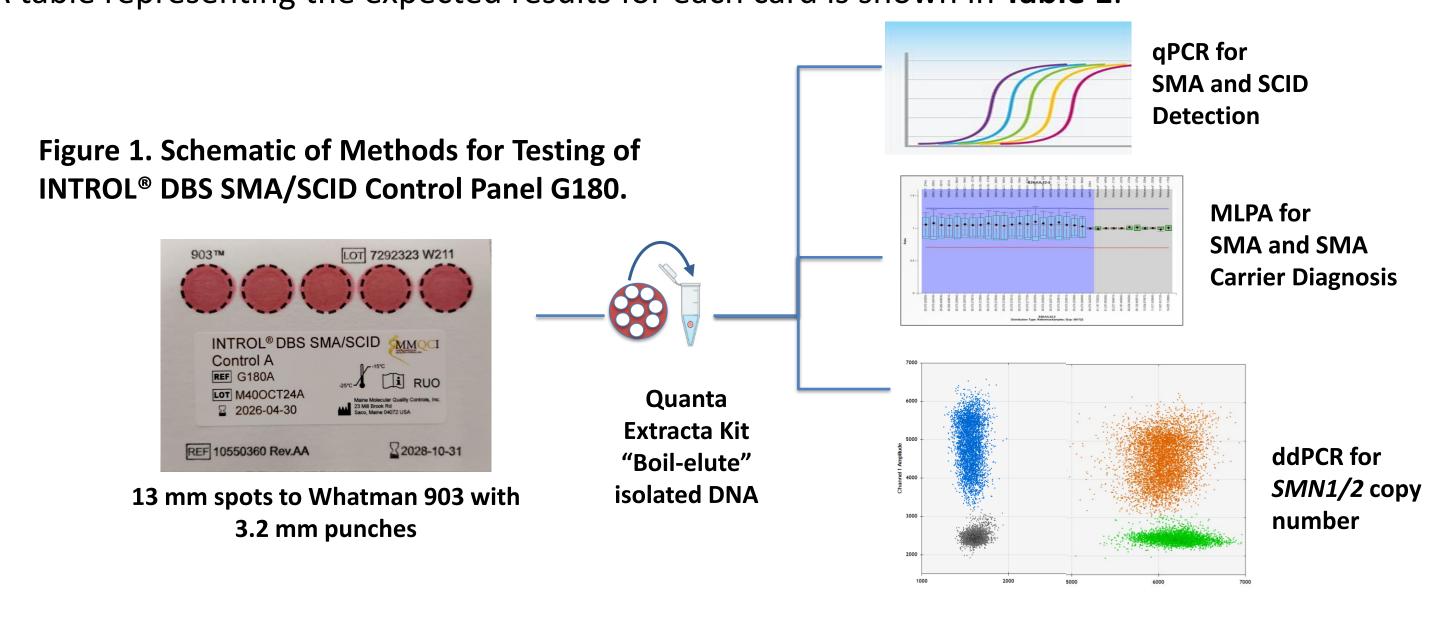


Table 1. Expected Results for INTROL DBS SMA/SCID Control Panel G180.

<u>-</u>	-		
Control	SMN1, SMN2 Copies	SMA Status	SCID Status
INTROL® DBS SMA/SCID Control A	2 SMN1	WT	TREC and KREC
INTROL DBS SIVIA/SCID COILLIOLA	2 SMN2	VVI	High
INTROL® DBS SMA/SCID Control B	1 SMN1	SMA Carrier	SCID Positive
INTROL DBS SIVIA/SCID COILLIOI B	3 SMN2	SIVIA Carrier	(Zero TREC and KREC)
INTROL® DBS SMA/SCID Control C	3 SMN1	SMA Silent Carrier	TREC and KREC
INTROL DBS SIVIA/SCID COILLIOI C	1 SMN2	SIVIA SHEIIL Carrier	Mid
INTROL® DBS SMA/SCID Control D	0 SMN1Ex7	SMA	TREC and KREC Low
INTROL DOS SIVIA/SCID CONTROLD	2 SMN2	SIVIA	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. ddPCR

