# Evaluation of the AmplideX® SMA Plus Kit for comprehensive *SMN1/SMN2* analysis in Spinal Muscular Atrophy

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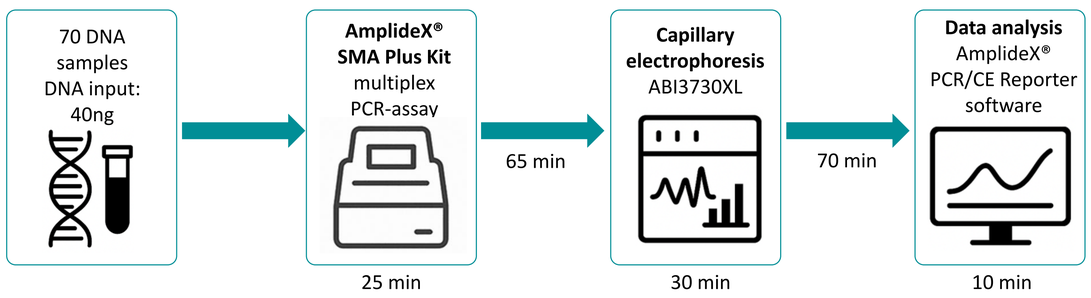
## Objectives |

- Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by progressive muscle weakness and atrophy caused by copy number variations or mutations in the *SMN1* gene. Disease severity is modulated by the number of *SMN2* gene copies.
- Recent studies have highlighted the potential influence of specific modifiers: the *SMN2* c.859G>C variant, which is associated with a milder phenotype, and the *SMN1* variants c.*3+80T>G and c.*211_*212del, which are linked to *SMN1* duplication and silent carrier status. However, the clinical significance of these *SMN1* variants is ethnicity-dependent, particularly important in Asian, Spanish and Ashkenazi Jewish populations.
- Accurate and fast molecular analysis is essential for confirming diagnosis in symptomatic patients and facilitating early access to targeted therapies given that SMA is a leading genetic cause of infant mortality, and for identifying carriers in the context of reproductive counseling as ~1 in 45 individuals in the Caucasian population is a SMA carrier.

This study aimed to evaluate the performance of the AmplideX® SMA Plus Kit (Asuragen, CE-IVD) for simultaneous ***SMN1/SMN2* copy number analysis** and detection of **clinically relevant variants** and **hybrid genes.**

## Methods |




- Data analysis was performed using AmplideX® PCR/CE Reporter software with a user-defined calibration and control sample; and user-defined bin settings to accomodate for the used extraction method (MagCore DNA extraction).
- Copy number results of *SMN1* and *SMN2* were compared with MLPA results (P021 kit, MRC Holland).

## Results and discussion |

### Copy number determination

Accurate identification of *SMN1* and *SMN2* copy number:
0 (SMA diagnosis), 1 (carrier), 2, 3, >4

**SMN1 copy number result (AmplideX® SMA Plus Kit)** vs **SMN1 copy number result (MLPA P021 kit)**

| | 0 | 1 | 2 | 3 | 4 | |
|---|---|---|---|---|---|---|
| 0 | 10 | | | | | 10 |
| 1 | | 11 | | | | 11 |
| 2 | | 1 | 36 | | | 37 |
| 3 | | | | 7 | | 7 |
| 4 | | | | | 1 | 1 |
| | 10 | 12 | 36 | 7 | 1 | 66 |

98% concordance with MLPA in determining *SMN1* copy number

**SMN2 copy number result (AmplideX® SMA Plus Kit)** vs **SMN2 copy number result (MLPA P021 kit)**

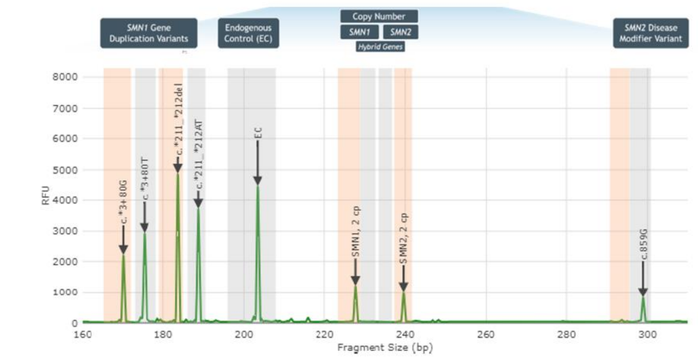
| | 0 | 1 | 2 | 3 | 4 | |
|---|---|---|---|---|---|---|
| 0 | 8 | | | | | 8 |
| 1 | | 19 | | | | 19 |
| 2 | | | 24 | | | 24 |
| 3 | | | | 8 | | 8 |
| 4 | | | | | 7 | 8 |
| | 8 | 19 | 24 | 8 | 7 | 66 |

100% concordance with MLPA in determining *SMN2* copy number

Analysis of **70 DNA samples**:

