# A Rapid, High-Throughput Multiplex PCR/CE Assay that Reliably Quantifies *SMN1* Copy Number

**Walairat Laosinchai-Wolf, Keri Jefferson, Ion Beldorth, Kimberly Nicholson, Blake Printy, Stela Filipovic-Sadic, Jacob Ashton, and John Milligan**

**Asuragen, Inc., Austin, TX**

## Summary

- Spinal Muscular Atrophy (SMA) is a lethal disease caused by homozygous loss of function of the *SMN1* gene that represents the leading genetic cause of infant death with an incidence of ~1/10,000.
- Recent updates to testing ACOG guidelines, which recommend screening for all women considering pregnancy, demand a simple, fast, and high-throughput testing solution.
- Herein, we present data from the AmplideX® PCR/CE *SMN1* Kit†, which generates *SMN1* copy numbers with a turnaround time of less than three hours, is compatible with blood and buccal cells, includes automated analysis software, and demonstrates >99% agreement compared to other methods.

## Introduction

Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease caused by loss of *SMN1* gene function, and is the primary genetic cause of infant death (Stabley et al. 2015). SMA has an incidence of ~1/10,000 live births and a carrier rate of ~1/50. *SMN1* is typically detected at exon 7, which is absent in ~95% of patients with SMA (Prior et al. 2011). Carriers lack a functional *SMN1* copy on a single chromosome and often have one functional *SMN1* copy (1+0), though other carrier genotypes (2+0) are known to occur (Prior et al. 2011). Current ACOG carrier screening guidelines recommend offering SMA carrier screening to all women who are pregnant or are considering pregnancy. This broad recommendation requires rapid, accurate, and high-throughput *SMN1* copy number determination. Herein we report the performance of the AmplideX® PCR/CE *SMN1* Kit†, a quantitative PCR/Capillary Electrophoresis (CE) assay for research use that quantifies *SMN1* copy number.

## Methods

The AmplideX® PCR/CE *SMN1* Kit† is a multiplexed PCR assay used to amplify exon 7 of the *SMN1* gene along with an endogenous control (EC) gene from 20-80 ng of genomic DNA (gDNA) purified from blood or buccal samples. PCR products are represented by two dye-labeled amplicons (EC and *SMN1*) that are resolved by capillary electrophoresis using an Applied Biosystems™ 3130, 3730, or 3500 series Genetic Analyzer. Peak areas are used to calculate an area ratio of *SMN1* to EC and normalized using the included SMN Calibrator to automatically determine *SMN1* genotypes (0, 1, 2, 3, or >3 genomic *SMN1* copies) using the AmplideX® PCR/CE Reporter Software†. Where included, *FMR1* triplet repeat amplification was performed using the AmplideX® PCR/CE *FMR1* kit† (Asuragen) following the manufacturer's protocol. A total of 2 µL each of *FMR1* and *SMN1* PCR amplicons were then combined with 9 µL Hi-Di™ and 2 µL Asuragen ROX 1000 Ladder (15 µL total) and injected for 20 sec/2.5 kV and run for 40 minutes/19.5kV on a 3500xL Genetic Analyzer.


**Figure 1. Assay Workflow.** The workflow is streamlined from sample-to-answer. The assay can be performed in less than 3 hours with 45 minutes of total hands-on time. CE instrument time is for a single injection, or 24 samples using an Applied Biosystems™ 3500xL Genetic Analyzer.

