Dried Blood Spot Testing with AmplideX® SMA Plus* Kit Resolves *SMN1* and *SMN2* Exon 7 Copy Numbers and More

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SUMMARY

- Newborn screening for SMA typically involves studying dried blood spot (DBS) samples with a qPCR test for presence/absence of *SMN1*, followed by a confirmatory diagnostic test from whole blood to confirm absence of *SMN1* and determine *SMN2* copy number.
- We demonstrate the feasibility of a single-reaction test to determine *SMN1* and *SMN2* copy numbers and identify disease modifier variants directly from DBS in less than four hours.
- This approach reduces overall turnaround time by simplifying the workflow and providing more information during initial DBS testing, potentially enabling earlier treatment and improved patient outcomes.

INTRODUCTION

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder commonly caused by homozygous absence of *SMN1*. *SMN2*, an *SMN1* paralog, modulates SMA severity. Breakthrough therapies rely on rapid quantification of *SMN1* and *SMN2* copies, and newborn screening using dried blood spot (DBS) samples has become a public health priority. However, most screening assays only determine presence/absence of *SMN1* exon 7, excluding copy numbers (CN) for *SMN1* and *SMN2* and disease-modifier variants (*SMN2*(NM_017411):c.859G>C, abbreviated as c.859G<C). Here, we present data from the AmplideX SMA Plus* Kit from 378 DBS measurements, demonstrating feasibility of a rapid, comprehensive, single-tube method that provides valuable information for both newborn screening and to aid in diagnosis directly from DBS samples.

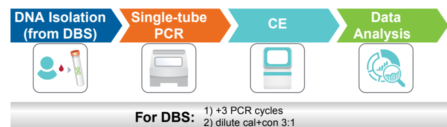
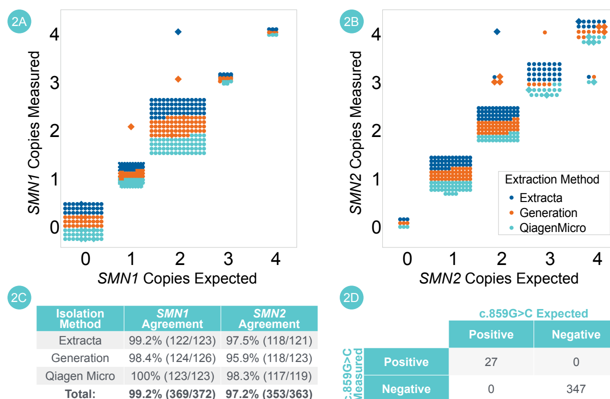


Figure 1. AmplideX SMA Plus DBS Workflow. Same protocol with minor changes.

MATERIALS AND METHODS

We tested 42 DBS samples with three DNA isolation methods on three genetic analyzer models (378 measurements total) using the AmplideX® SMA Plus* Kit, which quantifies *SMN1* and *SMN2* exon 7 CN and detects c.859G>C in a single-tube workflow. Samples consisted of blood spotted on Whatman® 903 filter paper (n=20) or Whatman FTA® cards (n=22). Samples were isolated with three methods: Quantabio Extracta DBS, Qiagen Generation DNA purification and elution solutions, and Qiagen QIAamp® DNA micro columns. Samples were tested using 2 ul of extraction eluate per PCR reaction as input following the kit protocol with two modifications: 1) 3 PCR cycles were added, and 2) The Calibrator and Control were diluted 3 to 1 using kit Diluent (See Figure 1). All 126 isolations were analyzed in singleton on ABI 3500, 3730, and SeqStudio Genetic Analyzers. Genotypes were determined using AmplideX PCR/CE Reporter software. Reference values were determined using matched whole blood with the AmplideX SMA Plus Kit (n=20) or MLPA and sequencing (n=22). QC failures were excluded from calculations.

