A Rapid Diagnostic and Screening System for Spinal Muscular Atrophy that Reports Copy Number Changes, Single Nucleotide Variants and Small Indels

Huiping Zhu[1], Stela Filipovic-Sadic[1], Melissa Church[1], Justin Janovsky[1], Kevin Kelnar[1], Lawrence Manzano[1], Lando Ringel[1], Jessica L Larson[1], Brian C Haynes[1], Na Liang[1], Yih-Yuan Chang[2], Chien-Hao Huang[2], Tsang-Ming Ko[2], Ya-Wen Huang[2], Caren Gentile[3], Alicia M Carlin[3], Sarah E Herlihy[3], Vivianna Van Deerlin[3], Gary J Latham[1]

[1]Asuragen, Inc., Austin, TX; [2]GenePhile Bioscience Laboratory, Ko's OBS/GYN Clinic, Taipei Citi 100, Taiwan; [3]Hospital of the University of Pennsylvania, Philadelphia, PA

Summary

- Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease that results from mutation of the survival motor neuron 1 gene (*SMN1*), where disease severity is modulated by the *SMN2* copy number.
- We developed a streamlined, single-tube PCR/CE prototype assay* which determines *SMN1* and *SMN2* copies, along with identification of two gene duplication variants and an SMA disease modifier SNV.
- Evaluation of AmplideX® PCR/CE *SMN1/2* Plus* prototype assay produced consistent results for 130 residual clinical samples across three laboratories, under a faster and simpler workflow than other commonly used methodologies.

Introduction

Approximately 95% of SMA cases are caused by homozygous deletion of *SMN1* exon 7. Although assays to diagnose the common *SMN1* exon 7 deletion are available, improved methods are needed that can expand the identification of carriers and patients with variations in disease severity that are relevant to existing and emerging molecular medicines. To this end, we report the performance of a prototype PCR/CE reagents and software that quantify *SMN1/2* copy numbers and genotype "silent carrier"-associated markers and a critical disease-modifier variant.

Materials and Methods

We developed a prototype *SMN1/2* assay that includes detection of two gene duplication variants (c.*3+80T>G and c.*211_*212del) and the c.859G>C disease modifier. PCR products were separated using Applied Biosystems™ 3500 or 3130 Series Genetic Analyzers (Thermo Fisher Scientific). Assay accuracy, specificity and sensitivity was assessed using cell-line and blood DNA samples with varying *SMN1/2* copies and positive and negative variants. The copy number of *SMN1* or *SMN2* was calculated as the peak area ratio of the target gene to an endogenous control, normalized to a calibrator sample. Normalized ratios were further binned into prototype copy number specific bins (separated by gray zones) and results were reported for 0, 1, 2, 3, or ≥4 copies of exon 7. An in-development analysis module enabled automated interpretation of electropherograms across peak detection and size-based classification, copy-number quantification, sample- and batch-level QC, and report generation. Analysis of 96 samples required <5 minutes, resulting in a total PCR/CE assay workflow of under 4 hours.

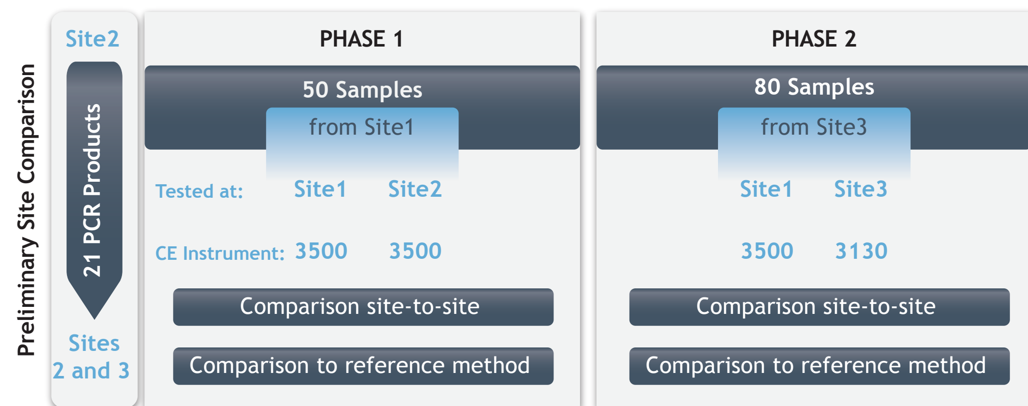

Figure 1. Study Overview. A total of 130 samples, including 50 from Hospital of the University of Pennsylvania (HUP) and 80 from GenePhile, were successfully genotyped by three laboratories (Site 1: HUP, Site 2: Asuragen, and Site 3: GenePhile). The results from the prototype PCR/CE *SMN1/2* Plus assay were compared site-to-site, and with reference methods where available (*SMN1/2* copy number: MLPA; two gene duplication markers: MLPA or Sanger sequencing; *SMN2* disease modifier SNV: Sanger sequencing).

