# Buccal Swab Testing with the AmplideX PCR/CE *SMN1/2* Plus Kit that Assesses Copy Number and Critical Mutations for SMA

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## Summary

- Carrier screening and SMA diagnostics may be facilitated by the use of less invasive sampling, such as buccal swab collection, as an alternative to blood draws.
- DNA isolates from 60 buccal swab samples were tested with the AmplideX® PCR/CE *SMN1/2* Plus Kit, and overall results were >98% concordant to reference results for *SMN1* and *SMN2* exon 7 copy number, and 100% concordant for the detection of variants c.*3+80T>G, c.*211_*212del, and c.859G>C.
- Although the final, binned copy number calls were not affected, analysis of 43 matching buccal and whole blood specimens revealed a difference in the normalized ratio distributions between buccal swab and whole blood sample types. This difference was minimized by using user-defined calibration (UDC) and calibrating to a buccal reference sample as outlined in the kit protocol guide.

## Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease that results from mutation of the survival motor neuron 1 gene (*SMN1*), and the most common genetic cause of infant death. SMA treatments SPINRAZA®, Evrysdi™, and ZOLGENSMA® achieve profound benefits on survival and motor milestones by modifying *SMN2* splicing or using gene replacement with functional SMN genes. Early detection of SMA (including *SMN2* copy number status) and identification of at-risk couples through carrier screening is critical to aid in early intervention and family planning decisions.

We developed an accurate and robust single-tube PCR assay and companion software (AmplideX PCR/CE *SMN1/2* Plus Kit*) that uses capillary electrophoresis (CE) to quantify *SMN1* and *SMN2* copy numbers (0 to ≥4). This kit also determines the presence/absence of the two *SMN1* gene duplication "silent carrier" variants, c.*3+80T>G and c.*211_*212del, and the *SMN2* disease modifier variant c.859G>C. The *SMN1/2* Plus Kit has been previously validated for use with DNA isolated from blood. Here, we show that DNA isolated from buccal swabs can also be used to determine *SMN1* and *SMN2* copy number and other variants using this kit.

## Materials and Methods

A total of 60 DNA samples isolated from buccal swabs, with varying *SMN1/2* copies and variant status, were tested using the *SMN1/2* Plus kit at a single site (Asuragen). Samples were tested in two cohorts: an initial cohort containing 17 samples isolated from buccal swabs using column- or magnetic bead-based methods, and a second cohort of 43 samples isolated from matched blood and buccal samples using column-based methods. PCR products were generated using a Veriti thermal cycler and resolved on Applied Biosystems™ 3500xL, 3130*xl*, 3730*xl*, and SeqStudio™ Genetic Analyzers. Raw electrophoresis data (.fsa) files were directly imported into an assay-specific analysis module of the AmplideX Reporter software for genotyping. This software automates peak detection and size-based classification, and performs *SMN1* and *SMN2* exon 7 copy number quantification, detection of gene duplication and disease modifier variants, and sample- and batch-level quality control checks. Samples were analyzed using both default calibration and user-defined calibration (UDC) as described in the protocol.

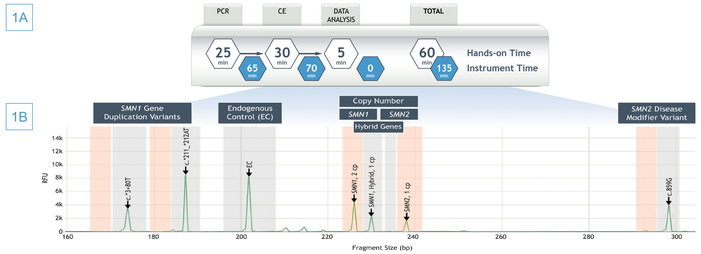


**Figure 1. Assay Workflow, Electropherogram Output, and Automatic Results Generation. A)** Assay workflow involves PCR followed by resolution of amplicons via capillary electrophoresis (CE) and automatic data analysis by the companion software. **B)** Fluorescently-labeled PCR amplicons are detected and quantified on a genetic analyzer; the electropherogram peaks are categorized by size (in base pairs) and, following batch-specific and replicate-specific calibration, normalized ratios (NR) generated by the companion software are binned to determine *SMN1* and *SMN2* exon 7 copy numbers. Mutation-specific peaks are used for positive/negative detection of gene duplication and disease modifier variants.