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Card Type	SMN1 Copies	SMN2 Copies	SMA Status	Lot	SMN1 Ave CNV	SMN1 STDV	SMN2 Ave CNV	SMN2 STDV	
				E28JUL22A	1.9	0.0889	1.9	0.0785	
INTROL DBS				B13JUN23A	1.9	0.0440	1.9	0.0754	
SMA/SCID Control A	2	2	WT	M47NOV23A	1.9	0.0566	1.9	0.0463	
(G180A)				M40OCT24A	1.9	0.0505	1.9	0.0596	
				M41OCT24A	2.0	0.0560	2.0	0.0453	
INTROL DDC				L04AUG22A	1.0	0.0356	3.1	0.1032	
INTROL DBS	1	3	SMA carrier	E07JUN23A	0.9	0.0291	2.9	0.0439	
SMA/SCID Control B	T	5		M49DEC23A	0.9	0.0561	2.9	0.1209	
(G180B)				M41APR25A	1.0	0.0254	3.1	0.0607	
INTROL DBS			SMA Silent Carrier	T10AUG22A	3.0	0.1184	1.0	0.0393	
SMA/SCID Control C	3	1	g.27134T>G	B12JUN23A	2.8	0.0660	0.9	0.0329	
(G180C)			g.27706-27707delAT	M50DEC23A	3.0	0.1385	1.0	0.0541	
INTROL DDC				S10AUG22A	0.0	0.0007	2.1	0.0541	
INTROL DBS		3	CNAA	E09JUN23A	0.0	0.0007	2.1	0.0933	
SMA/SCID Control D	0	2	SMA	M51DEC23A	0.0	0.0013	2.0	0.0797	
(G180D)				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.

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Card Type	SMN1 Copies	SMN2 Copies	SMA Status	SCID Status	Lot	SMN1 Ave Ct	SMN1 STDV	RPP30 Ave Ct	RPP30 STDV	TREC Ave Ct	TREC STDV					
					E28JUL22A	23.2	0.4671	23.8	0.1783	25.7*	0.1776					
INTROL DBS				High-level (TREC	B13JUN23A	22.8	0.2880	22.6	0.2913	27.5	1.3006					
SMA/SCID Control A	2	2	WT	and KREC Normal-	M47NOV23A	22.9	0.3388	22.5	0.3773	27.9	0.9308					
(G180A)				High)	M40OCT24A	22.7	0.3261	22.2	0.1592	27.4	0.2086					
					M41OCT24A	22.6	0.1583	22.4	0.1681	27.6	0.1481					
INITROL DRG			SMA carrier		L04AUG22A	24.0	0.7002	23.1	0.3515	0.0	0.0000					
INTROL DBS SMA/SCID Control B	1	3		SCID Positive (Zero	E07JUN23A	23.9	0.3880	23.1	0.2534	0.0	0.0000					
(G180B)	1	3	SIVIA Carrier	TREC and KREC)	M49DEC23A	23.9	0.8493	23.6	0.8264	0.0	0.0000					
(01805)					M41APR25A	24.8	0.2461	23.8	0.3112	0.0	0.0000					
INTROL DBS			SMA Silent Carrier	Mid level /TDEC and	T10AUG22A	24.5	0.5431	23.7	0.5260	32.0	0.5193					
SMA/SCID Control C	3	1	g.27134T>G	Mid-level (TREC and KREC Normal)	B12JUN23A	23.6	0.2932	22.4	0.2791	30.9	0.2579					
(G180C)			g.27706-27707delAT	KINEC NOTHIAL)	M50DEC23A	23.7	0.5106	22.6	0.2052	31.0	0.2843					
INTROL DRG					S10AUG22A	0.0	0.0000	24.2	0.2260	36.9*	0.4885					
INTROL DBS	_	,	CNAA	Low-level (TREC and	E09JUN23A	0.0	0.0000	23.1	0.6233	34.9	0.6242					
SMA/SCID Control D (G180D)	0	2	SMA	KREC Low)	M51DEC23A	0.0	0.0000	23.3	0.4071	36.8	0.6400					
(01800)					M41JAN25A	0.0	0.0000	23.1	0.1252	35.1	0.2951					

