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Technical Note

Performance and Compatibility of the Promega Spectrum Compact CE System for Performing AmplideX® Gene Variant Analysis

Summary

Genetic assay adoption by research and diagnostic laboratories depends on demonstrated equivalent results when performed on diverse instrumentation platforms employed across testing facilities. The AmplideX® PCR/CE family of genetic tests interrogates genomic DNA for a variety of pathogenic gene variants for which accurate analysis can be challenging. The Promega Spectrum Compact demonstrated comparable performance and excellent agreement with the Applied Biosystems 3500 DX CE platform when used to run four different AmplideX assay kits.

Key Points

- AmplideX® PCR/CE technology allows detection of difficult-to-resolve pathogenic variants, including triplet and hexanucleotide repeats, other STRs, SNVs, INDELs, and CNVs.
- Medical genetic test adoption by research and diagnostic laboratories necessitates reliable agreement in assay results across diverse instrumentation platforms.
- The Promega Spectrum Compact and Applied Biosystems 3500 Dx capillary electrophoresis (CE) systems, in combination with AmplideX PCR/CE family of genetic tests and AmplideX PCR/CE Reporter analysis software, demonstrated excellent agreement for *C9orf72*, *CFTR*, *FMR1,* and *SMN1/2* pathogenic variant identification and/or categorization.
- Concordant results from the Spectrum Compact and 3500 Dx CE systems, when interrogating multiple challenging genes using AmplideX PCR/CE family of genetic tests and AmplideX PCR/CE Reporter analysis software, indicates that these assays are amenable to consistent and accurate performance across labs with different CE instrumentation.
- The economical, user-friendly, and space-saving characteristics of the streamlined Spectrum Compact CE system might be attractive to laboratories that perform high-accuracy medium-throughput gene analysis.

Background

Molecular genetic assays demand accurate and reproducible performance when run on the diverse instrumentation platforms used in research and diagnostic laboratories worldwide. The Asuragen AmplideX® PCR/CE family of genetic tests are robust inherited disease testing tools that use capillary electrophoresis (CE) technology to resolve, analyze, and report gene sequence information contained in PCR-amplified fragments derived from genomic DNA. The AmplideX PCR/CE family of genetic tests analyze difficult gene targets including *C9orf72*, *CFTR*, *FMR1*, and *SMN1/2*,[1-4] which present a variety of challenges such as structural variation, pseudogenes, and GC-rich repeats.[5,6]

Advances in CE instrument design continue to improve user-friendliness and expand their suitability for easy adoption and operation in diverse laboratory settings. The Spectrum Compact CE System (Promega Corp., Madison, WI) may permit broader access to affordable CE technology for analyzing difficult gene targets.[7] The reduced-footprint Spectrum Compact unit has an intuitive guided user touchscreen interface that facilitates straightforward operation and maintenance regardless of experience level. Spectrum Compact CE analysis is streamlined by the availability of convenient prefilled consumables, including capillary arrays and anode & cathode buffer tanks. The Spectrum Compact is compatible with existing Sanger sequencing chemistries and fragment analysis using common 4-, 5-, 6-, and 8-dye STR kits available from Promega and other vendors.

It is essential that a DNA sample yields the same genotype when run across different CE systems that may exist in different laboratories. We compared analytical performance of the Promega Spectrum Compact versus the Applied Biosystem® 3500 Dx CE instrument[8] (Thermo Fisher Scientific, Waltham, MA) when interrogating ≈100 genomic DNA samples with four widely used inherited-disease assays from the AmplideX PCR/CE family of genetic tests. We demonstrate the concordant identification and classification of a broad range of challenging-to-resolve pathogenic variants between the Spectrum Compact CE System and the 3500 Dx platform.

Methods

***Overview*:** Genomic DNA samples were PCR-amplified using AmplideX PCR/CE family of genetic tests (*C9orf72*†, *CFTR*†, *FMR1*†,‡, and *SMN1/2* Plus†/*SMA* Plus‡), followed by CE resolution on both the Promega Spectrum Compact CE System† (Spectrum Compact) and the Applied Biosystems 3500 Dx (3500 Dx) Genetic Analyzer for direct comparison. Final assay results were obtained via automated analysis of CE electropherogram outputs with AmplideX Reporter.

