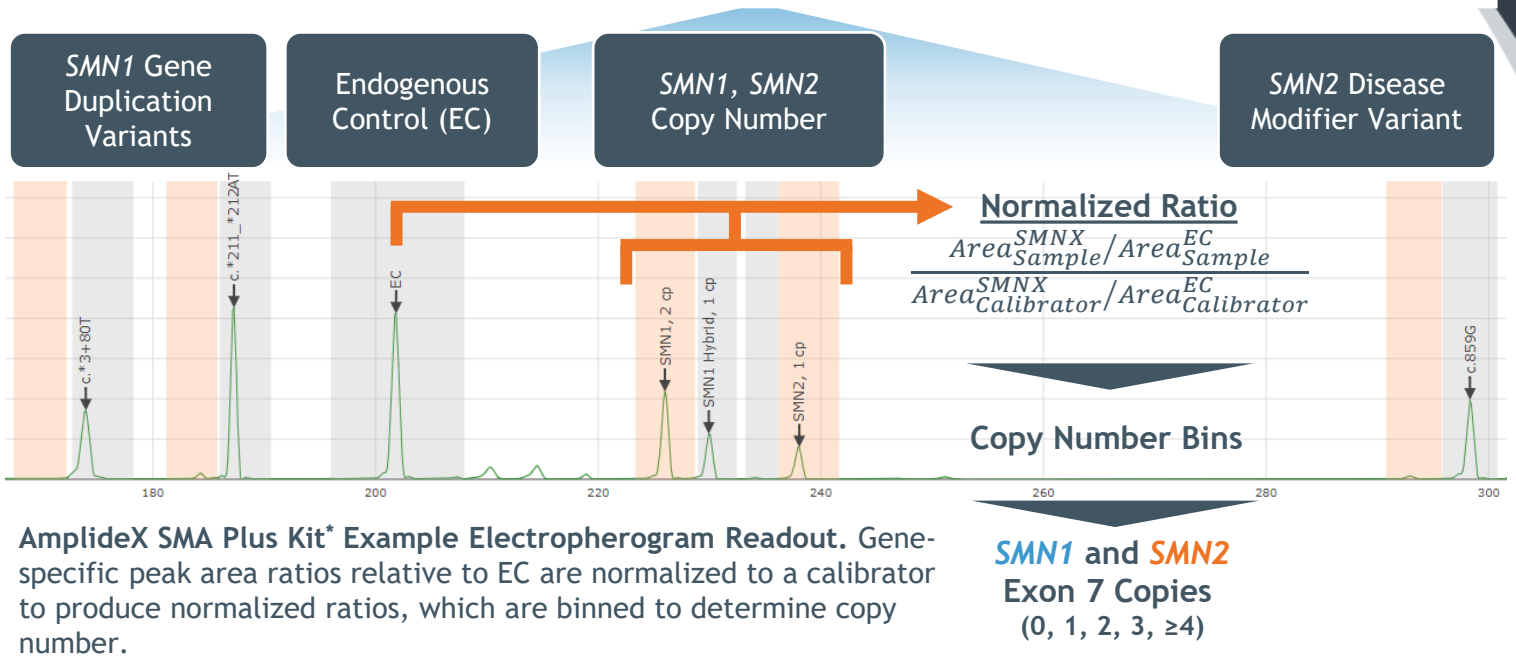
Analytical Validation of a Multiplex PCR/CE Assay for Simultaneous Determination of *SMN1/SMN2* Exon 7 Copy Number and Variant Status

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® SMA Plus Kit* is a one-tube PCR/CE assay that quantifies *SMN1* and *SMN2* copy numbers and genotypes gene duplication "silent carrier" markers (c.*3+80T>G and c.*211_*212del) and a disease modifier variant (*SMN2* c.859G>C).
- Includes reagents, controls, and automated, push-button data reporting via AmplideX Reporter software that reports results in under 5 minutes, with ~60 min of total hands-on time (<4 hours sample to result).
- We validated the kit by testing 122 unique samples to assess single-site precision, accuracy compared to reference methods, analytical specificity, DNA input range, reagent stability, and other factors



AmplideX SMA Plus Kit* Example Electropherogram Readout. Gene-specific peak area ratios relative to EC are normalized to a calibrator to produce normalized ratios, which are binned to determine copy number.