r											SALSA MLPA Probemix P060 SMA Carrier																					
				SA	LSA MI	LPA Pro	bemi	x P021	SMA					ı	NTROL	DBS		INTROL	DBS	ı	NTROL D	BS	INTROL DBS									
ard Type	ı	NTROL [DBS	II	NTROL D)BS	I	INTROL DBS I SMA/SCID Control C		INTROL DBS			INTROL DBS			INTROL DBS INTROL DBS SMA/SCID			Card Type	SMA	/SCID C	ontrol A	SMA	A/SCID C	ontrol B	SMA	/SCID Co	ntrol C	SMA/SCID Control D			
aru rype	SMA	/SCID Co	ntrol A	SMA	/SCID Co	ntrol B	SMA				Control D		SMN1 Copies		2			1			3											
N1 Copies		2			1			3			0		SMN2 Copies		2			3		1			2									
N2 Copies		2			3			1			2				WT			SMA car	rier	SM	A silent o	arrier	SMA									
		WT		S	MA carr	ier	SM	A silent o	arrier		SMA		SMA Status	Ave		Copy	Ave		Сору	Ave		Сору	Ave		Сору							
/IA Status	Ave	StDev	Сору	Ave	StDev	Сору	Ave	StDev	Сору	Ave	StDev	Сору		ratio	StDev	numbe	- 1	Supev	number		StDev	number		StDev	number							
	ratio	Sibev	number	ratio	Sidev	number	ratio	Sibev	number	ratio	JUEV	number	SMN1-7	1.01	0.0709	2.01	0.58		1.16	1.47	0.0436	2.94	0.00	0.0000	0.00							
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	SMN1-8	1.01	0.0100	2.02	1.15		2.29	1.00	0.0351	2.01	0.00	0.0000	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	SMN2-7	1.00	0.0208	1.99	1.60		3.20	0.59	0.0208	1.19	1.06	0.0321	2.13							
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	SMN2-8	1.01	0.0252	2.01	1.10		2.19	1.06	0.0252	2.13	1.10	0.0306	2.19							
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						_														
MN1&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	Table 5	. INTROL DBS SMA/SCID Controls evaluated by SALSA																		
MN1&2-1	1.00	0.0896	2.01	1.22	0.0153	2.45	1.17	0.0361	2.34	1.13	0.0451	2.27	MLPA F	Prob	emix	P060) SN	/IA Ca	rrier a	assa	v ³ . Co	orrect	t copy number									
/IN1&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		ns reported for exons 7 and exon 8 of SMN1 and SMN2																		
/N1&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	variatio	ns r	epor	tea r	or e	xons .	/ and	exc	n & c	ot SIVI	INT	and S)VINZ							
/N1&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	across a	all 4	card	type	s N	ormal	lizatio	n w	as nei	ned against the										
MN1&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				, .					•		Ca c	Barris	, с сп с							
MN1&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	referen	ce sa	<u>ampl</u>	es wit	th a	norm	al cob	v nu	ımber	of 2.										
MN1&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				SALS	A ML	PA Prob	emix P	460 9	SMA (Si	lent) C	arrie	r								
MN1&2-5 MN1&2-6	1.01	0.1044	2.02	0.90	0.0361	2.46 1.81	1.16	0.0300	2.32	1.16	0.0400	2.32		II	NTROL D			NTROL D			NTROL D			NTROL [DBS							
N1&2-Int 6	1.01	0.1102	2.01	1.25	0.0379	2.50	1.20	0.1044	2.41	0.61	0.0208	1.22	Card Type	_		ontrol A		/SCID Co	I		/SCID Co			_	ontrol D							
N1&2-Int 7			2.01		0.0115	2.45	1.16		2.32	0.62	0.0208	1.23	SMN1 Copies		2	71161 01 71	31117	1		311171	3		31117	0	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,							
		0.1044	2.02		0.0173	2.32	1.15		2.31	0.59	0.0173	1.18	SMN2 Copies					- 3			1											
N1&2-Int 7			2.02		0.0557	2.66	1.34	0.1332	2.69	0.53	0.0058	1.07	Sivilva Copies		WT			SMA carr	ior	CNA	A silent o	arrior		SMA								
		0.1021	2.01		0.0208	2.31	1.16	0.0361	2.32	0.62	0.0115	1.23	CNAA Status	Λια	VVI	Conv	_	SIVIA CALI	_	_	4 SHELL C		۸۷۰	SIVIA	_							
		0.0839	2.01		0.0231	2.35	1.17	0.0265	2.34	0.61	0.0200	1.22	SMA Status		StDev	Copy		StDev	1	Ave	StDev	Copy		StDev	Copy							
		0.0917	2.02		0.0300	2.34	1.13		2.27	0.61	0.0058	1.21		ratio		number			number			number			number							
													SMN1-7		0.0493	2.01	0.59	0.0252	1.17		0.0289	3.05	0.01	0.0115	0.01							
able 4.	IN	ΓROL	DBS	SMA	A/SCI	D Co	ntro	ls ev	aluate	ed b	ov SA	LSA	SMN1-8	1.00	0.0058	2.01	1.14	0.0153	2.29	1.09	0.0265	2.18	0.00	0.0000	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.