Samples: Approximately 100 DNA samples were obtained from newborn dried blood spot (DBS; for *CFTR* only), adult peripheral venous blood, commercially available cell lines, and plasmid mixes (*CFTR* only). Samples were selected to cover all genotype categories including normal, intermediate, premutation and full mutation for *FMR1*; normal, intermediate and expanded for *C9orf72*; SNP, STR and INDEL variants for *CFTR*; 0 to ≥4 copy number variants for *SMN1* and *SMN2*, and 3 unique variants of *SMN1/2* related to carrier risk or disease prognosis.

PCR/CE and Analysis: Genomic DNA samples were amplified by PCR using AmplideX reagents, per assay instructions, followed by CE on both the 3500 Dx and the Spectrum Compact systems for direct comparison. Notable characteristics of both CE instruments are presented in Table 1. In a preliminary study,[10] the run parameters for both CE units were optimized to obtain expected peak morphology and resolution for each assay. Injection and run conditions for the Spectrum Compact instrument are shown in Table 2. Spatial and spectral calibrations were performed in accordance with the respective operating manual.[11,12] Both instruments were calibrated using a DS-30 matrix dye standard per assay instructions.[13] Final assay results were generated via analysis of CE electropherograms with AmplideX Reporter. Runs that were identified as QC failures by AmplideX Reporter were noted but excluded from analysis.

†For research use only. Not for use in diagnostic procedures.
‡CE-IVD. For US export only.

Table 1. Characteristics of the Promega Spectrum Compact and Applied Biosystems 3500 Dx CE instruments.

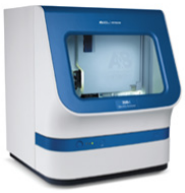
	CE System	
Parameter	Spectrum Compact 	3500 Dx 
Capillaries, n	4	8 standard, 24 available
Array length	36 cm	36 and 50 cm
Sample capacity, n	32 (4 × 8-well strips)	32 (strips), 96- and 384-well plates
Throughput level	Moderate	Moderate-to-High
Polymers available	Polymer4, Polymer7	POP-4, POP-6, POP-7
Resolution	1 bp	1 bp
Footprint	40 cm W × 60 cm D (60 cm H)	61 cm W × 61 cm D (72 cm H)
Weight	41 kg	82 kg
Controller	Integrated touchscreen	External PC (vendor supplied)
Consumables tracking	2D barcode scanning	RFID

Table 2. Optimized AmplideX run parameters with the Spectrum Compact CE system.

AmplideX Assay	Analysis Module Version	Run Time*	Run Voltage	Injection Time	Injection Voltage
CFTR	3.0.4	1500 s	13 kV	20 s	2.5 kV
SMN1/2 Plus	1.1.5	1500 s	15 kV	20 s	2.5 kV
FMR1	2.0.1, 3.0.5	1500 s	18 kV	20 s	2.5 kV
C9orf72	1.0.1	1500 s	13 kV	20 s	1.6 kV

*For the 3500 Dx, run time was 1500 sec for *CFTR* and *SMN1/2*, and 2400 sec for *C9orf 72* and *FMR1*. Polymer7 was used in all Spectrum Compact runs (Promega CE2404).

Results

CE System Differences: The Spectrum Compact CE system has a footprint that is 36% smaller than the 3500 Dx and a height that is 17% lower (**Table 1**), which are attractive advantages for laboratories with space constraints. The Spectrum Compact is controlled using an integrated touchscreen and, unlike the 3500 Dx, does not require an auxiliary PC connection for instrument operation. Additionally, at 41 kg (90 lbs), the Spectrum Compact is half the weight versus the 3500 Dx. Whereas the 3500 Dx has a higher throughput capacity than the Spectrum Compact, this surplus capability may be excessive and underutilized by laboratories that have only moderate-throughput sample processing requirements. Optimized electrophoresis run times (**Table 2**) on the Spectrum Compact were 1500 s (25 min) for all four AmplideX kits that were tested; with the 3500 Dx, *CFTR* and *SMN1/2* analysis also used 1500 s runs, while optimal run times for *C9orf72* and *FMR1* analysis were 60% longer at 2400 s (40 min). In terms of instrument maintenance, replacement of consumables and capillary arrays on the Spectrum Compact is easy and straightforward, and the rigid structure of the capillary makes it less prone to accidental damage during installation compared to older CE instrument models. Beyond consumable replacement, instrument maintenance requirements are minimal. These features make the Spectrum Compact accessible for new users less familiar with CE instruments and simplifies training and maintenance for experienced labs.