* CE-IVD under review.

AmplideX® SMA Plus Kit*

Complete testing solution offering comprehensive analysis of *SMN1* and *SMN2*

AmplideX®
SMA Plus Kit

Kit Components

2X PCR Mix	*SMN* Control
SMN1/2 HEX Primer Mix	Diluent
SMN Calibrator	ROX 1000 Size Ladder

REACTIONS PER KIT
50, 100

SAMPLE TYPES
Blood

DNA INPUT
20–70 ng SeqStudio/3500/3730 Genetic Analyzers
20-60 ng 3130 Genetic Analyzers

CE INSTRUMENTATION
Applied Biosystems® Genetic Analyzers

TIME-TO-RESULT
<4 HRS

HANDS-ON-TIME
60 MINS

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AmplideX® PCR/CE Reporter Software

Simple and streamlined sample reporting at the push of a button

ILLUSTRATIVE

FEATURES INCLUDE

- Quickly determine *SMN1* and *SMN2* copy number and detect silent carrier and disease modifier variants
- Batch-level QC and run metrics
- Project Management tool
- Export results in PNG and LIMS-compatible formats (Excel/CSV)

Comparison to Multiple Common Reference Methods Shows Consistent Copy Number Results for *SMN1* and *SMN2*

Measured *SMN1* Copy Number (AmplideX)	Consensus *SMN1* Copy Number (MLPA + ddPCR)						
	0	1	2	3	4	Sum
0	15	0	0	0	0	15
1	0	16	0	0	0	16
2	0	0	60	1	0	61
3	0	0	0	20	0	20
4	0	0	0	0	10	10
Sum	15	16	60	21	10	122

***SMN1*: 99.2%** (121/122)

Measured *SMN2* Copy Number (AmplideX)	Consensus *SMN2* Copy Number (MLPA + ddPCR)						
	0	1	2	3	4	Sum
0	25	0	0	0	0	25
1	0	40	0	0	0	40
2	0	0	33	0	0	33
3	0	0	1	15	0	16
4	0	0	0	1	6	7
Sum	25	40	34	16	6	121

***SMN2*: 98.3%** (119/121)

Target	Percent Agreement
c.*3+80T>G	100% (132/132)
c.*211_*212del	100% (118/118)
c.859G>C	100% (132/132)

- 134 DNA samples (116 unique specimens, 7 unique isolation methods) measured in singleton on a 3500xL Genetic Analyzer and analyzed with AmplideX Reporter Software
  - Precision QC failures (PR, n=4 for *SMN1* and *SMN2*) were excluded from analysis
- Two reference methods were used to measure *SMN1* and *SMN2* exon 7 copy numbers (MLPA and ddPCR)
  - Samples with disagreement or ambiguous results in either method were excluded from analysis
- **Results were over 98% concordant to reference results for both *SMN1* and *SMN2***
  - Fewer QC failures observed in AmplideX® SMA Plus Kit* than in ddPCR, which caused majority of ambiguous reference method results (n=6 of 9 for *SMN1*, n=8 of 10 for *SMN2*)

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Single-site Precision Shows Low Variability Across Multiple Runs, Days, Reagent Lots, and Operators

Sample	Sample Type	*SMN1* Copies	*SMN1* Copy Number Percent Agreement	*SMN1* %CV (NR)	*SMN2* Copies	*SMN2* Copy Number Percent Agreement	*SMN2* %CV (NR)
1	Blood	0	100% (40/40)	NA	2	100% (40/40)	8.0
2	Blood	1*	97.5% (39/40)	5.8*	3	96.8% (30/31)	5.8
3	Blood	1	100% (40/40)	6.3	4	100% (38/38)	5.8
4	Blood	4	100% (40/40)	4.5	1	100% (40/40)	6.3
5	Cell Line	0	100% (40/40)	NA	2	100% (40/40)	5.4
6	Cell Line	1	100% (40/40)	6.7	3	97.4% (37/38)	6.9
7	Cell Line	3	100% (38/38)	5.6	0	100% (39/39)	NA
8	Cell Line	2	100% (40/40)	7.9	2	100% (40/40)	8.4
9	Cell Line	2	100% (40/40)	6.9	2	100% (40/40)	5.8
		Total:	**99.7% (357/358)**		**Total:**	**99.4% (344/346)**	