## Results

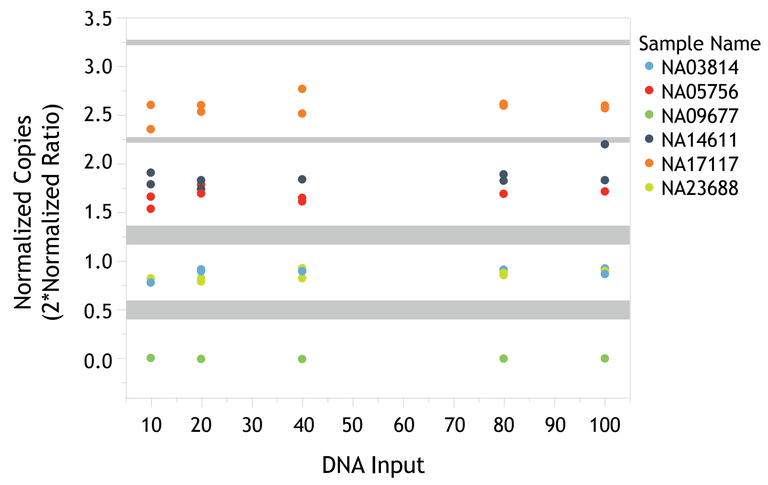



**Figure 2. DNA Input.** Normalized copy number across cell-line samples with 10-100 ng/reaction DNA input. White bars define *SMN1* copy number bins used to determine *SMN1* copy number. Samples were tested on a 3500xL Genetic Analyzer. 100% (60/60) of sample measurements produced expected copy number results on the 3500xL based on reference method measurements using an independent assay. Similar results were obtained using a 3730 and 3130xl Genetic Analyzer (data not shown).

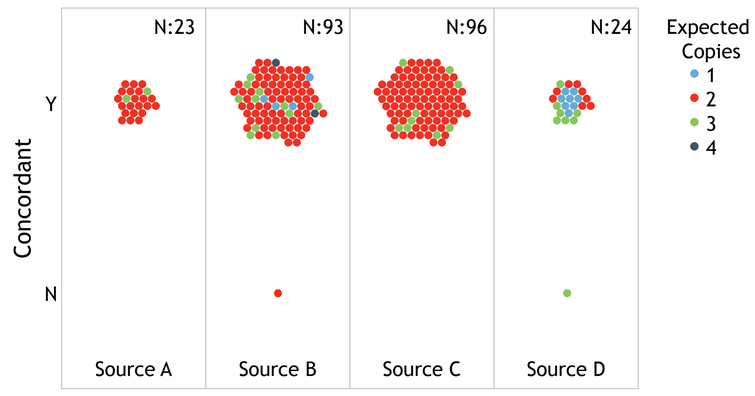
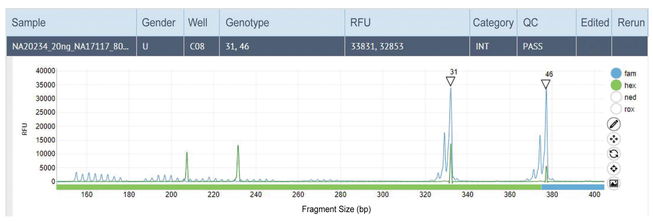



**Figure 3. Concordance of Clinical Samples.** 236 samples collected from four sources comprised of DNA purified from either blood or buccal samples using spin column, precipitation, or magnetic bead-based purification methods were tested. Expected *SMN1* copy numbers established by an independent comparator method ranged from 1 to >3 *SMN1* copies (shown here as 4 *SMN1* copies). A total of 99.2% (234/236) of sample measurements produced expected copy number results. Positive percent agreement for independently annotated single-copy *SMN1* samples was 100% (12/12), while the negative percent agreement was 99.6% (223/224).

***FMR1* Analysis**

20 ng *FMR1* input (31/46 repeats), 80 ng *SMN1* input (3 *SMN1* copies)




***SMN1* Analysis**

20 ng *SMN1* input (3 *SMN1* copies), 80 ng *FMR1* input (31/46 repeats)

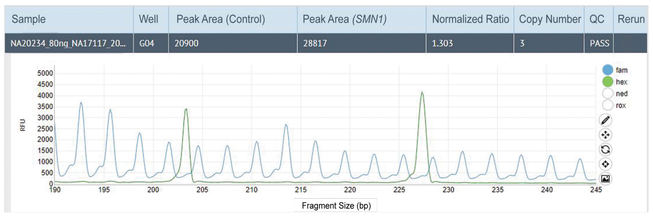



**Figure 4. Co-injection of *FMR1* and *SMN1*.** Samples were amplified via PCR using the AmplideX® PCR/CE *FMR1* kit† or *SMN1* kit† in separate PCR reactions using either 20 or 80 ng of DNA input and analyzed using the AmplideX® PCR/CE Reporter Software†. A total of four samples each for the *FMR1* and *SMN1* assays (along with appropriate calibrators and controls) were analyzed using both 20 ng and 80 ng DNA input combined in all possible combinations with the co-injection methodology, all of which (96/96) produced correct genotype results in both assays.