C9orf72 Overview: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are proteinopathic neurodegenerative diseases linked to aberrantly expanded hexanucleotide repeat GGGGCC (G4C2) sequences in a non-coding region of the *C9orf72* (*chromosome 9 open reading frame 72*) gene.[14] Normal individuals carry 2–10 G4C2 repeats.

C9orf72 Genotype Categorization: The AmplideX *C9orf72* PCR/CE Kit assay categorizes DNA samples into three groups (Normal, Intermediate, and Expanded) based on the number of G4C2 repeats (≤19, 20–30, and >30, respectively) detected in *C9orf72*.[1] Through 110 total repeat category calls made using 46 unique samples, the Spectrum Compact showed 99.1% agreement with the 3500 Dx output (**Table 3**). The single discrepancy occurred when a noise peak was incorrectly called a 23-repeat peak on data generated by the 3500 Dx. The genotype category agreement between the Spectrum Compact and 3500 Dx systems is 100% if the erroneous call with the 3500 Dx is excluded.

Table 3. *C9orf72* genotype category agreement between the Spectrum Compact and 3500 Dx CE systems.

		3500 Dx		
	C9orf72	Normal	Intermediate	Expanded
Spectrum Compact	Normal	21	1†	0
	Intermediate	0	35	0
	Expanded	0	0	53

†Discrepancy due to false positive call of noise peak on 3500 Dx output.

C9orf72 G4C2 Repeat Sizing: Because the *C9orf72*-associated disease phenotype is aligned with the number of G4C2 repeat sequences present,[14] accurate repeat size determination is essential. There was excellent overall concordance in repeat size determination between data generated on the Spectrum Compact and the 3500 Dx (**Figure 1**). Although expanded G4C2 variants are not clearly visible on *C9orf72* electropherograms as pileup peaks with the Spectrum Compact (**Figure 2**), they are accurately identified and genotyped due to the presence of the characteristic stutter pattern caused by the repeat-binding primer in the expanded repeat size region.

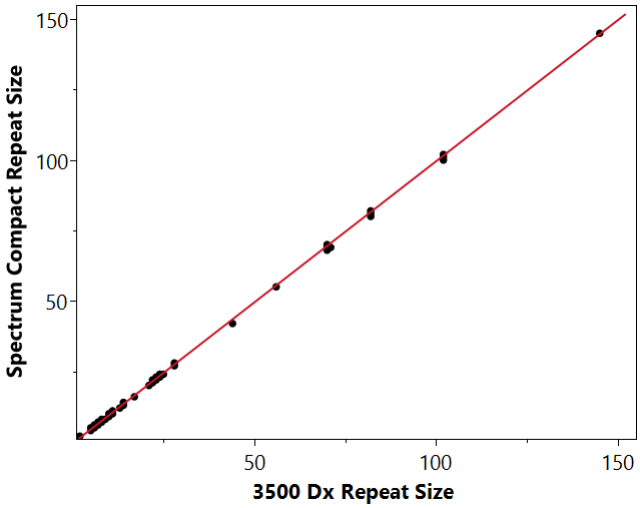


Figure 1. Agreement in *C9orf72* hexanucleotide G4C2 repeat sizing between the Spectrum Compact and 3500 Dx CE systems. Logistic regression shows high concordance between repeat size determined by both CE platforms (N=110), with r^2=1.000 and y = 1.002(x) – 0.5987. Identified full mutations with repeat sizes greater than the assay sizing limit (145 repeats) assigned value 145 for the logistic regression.