*copy number and NR measurements based on *SMN1* hybrid

Target	Percent Agreement
c.*3+80T>G	100% (279/279)
c.*211_*212del	100% (279/279)
c.859G>C	100% (279/279)

- Study consisted of 9 DNA samples tested in duplicate in 20 batch runs over 10 days using two operators, three reagent lots, two thermal cyclers on a 3500xL Genetic Analyzer
  - QC failures were excluded from analysis
- Percent CV for normalized ratios (NR) and percent agreement with the mode were calculated by sample for both *SMN1* and *SMN2*
- **Across all measurements, copy numbers were over 99% concordant for both *SMN1* and *SMN2***

* CE-IVD under review.

Results Are Consistent Across Full DNA Input Range Between Reagent Lots and CE instruments for All Relevant Genotypes

DNA Input	*SMN1* Copy Number Percent Agreement	*SMN2* Copy Number Percent Agreement
10	96.8% (121/125)	100% (123/123)
20	100% (128/128)	100% (128/128)
50	99.2% (126/127)	98.4% (124/126)
60	99.2% (123/124)	98.4% (124/126)
70	100% (124/124)	98.3% (118/120)
80	100% (89/89)	100% (95/95)
Total	**99.2% (711/717)**	**99.2% (712/718)**

Target	Percent Agreement
c.*3+80T>G	100% (735/735)
c.*211_*212del	100% (735/735)
c.859G>C	100% (735/735)

- Study consisted of 8 DNA samples tested in duplicate at 10, 20, 50, 60, 70, and 80 ng total DNA input using two reagent lots on the following Genetic Analyzers: 3500xL, 3730*xl*, 3130*xl*, and SeqStudio
- Sample panel covered 0, 1, 2, 3 copies of *SMN1*, 0, 1, 2, 3, ≥4 copies of *SMN2*
- Assay DNA Input range: 20-70 ng (3500xL, 3730*xl*, SeqStudio), 20-60 ng (3130*xl*)
    - QC failures (all instruments) and 80 ng measurements (3130*xl* only; above intended input range) were excluded from analysis
- Percent agreement with expected copy number results were calculated for each DNA input for both *SMN1* and *SMN2*
- **Across all measurements, copy numbers were over 99% concordant for both *SMN1* and *SMN2***

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Overall Performance and Conclusions

CE Platform	*SMN1* Copy Number Percent Agreement	*SMN2* Copy Number Percent Agreement	c.*3+80T>G Percent Agreement	c.*211_*212del Percent Agreement	c.859G>C Percent Agreement
3500xL	99.5% (739/743)	99.2% (723/729)	100% (781/781)	100% (767/767)	99.9% (780/781)
3730*xl*	100% (165/165)	100% (161/161)	100% (191/191)	100% (191/191)	100% (191/191)
3130*xl*	97.5% (154/158)	99.3% (152/153)	100% (191/191)	100% (191/191)	100% (191/191)
SeqStudio	100% (167/167)	100% (168/168)	100% (192/192)	100% (192/192)	100% (192/192)
Total	**99.4% (1225/1233)**	**99.4% (1204/1211)**	**100% (1355/1355)**	**100% (1341/1341)**	**99.9% (1354/1355)**

- Across all analytical validation studies, percent agreement was over 99% for *SMN1* and *SMN2* copy numbers and all variants detected
  - Including studies shown here as well as reagent stability and thermal cycler equivalency studies not shown
  - Consensus between ddPCR and MLPA as reference values for *SMN1* and *SMN2*, Sanger sequencing as reference for variants
- Analytical specificity (exclusivity) from method comparison, precision, and DNA input studies for samples with 0 copies of *SMN1* or *SMN2* and for all variants detected by the assay was 100%
- The expanded content of the AmplideX SMA Plus Kit* combined with its convenience and versatility may provide an all-in-one option for laboratories interested in SMA carrier screening and diagnostics.
- Visit https://asuragen.com/eshg-20/ to download a copy of this presentation

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