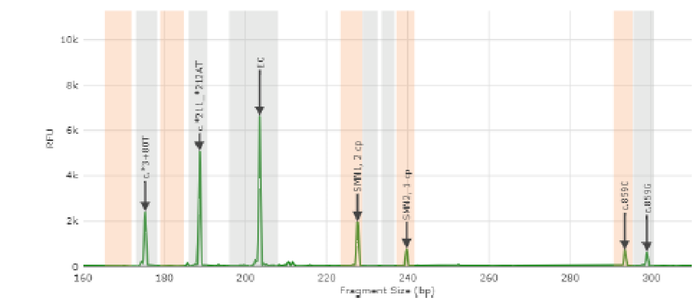
- for 66 samples the copy number for *SMN1* and *SMN2* was calculated
  - 1 sample showed a discordant *SMN1* result compared to MLPA: SNP analysis is in progress
- 1 sample showed a *SMN1* hybrid peak
- 3 samples were 'Marked for Rerun' during data analysis
  - 1 sample had insufficient DNA concentration
  - 2 samples showed *SMN1* normalized ratios outside defined BIN thresholds:
    - one extracted from a Coriell cell line (different source material)
    - one with 4 *SMN1* copies confirmed by MLPA showing a normalized ratio between 3 and 4 copies

### Variant detection




| Well | EC Area | SMN1 Area | SMN1 Normalized Ratio | SMN1 Exon 7 Copy Number | c.*3+80T>G | c.*211_*212del | SMN2 Area | SMN2 Normalized Ratio | SMN2 Exon 7 Copy Number | c.859G>C | Hybrid Peak |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E5 | 26770 | 7753 | 1,012 | 2 | Positive | Positive | 6412 | 0,969 | 2 | Negative | - |

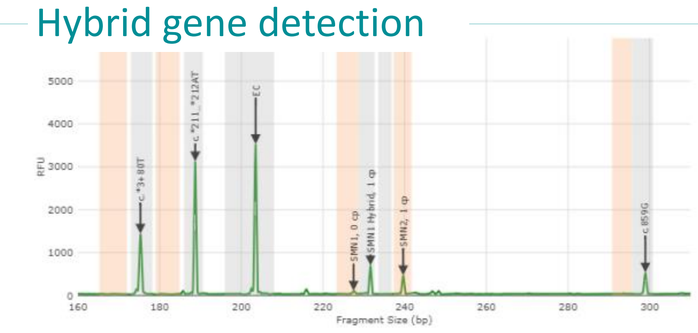
*SMN1* variants c.*3+80T>G and c.*211_*212del, associated with increased risk of silent carrier status, were observed in 4 samples. Further evaluation of family members is recommended to confirm carrier status.




| Well | EC Area | SMN1 Area | SMN1 Normalized Ratio | SMN1 Exon 7 Copy Number | c.*3+80T>G | c.*211_*212del | SMN2 Area | SMN2 Normalized Ratio | SMN2 Exon 7 Copy Number | c.859G>C | Hybrid Peak |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D2 | 42115 | 12904 | 0,821 | 2 | Negative | Negative | 5295 | 0,426 | 1 | Positive | - |

*SMN2* variant c.859G>C detected in 1 sample: in affected patients this variant is associated with a milder phenotype.
In this sample *SMN1* copy number was normal.

### Hybrid gene detection




| Well | EC Area | SMN1 Area | SMN1 Normalized Ratio | SMN1 Exon 7 Copy Number | c.*3+80T>G | c.*211_*212del | SMN2 Area | SMN2 Normalized Ratio | SMN2 Exon 7 Copy Number | c.859G>C | Hybrid Peak |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B5 | 22282 | 1718 | 0,000 | 1 | Negative | Negative | 3320 | 0,534 | 1 | Negative | SMN1 Hybrid |

*SMN1-SMN2* hybrid peak detected (chimeric gene with both *SMN1* and *SMN2* sequences) in 1 sample.
Further evaluation is ongoing (discordant result with MLPA – total of 2 *SMN1* copies).

## Conclusions and future perspectives |

The implementation of the AmplideX® SMA Plus Kit streamlines SMA genetic analysis by providing a comprehensive, fast, and high-throughput solution for both **diagnostic and carrier screening**.

Our evaluation of the AmplideX® SMA Plus Kit in a routine diagnostic laboratory setting demonstrated the following key findings:

- Comprehensive SMA genotyping in 1 single assay, enabling detection of both copy number variations and variants associated with milder phenotype or silent carrier status.
- End-to-end solution including all reagents in 1 kit and automated result calling using AmplideX® PCR/CE Reporter software.
- Higher reagent costs, but significantly shorter workflow compared to MLPA (same-day results; turnaround time <4 hours from DNA to result),
- Ongoing validation on additional samples, as well as in-depth evaluation of discordant results and assessment of inter- and intra-run precision will be essential before implementation can be considered in routine diagnostics and carrier screening programs, including the confirmation of positive newborn screening results.

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