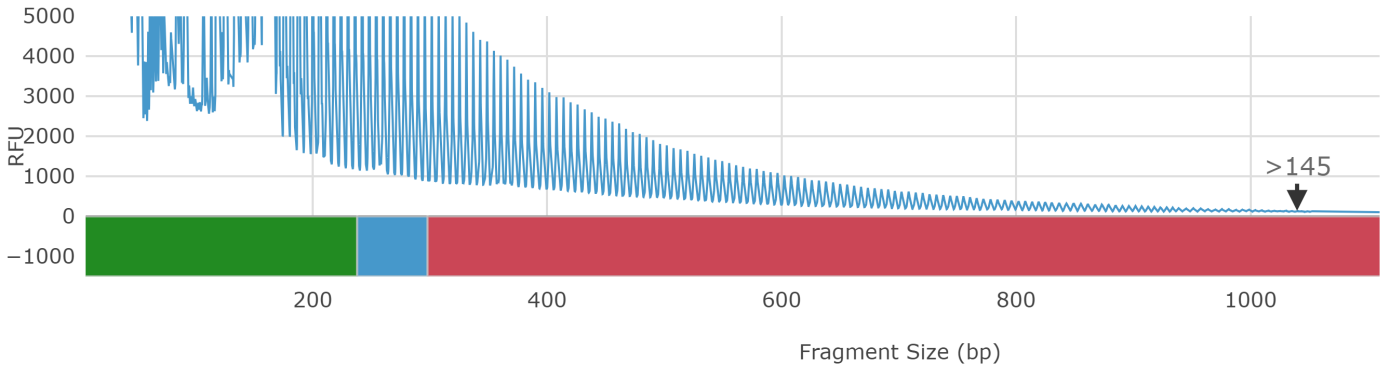


Figure 2. *C9orf72* expanded mutations are not clearly visible as pileup peaks on Spectrum Compact electropherograms but are correctly sized using repeat stutter.

CFTR Overview: Cystic fibrosis is a potentially lethal autosomal recessive disease characterized by severe impairment of the respiratory system and digestive tract.[15] More than 400 pathogenic variants of cystic fibrosis transmembrane conductance regulator (*CFTR*) gene are known.[16] Carrier frequency in the U.S. for CF-associated *CFTR* variants ranges from ≈1:30 in non-Hispanic whites to ≈1:90 in Asians.[17] The AmplideX *CFTR* PCR/CE Kit assay evaluates 65 disease–causing *CFTR* variants, providing 92% coverage for the U.S. populace and an estimated 95% coverage worldwide.[2,18]

Samples for *CFTR* analysis included 28 whole or surrogate blood specimens, 32 DBS or surrogate specimens, and 4 plasmid mixes[10] which together covered all 65 kit-assessed pathogenic variants. PCR was performed on the GeneAmp 9700 (Applied Biosystems, gold-plated sample block), VeritiPro (Applied Biosystems), and/or C1000 (Bio-Rad) thermal cyclers.

CFTR Variant-Level Genotyping: *CFTR* variant-level metrics were in good agreement between the Spectrum Compact and 3500 Dx CE systems (**Table 4**). Each sample measurement generates 63 unique variant calls. PolyT/TG agreement was evaluated separately. All incorrect results impacting PPV were tested using 60 ng input on the GeneAmp 9700 thermal cycler, while the same samples tested at 20 ng were concordant. This suggests that lower inputs (20-30 ng total DNA input) may be better suited for this thermal cycler when analyzed with the Spectrum Compact. For PolyT/TG concordance, all PolyT results were 100% correct; incorrect results were all due to mis-sizing of the PolyTG tract, for which the Spectrum Compact consistently sized repeats as N-1 compared to the 3500 DX. For this reason, Spectrum Compact users may wish to turn off PolyTG reporting when using this assay.

Table 4. *CFTR* Variant-Level Metrics on Spectrum Compact versus 3500 Dx CE systems.

Measure	N	Percent Concordant
PPV	139	97.1%*
PPA	139	97.1% (99.3%†)
NPA	5670	99.9%
OPA	5809	99.9%
PAz	5809	99.9%
PA_{TTG}	188	75.5%

Abbreviations: PPV = positive predictive value, PPA = positive percent agreement, NPA = negative percent agreement, OPA = overall percent agreement, PA_z = zygosity agreement. PA_{TTG} = percent agreement PolyT/TG.