*This product is under development. Specifications have not been finalized.
Presented at AMP 2019

Results

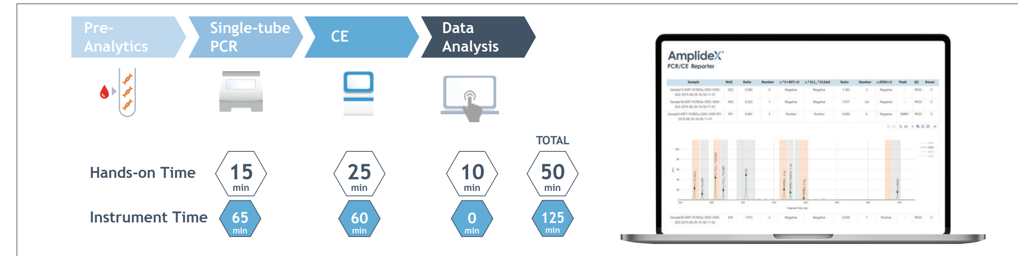

Figure 2. The *SMN1/2* Plus PCR/CE Assay Offers Streamlined, Sample-to-Answer Workflow. It includes reagents, controls, and automated, push-button data reporting via AmplideX Reporter software. The assay can be performed with a turnaround time of <3 hours for 1 capillary electrophoresis injection (4-24 samples) with ~50 min of total hands-on time.

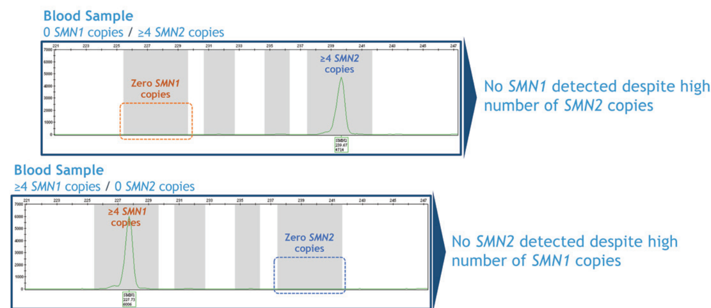

Figure 3. High Specificity for Both *SMN1* and *SMN2* Ensures Reliable Results. Elevated copies of either *SMN1* or *SMN2* have no effect on accurate reporting of copy number of these two highly homologous genes.

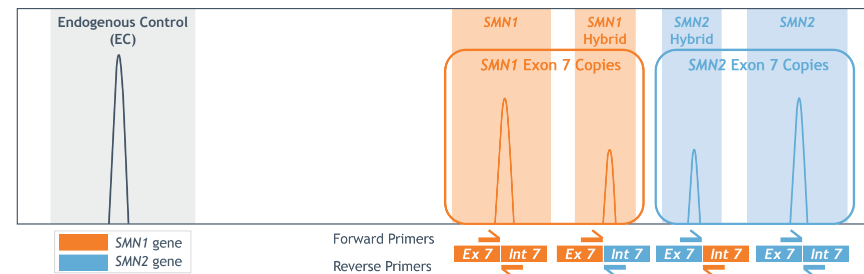

Figure 4. The Assay Detects and Differentiates SMN1-to-SMN2 and SMN2-to-SMN1 Gene Conversions Within Exon 7 and Intron 7 Regions. *SMN1-SMN2* and *SMN2-SMN1* gene conversion peaks are detected as unique peaks in the CE trace and their copy number quantification is based on detection of c.840C (*SMN1*) or c.840T (*SMN2*).