Table 6. INTROL DBS SMA/SCID Controls evaluated by SALSA MLPA Probemix P460 SMA (Silent) Carrier assay³. Correct copy number variations reported for exon 7 and 8 of SMN1 and SMN2, and accurate detection of the silent carrier risk factor polymorphisms in SMN1 (g.27134T>G and g.27706-27707delAT). Normalization was performed against the reference samples with a normal copy number of 2.

|SMN1-8 (MUT)| 0.00 | 0.0000 | 0.00 | 0.000 | 0.00 | 55% | 0.0153 | 55%

Acknowledgements

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.

INTROL DB	L 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	1.91	0.01	0.7	1.86	0.03	1.5	
INTROL DB	S SMA/SCID Contr	rol A	Qiagen Elution 2			.85 0.13	0.62	9.63E+07	22.13	80.0	0.36	1.97E+08	24.00	0.07	0.29	1.17E+08	1.90	0.00	0.3	1.86	0.01	8.0
INTROL DBS SMA/SCID Control A			One-s	tep (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	1.90	0.01	8.0	1.87	0.02	1.3
INTROL DB	S SMA/SCID Contr	rol A	One-step (DNA3 one was	sh) 21	.73 0.07	0.32	1.16E+08	22.10	80.0	0.36	2.25E+08	24.01	0.06	0.25	1.30E+08	1.90	0.01	0.6	1.94	0.07	3.8
INTROL DB	S SMA/SCID Contr	rol A	TritonX-M	gCI2 (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	not tested		,Or	est tested		
INTROL DB	S SMA/SCID Contr	rol A	TritonX-M	gC12 (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	not tested		,01	est tested		
INTROL DBS SMA/SCID Control A Quanta			ita 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	1.94	0.03	1.7	1.96	0.02	1.1	
INTROL DBS SMA/SCID Control A			Perkir	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	1.89	0.02	1.1	1.84	0.06	3.1	
INTROL DB	S SMA/SCID Contr	rol A	MRC H	olland 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	1.72	0.17	10	1.80	0.15	8.3
Frest	h Patient sample		Quan	ita 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	2.00	0.00	0.0	1.02	0.01	0.7
Patient	Patient sample (w/EDTA) Quanta Extracta					.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	1.95	0.04	2.2	1.95	80.0	4.0
B)		ı	Punch-to-	Punch va	ariability				(C) _				Card	-to-Ca	rd vari	abili	ity				
				SMN1												SMN1						
	N= 42 A		SD SD			nL SD	%c	v			N= 18		AVE. Cp		SD	%C\	v	AVE. Cps	/mL	SD	9/	6CV
	punch-to-punch	22.59		2.40E+0		39 17.8	8%			card-to-card		22.86 0.639		2.79%		2.11E+	<u> </u>	5822763 27		.63%		
										RPP30												
,						1																
	N= 42	AVE. Cp	SD	%cv	AVE. Cps/mL	SD	%C	v			N= 18	3 4	AVE. Cp		SD	%CV	AV	E. Cps/m	ι	SD	%	6CV