*For PPV, 4/4 false positives with Spectrum Compact were on a single thermal cycler model (Applied Biosciences GeneAmp 9700) at high input (60 ng); this exceeds the recommended 20–30 ng DNA input for Spectrum Compact with this cycler.
†For PPA, 3/4 false negatives with Spectrum Compact were due to erroneous positive calls on the 3500 Dx instrument. PPA would be 99.3% excluding the incorrect 3500 Dx results.

FMR1/Fragile X Syndrome Overview: Fragile X syndrome is an inherited CNS disorder caused by an expansion of the CGG triplet repeat within the *FMR1* (*fragile X messenger ribonucleoprotein 1*) gene on the X chromosome, which causes methylation-dependent *FMR1* promoter silencing.[19] This repeat size variation results in a deficiency of the downstream protein (FMRP) that is responsible for normal synaptic development and architecture. Normally, there are between 5–44 CGG repeats; fragile X syndrome occurs with >200 repeats.[19] Triplet copy numbers ranging between 45–54 repeats are considered an 'intermediate' grey zone, and 55–200 repeats are considered 'premutation'; the risk of women having children with fragile X syndrome or associated disorders rises with increased repeat sizing.[20] The AmplideX *FMR1* PCR/CE Kit reagents are designed to identify and accurately size CGG repeat alleles up to 200 repeats, identify all allele expansions including low-abundance full mutation size mosaics up to 1300 CGG repeats, and accurately resolve female *FMR1* zygosity.[3]

Samples for *FMR1* analysis included 28 whole blood samples and two controls, each run 1–5 times using both CE platforms.

FMR1 Genotype Categorization: The AmplideX *FMR1* PCR/CE Kit categorizes DNA samples into four groups (Normal, Intermediate, Premutation, and Full Mutation) based on the number of CGG sequences detected in *FMR1*.[3]

Across 110 total repeat category calls made using 46 unique samples, the Spectrum Compact showed 95.5% agreement (105/110) with 3500 Dx output (**Table 5**). The 5 discrepancies were all due to small differences in repeat size determinations on the Spectrum Compact compared to the 3500 Dx; 4 at the 45-CGG repeat boundary between normal and intermediate categories per EMQN Guidelines[20] (44 repeats identified on the Spectrum Compact versus 45 repeats on the 3500 Dx), and one at the 200-repeat boundary between Premutation and Full Mutation (199 repeats on the Spectrum Compact versus >200 repeats on the 3500 Dx). All five discrepancies were within precision allowance when considering EMQN-allowable size tolerances (±5% of repeat size)[20] and performance claims (±1 repeat for <70 CGG repeats, ±5% of repeat size for repeats >120).[21]

Table 5. *FMR1* genotype category agreement between the Spectrum Compact and 3500 Dx CE systems.

		3500 Dx			
	FMR1	Normal	Intermediate	Premutation	Full Mutation
Spectrum Compact	Normal	26	4†	0	0
	Intermediate	0	15	0	0
	Premutation	0	0	23	1††
	Full Mutation	0	0	0	41

Note: All discrepancies (n=5) due to repeat size differences on the Spectrum Compact versus the 3500 Dx were within EMQN precision tolerances and AmplideX FMR1 PCR/CE Kit performance claims.
†4 ±1 CGG repeat size discrepancies at Normal/Intermediate boundary (45 repeats). ††±5% CGG repeat size discrepancy at Premutation/Full Mutation boundary (200 repeats).

FMR1 Repeat Sizing: Because the risk of Fragile X and associated disorders is dependent on the number of repeat CGG sequences present in *FMR1*,[20] accurate repeat size determination is essential. There was excellent overall concordance between repeat sizing determined by the Spectrum Compact and 3500 Dx instruments (**Figure 3**).

FMR1 Large Repeat Resolution: Excellent resolution of full-mutation *FMR1* alleles is achievable with the Spectrum Compact CE System (**Figure 4**).