RESULTS



2C

Isolation Method	*SMN1* Agreement	*SMN2* Agreement
Extracta	99.2% (122/123)	97.5% (118/121)
Generation	98.4% (124/126)	95.9% (118/123)
Qiagen Micro	100% (123/123)	98.3% (117/119)
Total:	**99.2% (369/372)**	**97.2% (353/363)**

2D

		c.859G>C Expected	
		Positive	Negative
c.859G>C Measured	Positive	27	0
	Negative	0	347

Figure 2. *SMN1* and *SMN2* Copy Number and Disease Modifier Agreement for DBS Samples Across all CE Instruments and Extraction Methods. A) *SMN1* exon 7 copy number agreement. Diagnostic accuracy for SMA for confirmed patients was 100% (90/90). **B)** *SMN2* exon 7 copy number agreement. **C)** *SMN1* and *SMN2* agreement by extraction method. **D)** c.859G>C agreement was 100% (374/374). In A and B, color represents extraction method, and diamond shapes indicate clinical samples with endogenous control (EC) peaks <1000 RFU, indicative of low signal. Most copy number misses were in clinical samples with low signal. Repeat testing resolved most discrepancies; after repeating QC failures and discrepancies for *SMN2*, agreement was 99.2% (369/372).

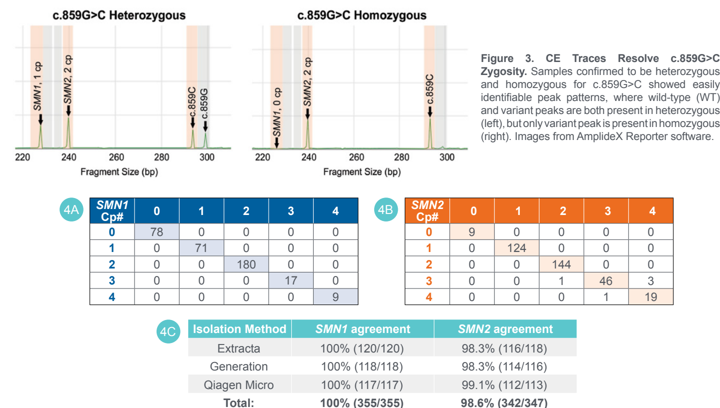


Figure 3. CE Traces Resolve c.859G>C Zygosity. Samples confirmed to be heterozygous and homozygous for c.859G>C showed easily identifiable peak patterns, where wild-type (WT) and variant peaks are both present in heterozygous (left), but only variant peak is present in homozygous (right). Images from AmplideX Reporter software.

4A

SMN1 Cp#	0	1	2	3	4
0	78	0	0	0	0
1	0	71	0	0	0
2	0	0	180	0	0
3	0	0	0	17	0
4	0	0	0	0	9

4B

SMN2 Cp#	0	1	2	3	4
0	9	0	0	0	0
1	0	124	0	0	0
2	0	0	144	0	0
3	0	0	1	46	3
4	0	0	0	1	19

4C

Isolation Method	*SMN1* agreement	*SMN2* agreement
Extracta	100% (120/120)	98.3% (116/118)
Generation	100% (118/118)	98.3% (114/116)
Qiagen Micro	100% (117/117)	99.1% (112/113)
Total:	**100% (355/355)**	**98.6% (342/347)**

Figure 4. *SMN1* and *SMN2* Copy Number Agreement for DBS Samples with EC Peak Heights ≥1000 RFU. A) *SMN1* exon 7 copy number agreement. **B)** *SMN2* exon 7 copy number agreement. **C)** *SMN1* and *SMN2* agreement by extraction method. Samples with <1000 RFU in the endogenous control (EC) peak indicative of low signal (Figure 2 A and B, diamonds) were less accurate than samples with strong signal (≥1000 RFU). For samples above this cutoff, agreement for *SMN1* was 100% (355/355) and agreement for *SMN2* was 98.6% (342/347). Below this cutoff, agreement for *SMN1* was 82.3% (14/17) and agreement for *SMN2* was 70.6% (12/17).

CONCLUSIONS

- We demonstrated the feasibility of accurately resolving *SMN1* and *SMN2* copy numbers and disease modifier variant status directly from DBS samples in a single reaction with the AmplideX SMA Plus* Kit.
- Agreement was high (97-99%) for both *SMN1* and *SMN2* copy numbers; manual application of a signal threshold of 1000 RFU to the endogenous control (EC) peak improved accuracy (~99-100%), especially for the Generation isolation method.
- Accuracy for the positive disease modifier variant c.859G>C was 100% (27/27) in confirmed patients, and unique profiles in heterozygous and homozygous samples suggest the AmplideX SMA Plus Kit may be useful for resolving c.859G>C zygosity.
- Detection of *SMN1* and *SMN2* copy numbers and disease modifiers directly from DBS samples in a single working shift may enable earlier treatment and improved patient outcomes by eliminating need for follow-up blood collection or lengthy confirmatory diagnostic assays.

**CE-IVD. For US export only.
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