Multisite Evaluation of a Single-tube *SMN1/2* PCR/CE Assay System that Assesses Copy Number and Expanded Content for Spinal Muscular Atrophy

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Abstract ID 1346
Poster # 646/PF

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.
- The AmplideX® PCR/CE *SMN1/2* Plus Kit is a one-tube PCR that quantifies *SMN1* and *SMN2* copy numbers and genotypes gene duplication "silent carrier" markers (c.*3+80T>G and c.*211_*212del) as well as a disease modifier variant (*SMN2* c.859G>C).
- The integrated AmplideX Reporter software module directly processes .fsa files and reports copy-number values and variant genotypes from a 96-well plate in less than 5 minutes.
- Four laboratories evaluated the *SMN1/2* Plus kit by testing a total of 471 samples and achieving ≥98% agreement with reference results across copy number and other gene variants.
- The expanded content of the kit combined with its convenience, usability and versatility may provide an all-in-one option for laboratories interested in SMA carrier screening and diagnostics.

Introduction

Approximately 95% of SMA cases are caused by a deletion in both alleles of exon 7 in the *SMN1* gene. Although *SMN1/2* copy-number assays are available, improved diagnostic methods are needed to expand the identification of carriers and patients with genetic variations relevant to existing (Spinraza® and Zolgensma®) and emerging (Risdiplam) molecular medicines. No current assay, however, combines *SMN1/2* copy number detection with gene duplication and disease modifier variants in an easy-to-run, streamlined workflow that is broadly accessible to laboratories. To this end, we describe the multisite performance of a single-tube PCR/CE kit, including companion software that reports such expanded content.

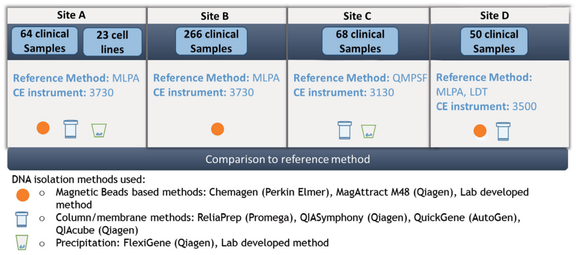

Figure 1. Study Overview. A total of 448 residual clinical samples were tested. Among these, 64 were tested at site A, 266 at site B (GenePhile), 68 at site C (CHU Rouen); 50 samples from Hospital of the University of Pennsylvania, HUP were tested at site D (Asuragen). In addition, 23 cell line DNA samples were tested at Site A. The results from the PCR/CE *SMN1/2* Plus Kit were compared with reference methods where available (*SMN1/2* copy number: MLPA or QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments); two gene duplication markers: MLPA or Sanger sequencing; *SMN2* disease modifier: Sanger sequencing).

Materials and Methods

The AmplideX PCR/CE *SMN1/2* Plus Kit is a one-tube PCR that quantifies *SMN1* and *SMN2* copy numbers and genotypes gene duplication "silent carrier" markers as well as a disease modifier variant in *SMN2*. A total of 448 residual clinical whole blood DNA samples and 23 cell line samples with varying *SMN1/2* copies and variant genotypes were tested at four independent laboratories. These samples were prepared using nine different common DNA isolation kits/procedures across precipitation, column/membrane-based and magnetic beads-based methods. PCR products were separated using an Applied Biosystems™ 3500, 3130 and 3730 Genetic Analyzers (Thermo Fisher Scientific). Raw electrophoresis data (.fsa) files were directly imported into an assay-specific analysis module of AmplideX® Reporter software that automates peak detection and size-based classification, copy-number quantification, detection of gene duplication and disease modifier variants, and sample- and batch-level quality control checks, and generates a summary report.

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Presented at ACMG Digital 2020 - ID 1346P- 646/PF

Results

Figure 2. The AmplideX *SMN1/2* Plus PCR/CE Kit Offers a Streamlined, Sample-to-Answer Workflow. It includes reagents, controls, and automated, push-button data reporting via AmplideX Reporter software. The kit is compatible with common DNA isolation methods and does not require a method-specific copy number calibrator. The assay can be performed with a turnaround time of <4 hours for one capillary electrophoresis injection (4-24 samples) with ~60 min of total hands-on time.

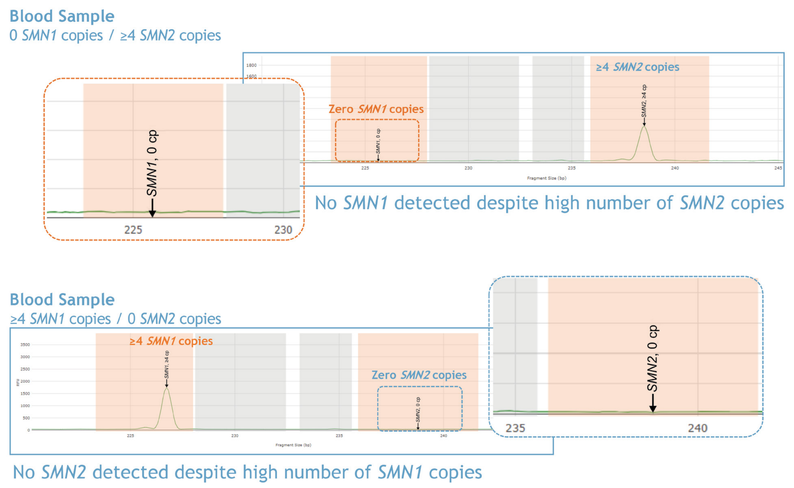

Figure 3. High Specificity for Both *SMN1* and *SMN2* Ensures Reliable Results. Elevated copies of either *SMN1* or *SMN2* have no effect on accurate reporting of copy number of these two highly homologous genes.