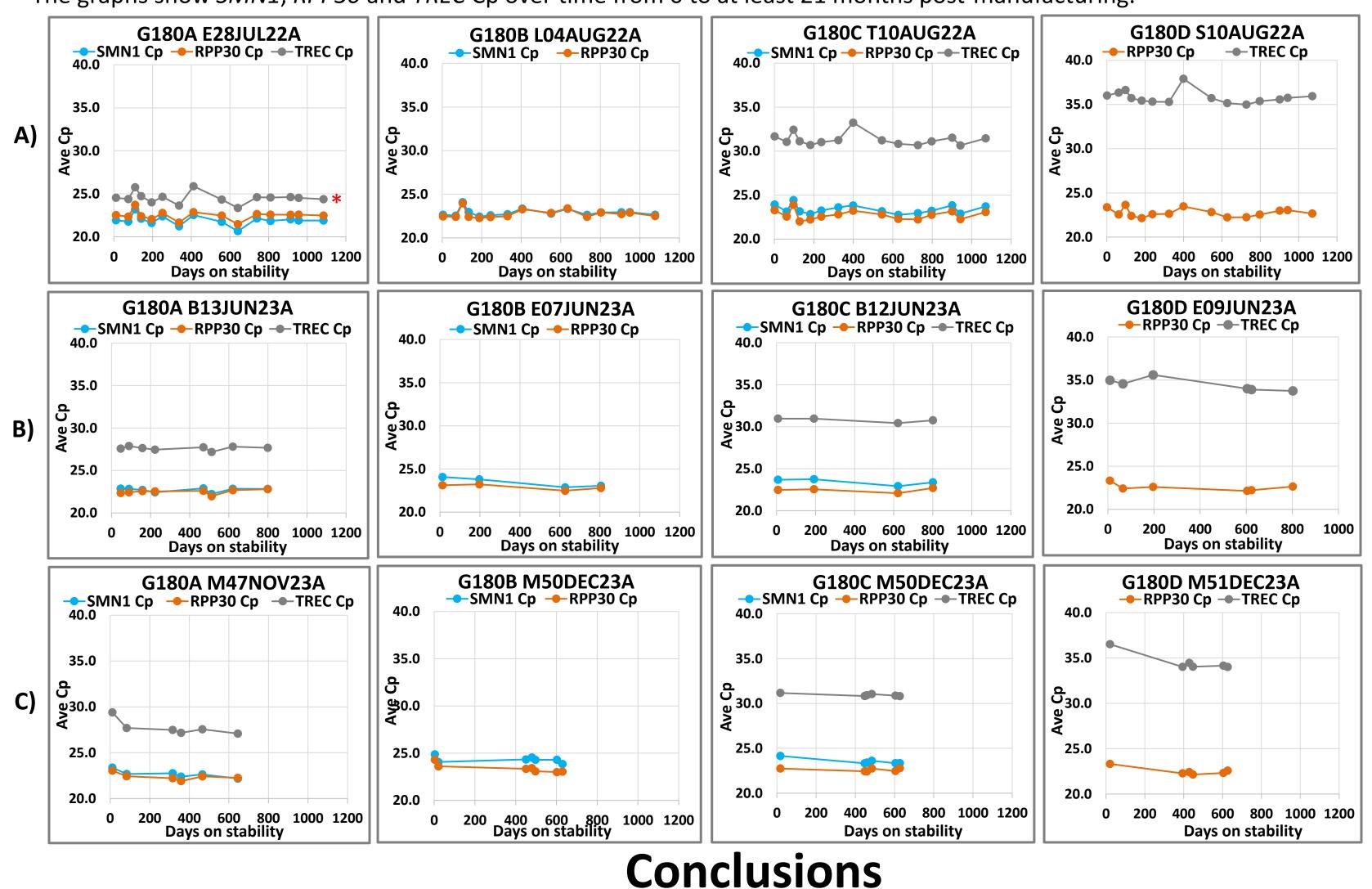
%CV AVE. Cps/mL

0.94%

Figure 2. Real-time stability studies demonstrate >24 months stability when stored at -20°C. Real-time stability was measured for at least 3 consecutive lots of controls A) JUL-AUG22, B) JUN23, and C) NOV-DEC23 with a similar formulation. To assess stability of SMN1, RPP30 and TREC Cp levels over time, samples across all control levels from the panel were run in triplicates. The graphs show SMN1, RPP30 and TREC Cp over time from 0 to at least 21 months post-manufacturing.

25.32 0.737

2.91%



- > The INTROL DBS SMA/SCID Control Panel G180 demonstrated reproducible compatibility across multiple platforms, qPCR, ddPCR and MLPA test methods with 100% correct calls across multiple lots.
- The INTROL DBS SMA/SCID Control Panel G180 demonstrated reproducible results when evaluated with 10 extraction methods with %CV <10% by qPCR and <5% by ddPCR; and homogenous distribution across spots and punches (Cp SD<1 and %CV<5%).
- > The INTROL DBS SMA/SCID controls provide the ability to assess routine monitoring of assay determination of copy number of SMN1 and SMN2, and the ability to monitor the performance of quantification of TREC/KREC levels for detection of SCID using a multiplex SMA/SCID qPCR assay. Stability data for INTROL DBS SMA/SCID controls supports stability for 24 months when stored at -20°C.
- > The INTROL DBS SMA/SCID Control Panel G180 enables the monitoring of proper detection of clinically relevant variants by SMA and SMA Carrier diagnostic DBS assays.