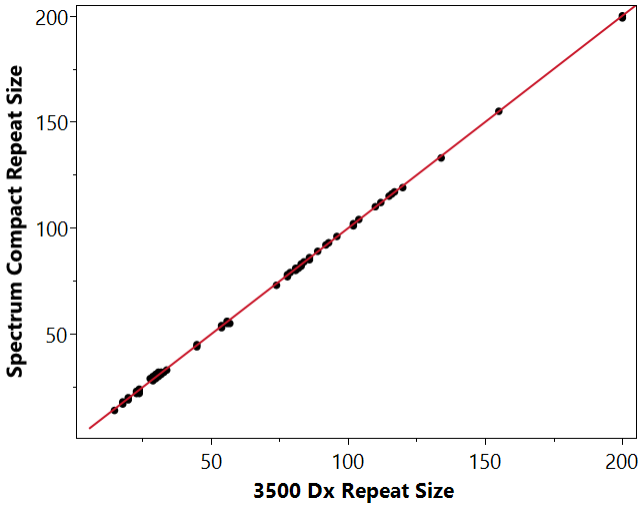


Figure 3. Agreement in *FMR1* trinucleotide CGG repeat size determined by the Spectrum Compact and 3500 Dx CE systems. Logistic regression shows high concordance between repeat size determined by both CE platforms (N=110), with r^2=1.000 and y = 1.002(x) – 0.355. Identified full mutations with repeat sizes greater than the assay sizing limit (200 repeats) assigned value 200 for the logistic regression.

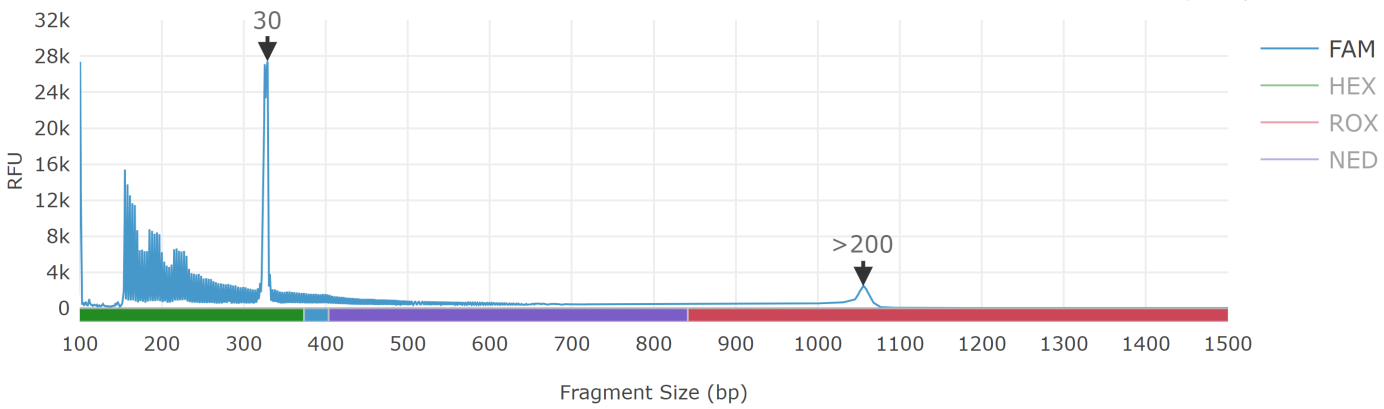


Figure 4. *FMR1* Full Mutation alleles are easily resolved on the Spectrum Compact CE system as pileup peaks at the expected repeat size.

SMN1/2 Overview: Copy number variations in *SMN1* and its paralog *SMN2* (both encoding survival motor neuron protein) are associated with the onset and severity of spinal muscular atrophy (SMA).[22] Carrier risk and disease severity may also be impacted by the presence of *SMN1/2* gene duplication variants and a disease modifier variant in *SMN2*, respectively.[23] The AmplideX PCR/CE *SMN1/2* Plus Kit assay identifies copy number variations for both *SMN1* and *SMN2*, quantifies *SMN1* exon 7 copy number, and detects relevant *SMN2* variants and gene hybrids, in a single reaction.[4]

Samples for *SMN1/2* analysis included 30 whole blood samples, with PCR performed on three thermal cyclers (Veriti, VeritiPro, and C1000), for a total of 1–9 runs on both CE platforms.