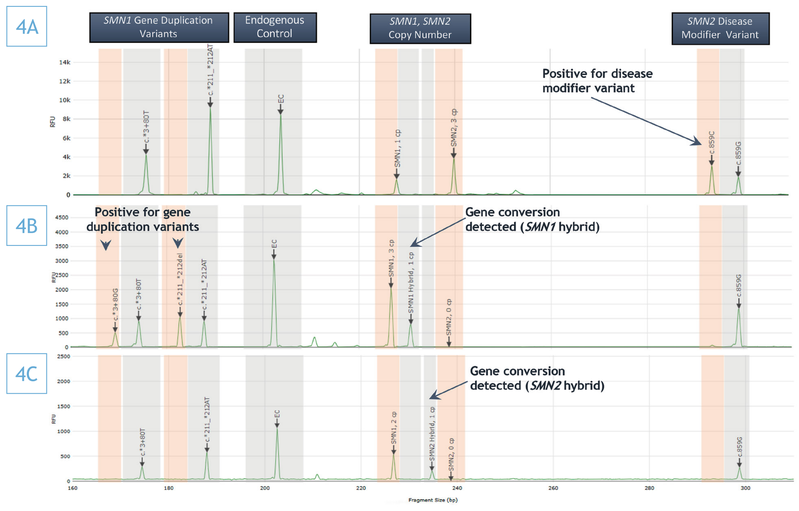

Figure 4. Example Electropherograms. A) Sample positive for the SMA disease modifier variant. **B)** Sample positive for two gene duplication variants and a gene conversion (*SMN1* hybrid, c.840C/+100G). **C)** Sample positive for a gene conversion (*SMN2* hybrid, c.840T/+100A). *SMN1* hybrid and *SMN2* hybrid 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*). The software reports total number of *SMN1* exon 7 by summing *SMN1* and *SMN1* hybrid copy numbers, reports total number of *SMN2* exon 7 by summing *SMN2* and *SMN2* hybrid copy numbers.

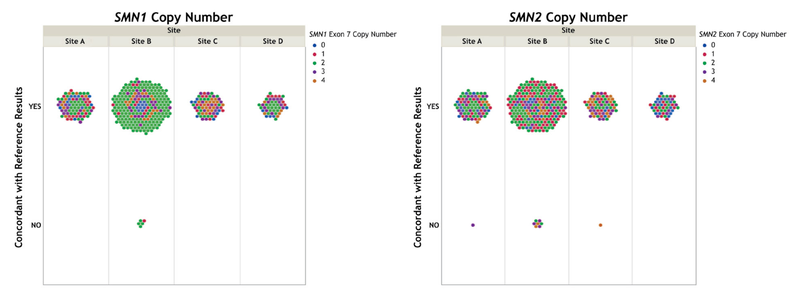

Figure 5. High Concordance Between *SMN1/2* Plus Kit and Reference Methods. Copy number agreement between *SMN1/2* Plus Kit and reference methods for each laboratory site were as follow: Site A: 100% for *SMN1*, 98.8% for *SMN2*; Site B: 98.0% for *SMN1*, 97.3% for *SMN2*; Site C: 100% for *SMN1*, 98.5% for *SMN2*; Site D: 100% for both *SMN1* and *SMN2*. Variant reference results were available for 205 unique samples, among which 39 were positive for *SMN1* c.*3+80T>G, 40 were positive for c.*211_*212del and 12 were positive for *SMN2* c.859G>C. Variant call results from *SMN1/2* Plus Kit were 100% concordant with reference results.

SMN1/2 Plus Kit

Reference Method: *SMN1* Cp#	0	1	2	3	4+	QC Failure
0	33	0	0	0	0	0
1	0	47	3	0	0	1
2	0	1	259	0	0	6
3	0	0	1	63	0	3
4	0	0	0	0	50	4
					% Agreement	98.9%	3%
					# Calls	457	14

SMN1/2 Plus Kit

Reference Method: *SMN2* Cp#	0	1	2	3	4+	QC Failure
0	45	0	0	0	0	0
1	0	124	2	0	0	4
2	0	0	183	3	0	7
3	0	0	0	58	3	1
4	0	0	0	1	38	2
					% Agreement	98.0%	3%
					# Calls	457	14

Table 1. *SMN1* and *SMN2* Copy Number Agreement with the Reference Method. Combined copy number data for 471 unique samples (448 residual clinical samples and 23 cell line samples) isolated with nine different methods and tested at four laboratories shows 98.9% agreement for *SMN1* and 98.0% for *SMN2* with the reference method. "4+" indicates samples with 4 or more copies of the designated gene. A total of 14 (3%) samples with QC failures (9 precision failure, 5 ROX ladder failure) were excluded.

Conclusions

- A total of 457/471 (97%) unique samples were successfully tested in a first pass using the kit at four laboratory sites in the US and abroad.
- *SMN1* (98.9%) and *SMN2* (98.0%) copy numbers were quantified over a range of 0 to 4 or more copies in excellent agreement with the reference method.
- Among the 205 unique samples with available variant reference results, 100% were in agreement for all variants using the kit. A total of 39 were positive for *SMN1* c.*3+80T>G, 40 were positive for c.*211_*212del, and 12 were positive for *SMN2* c.859G>C.
- The single-tube PCR/CE *SMN1/2* Plus kit accurately quantifies 0, 1, 2, 3, or ≥4 gene copies for both *SMN1* and *SMN2*, and reports the status of three clinically significant variants from blood specimens using a ~3 hour workflow and integrated calibrators, controls and software.

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