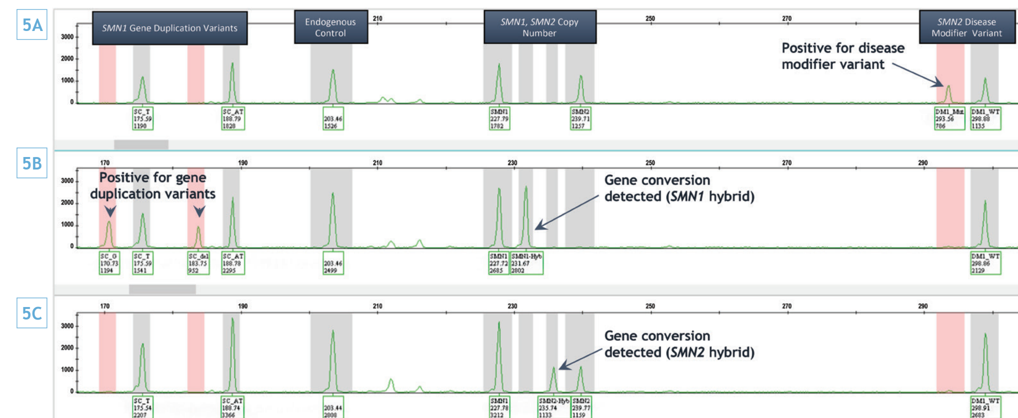

Figure 5. Example Electropherograms. A) Sample positive for the SMA disease modifier variant. **B)** Sample positive for two gene duplication variants and a gene conversion (*SMN1*-hybrid, c.840C/+100G). **C)** Sample positive for a gene conversion (*SMN2*-hybrid, c.840T/+100A). Internal testing with 71 samples with verified gene duplication and disease modifier status, showed 100% agreement with the reference methods. Further, all sample calls were in agreement for these same variants between sites.

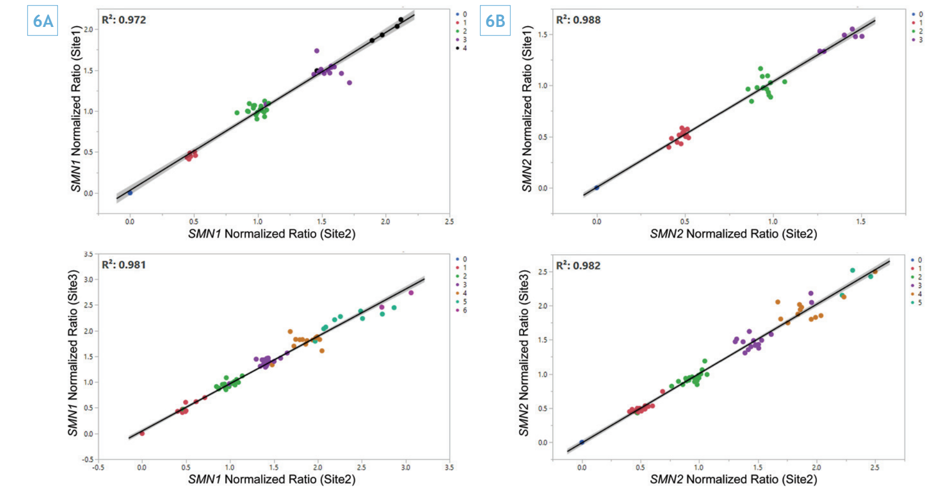

Figure 6. *SMN1* and *SMN2* Normalized Ratios are Correlated Across Test Sites (R >0.97). A) Comparison of results from Sites 1 and 2 (50 samples). **B)** Comparison of results from Sites 2 and 3 (80 samples). In addition, all calls for the gene duplications and disease modifier variant agreed between laboratories.

Combined Data for 130 Samples (Site1/3500, Site3/3130)

SMN1 Cp#	0	1	2	3	4+	out of bins
0	15	0	0	0	0	0
1	0	13	0	0	0	3
2	0	0	40	0	0	0
3	0	0	1	28	1	0
4	0	0	0	0	28	1
				%Agreement	98.4%	3%
				#Calls	126	4

SMN2 Cp#	0	1	2	3	4+	out of bins
0	24	0	0	0	0	0
1	0	33	1	0	0	0
2	0	0	31	3	0	1
3	0	0	0	20	2	0
4	0	0	0	0	15	0
				%Agreement	95.3%	1%
				#Calls	129	1

Table 1. *SMN1* and *SMN2* Copy Number Agreement with the Reference Method. Combined copy number data for 130 samples from two Sites (Site1/3500 and Site3/3130) shows 98.4% agreement for *SMN1* and 95.3% for *SMN2* with the reference method using an in-development analysis tool and bin settings.

Conclusions

- Using a single PCR, the AmplideX PCR/CE *SMN1/2* Plus* prototype kit integrates quantification of *SMN1* and *SMN2* copy number from 0 to ≥4 copies while also detecting two gene duplication markers and an *SMN2* disease modifier SNV.
- Both zero and one *SMN1* copy samples were accurately identified in every case. Across 130 unique samples, *SMN1* and *SMN2* copy numbers were quantified with excellent agreement to reference results and normalized ratios were highly correlated between laboratories.
- All gene duplication and disease modifier variant calls were reproducible and agreed with independent results.
- The simple and rapid workflow (<3 hours) of this assay, coupled with integrated controls, and automated companion software, may substantially reduce the complexity and time for *SMN1/2* testing and provide more comprehensive information than current assays to advance both diagnostic and screening applications.

Penn Medicine

Asuragen
www.asuragen.com