SMN1 and SMN2 Exon 7 Copy Number Agreement: Overall *SMN1* concordance was excellent at 98.9% (90/91 calls) between the Spectrum Compact and 3500 Dx systems (**Table 6**). The single discordant call occurred when the 3500 Dx identified an *SMN1* copy number as 3 instead of the expected value of 2. This sample had an endogenous control peak with <1000 RFU, indicating poor amplification that likely impacted the result.

Table 6. *SMN1* exon 7 copy number agreement between Spectrum Compact and 3500 Dx CE systems.

		3500 Dx				
	SMN1 Copy Number	0	1	2	3	4
Spectrum Compact	0	8	0	0	0	0
	1	0	10	0	0	0
	2	0	0	36	1†	0
	3	0	0	0	16	0
	4	0	0	0	0	20

†Single discordant call due to incorrect copy number determination on 3500 Dx.

Overall *SMN2* concordance was 97.7% (86/88 calls) between the two CE systems (**Table 7**). Both discordant calls occurred with the same sample and were due to inaccurate *SMN2* calls on the 3500 Dx. This same sample also generated the single *SMN1* miss with the 3500 Dx (**Table 6**), which had an endogenous control peak with <1000 RFU, indicating poor amplification that likely impacted the result.

Table 7. *SMN2* exon 7 copy number agreement between Spectrum Compact and 3500 Dx CE systems.

		3500 Dx				
	SMN2 Copy Number	0	1	2	3	4
Spectrum Compact	0	14	0	0	0	0
	1	0	13	2†	0	0
	2	0	0	30	0	0
	3	0	0	0	13	0
	4	0	0	0	0	16

†Both discrepancies due to incorrect copy number determination on 3500 Dx.

With the 3500 Dx, 92.0% of samples passed QC for *SMN1*; with the Spectrum Compact, 96.4% passed QC for *SMN1*. With the 3500 Dx, 92.0% of samples passed QC for *SMN2*; with the Spectrum Compact, 95.5% passed QC for *SMN2*. Approximately 20% of samples analyzed on the Spectrum Compact were flagged by AmplideX Reporter as "at risk" due to shouldering peak morphology for both *SMN1* and *SMN2*; however, this did not impact accurate *SMN1* and *SMN2* exon 7 copy number determination with the Spectrum Compact (**Figure 5**). This is likely due to the shoulders present in the wildtype c*3+80T peak (leftmost peak, **Figure 5**).

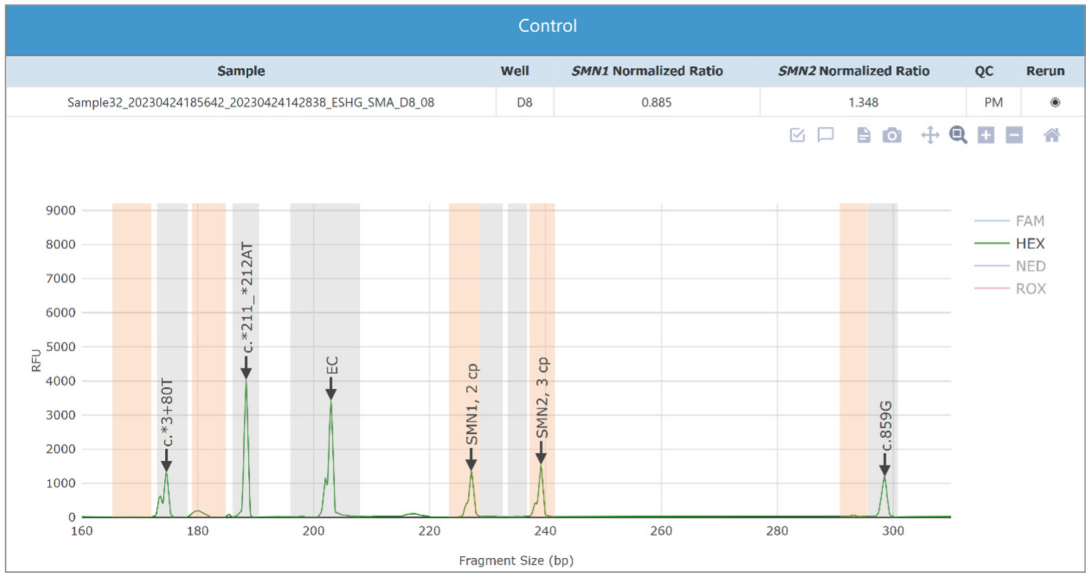


Figure 5. Shouldering peak morphology observed on the Spectrum Compact CE. The *SMN1* and *SMN2* peaks of some samples were flagged by the AmplideX Reporter as "at risk" but this did not interfere with accurate exon 7 copy number categorization and reporting. RFU = relative fluorescence units; EC = endogenous control.