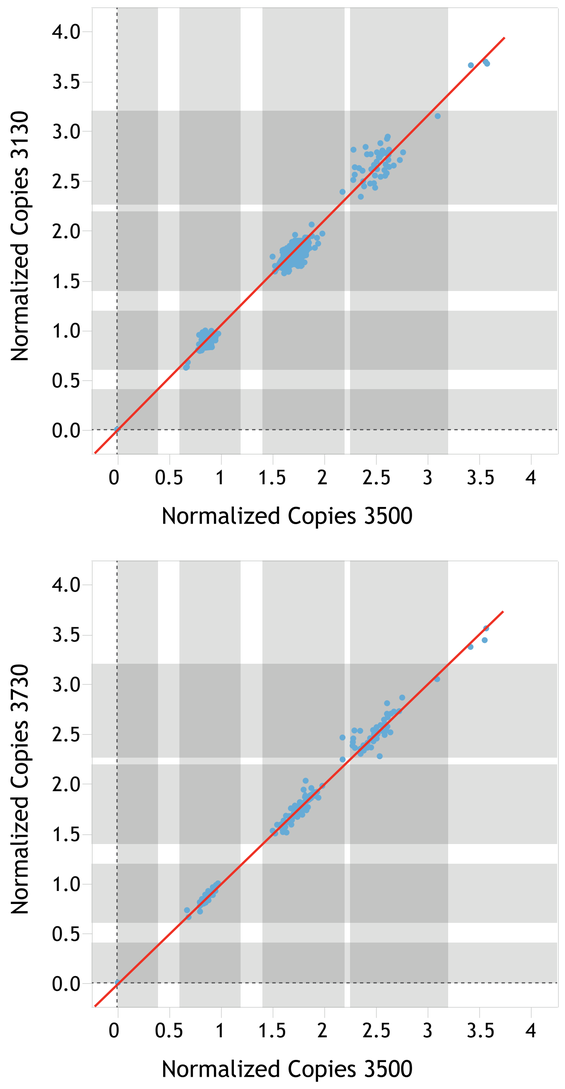



**Figure 5. Performance Comparison Between CE Platforms.** Over 200 sample measurements spanning all compatible DNA inputs, sample types, and *SMN1* copy number bins (0, 1, 2, 3, or >3) were analyzed on 3500xL, 3130xl, and 3730xl Genetic Analyzers to compare performance between platforms. For the 3130 vs. 3500 comparison, the fit equation was y= 1.062x – 0.023 with R2=0.995, with percent agreement of 99.6% (234/235). For the 3730 and 3500 comparison, the fit equation was y= 1.004x – 0.008 with R2=0.998, with percent agreement of 99.1% (233/235).

## Conclusions

- The assay supports an input range of 20-80 ng genomic DNA purified from whole blood or buccal swabs.
- The assay has an overall percent agreement of 99.2% (234/236), positive percent agreement for samples with 1 *SMN1* copy of 100% (12/12), and negative percent agreement of 99.6% (223/224) compared to independent methods.
- The assay can be co-injected with PCR products from the AmplideX® PCR/CE *FMR1* kit† to produce accurate results for both assays using a single software analysis tool.
- The assay has robust performance regardless of CE instrumentation, with over 99% agreement between the Applied Biosystems™ 3500, 3130, and 3730 Genetic Analyzers.

### References

1. Prior TW, Nagan N, Sugarman EA, Batish SD, and Braastad C. Technical standards and guidelines for spinal muscular atrophy testing. Genetics in Medicine. 2011;13(7): 686-694.
2. Stabley DL, Harris AW, Holbrook J, Chubbs NJ, Lozo KW, Crawford TO, Swoboda KJ, Funanage VL, Wang W, Mackenzie W, Scavina M, Sol-Church K, and Butchbach ME. *SMN1* and SMN2 copy numbers in cell lines derived from patients with spinal muscular atrophy as measured by array digital PCR. Molecular Genetics & Genomic Medicine. 2015; 3(4): 248-257.

**†Research Use only. Not for use in diagnostic procedures.**
**Presented at ASHG 2018**

Asuragen®
www.asuragen.com