SMN1/2 SNP Agreement: For *SMN1* SNPs associated with SMA and related pathologies, agreement between the Spectrum Compact and 3500 Dx systems was high (Table 8). For the *SMN1* duplication markers, agreement was 98.0% (97/99) for c.*3+80T>G and 99.0% (98/99) for c.*211_*212del. With c*3+80T>G, the two discordant calls were due to false negative calls on the Spectrum Compact. With c.*211_*212del, the single discordant call was due to a false positive on the Spectrum Compact. For the prognostic *SMN2* disease modifier variant c.859G>C, agreement was 99.0% (98/99); the single discordant call was from a false negative call on the 3500 Dx.

Table 8. Agreement in detecting *SMN1* and *SMN2* pathological single-nucleotide polymorphisms.

	3500 Dx			
Spectrum Compact	c.*3+80T>G	Negative	Positive	**Agreement**
	Negative	76	2	**98.0%**
	Positive	0	21	
	c.*211_*212del	Negative	Positive	
	Negative	74	0	**99.0%**
	Positive	1	24	
	c.859G>C	Negative	Positive	
	Negative	74	0	**99.0%**
	Positive	1	24	

Summary

The Spectrum Compact CE system demonstrated high concordance with the Applied Bioscience 3500 Dx instrument in reliably identifying and reporting diverse challenging genes in conjunction with the AmplideX PCR/CE family of genetic tests that deliver accurate sample-to-answer results within 4–6 hours. With *C9orf72*, there was >99% genotyping concordance between the Spectrum Compact and 3500 Dx, which would have been 100% if not for a single inaccurate call on the 3500 Dx. With *CFTR* variant-level genotyping, the Spectrum Compact offered >97% positive predictive value, and the few false positives generated by Spectrum Compact were attributed to sample overloading. *CFTR* positive percent agreement was 97.1% and would have been 99.3% if not for 3 false-positive calls on the 3500 Dx. There was 100% agreement with *FMR1* genotype categorization between the Spectrum Compact and 3500 Dx with allowable repeat sizing precision, and all discrepancies were due to small differences in repeat sizes at category boundaries. In general, resolution and sizing of large *FMR1* repeats was highly concordant between the two CE systems. With *SMN1*, exon 7 copy number resolution agreed in nearly 99% of calls, with the single mismatch attributed to an erroneous call from the 3500 Dx. Similarly, with *SMN2*, copy number resolution agreed nearly 98% of the time, with the two mismatches due to inaccurate calls on the 3500 Dx. All missed calls for both *SMN1* and *SMN2* were attributable to a single sample with low endogenous control peak height (<1000 RFU), indicative of poor amplification that likely impacted quantification. While a peak morphology flag was issued on approximately 20% of *SMN1/2* runs on the Spectrum Compact unit due to shouldering, this had no impact on call accuracy. *SMN1/2* genotyping agreement was 98–99% between the two CE platforms. Overall, the Spectrum Compact and 3500 Dx systems performed with high precision and in excellent concordance when analyzing diverse and challenging-to-resolve gene targets.

Conclusion

The Spectrum Compact CE system is a high-precision, affordable, small-footprint, user-friendly CE option that reliably interrogates genomic DNA for detection of difficult-to-resolve pathogenic variants in the *C9orf72*, *CFTR*, *FMR1*, and *SMN1/2* genes when coupled with AmplideX PCR/CE family of genetic tests and AmplideX PCR/CE Reporter analysis software. The Spectrum Compact platform is an attractive option for users who demand reproducibly accurate CE performance with high resolution, require medium-throughput capabilities, and are migrating from older CE models or are contemplating first purchase of a reliable, intuitive, and economical CE system.

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