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## Results

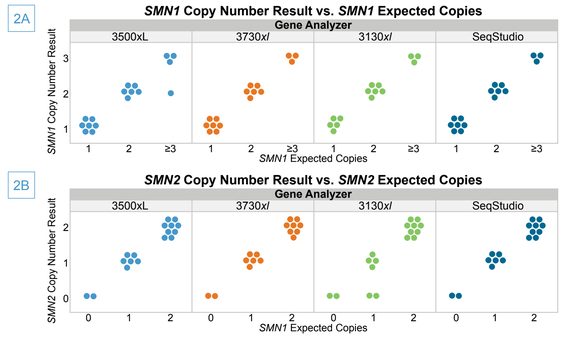


**Figure 2. *SMN1* and *SMN2* Copy Number Concordance of 17 Buccal Samples with MLPA Results.** Reference copy number results were determined using an MLPA-based assay that resolves *SMN1* and *SMN2* exon 7 copy number to 0, 1, 2, and ≥3 copies. Concordance by copy number from four genetic analyzers are shown. **A)** *SMN1* exon 7 copy numbers were concordant for 98.4% (62/63) of measurements (5 measurements excluded due to Precision (PR) QC failures). **B)** *SMN2* exon 7 copy numbers were concordant for 97.0% (65/67) of measurements (1 PR failure excluded). Four samples were positive for both c.*3+80T>G and c.*211_*212del on all CE instruments.

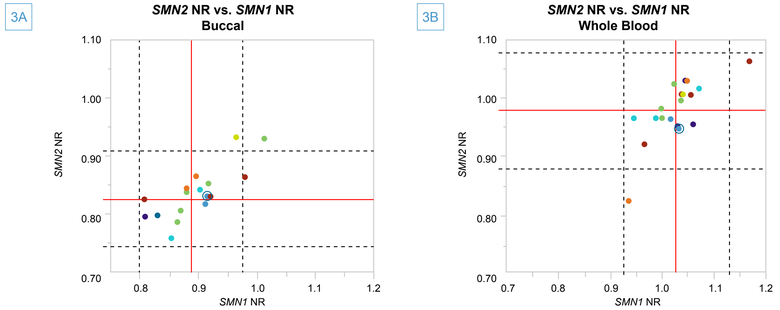


**Figure 3. Selection of User-Defined Calibrators for *SMN1/2* Copy Number Quantification.** In the second cohort of samples, 19 of 43 were determined to be potential user-defined calibrators (i.e. 2 copies of both *SMN1* and *SMN2* exon 7). For each sample type and for each genetic analyzer, the mean *SMN1* normalized ratio (NR) and mean *SMN2* NR were calculated (depicted as vertical and horizontal red lines, respectively). Results shown were generated on a SeqStudio with **A)** buccal swab, and **B)** whole blood samples from matching donors. Samples were determined to be suitable user-defined calibrators if both their *SMN1* and *SMN2* NR values consistently fell within 10% of the mean (range boundaries depicted as black dotted lines). Chosen UDC indicated with blue circle.

4A

| | Default Calibration | | | | |
|---|---|---|---|---|---|
| | 3500xL | 3730xl | 3130xl | SeqStudio | Total |
| *SMN1* Exon 7 | 100% (42/42) | 100% (43/43) | 100% (40/40) | 100% (42/42) | 100% (167/167) |
| *SMN2* Exon 7 | 100% (40/40) | 100% (43/43) | 97.3% (36/37) | 100% (42/42) | 99.4% (161/162) |
| c.*3+80T>G | 100% (43/43) | 100% (43/43) | 100% (40/40) | 100% (42/42) | 100% (168/168) |
| c.*211_*212del | 100% (43/43) | 100% (43/43) | 100% (40/40) | 100% (42/42) | 100% (168/168) |
| c.859G>C | 100% (43/43) | 100% (43/43) | 100% (40/40) | 100% (42/42) | 100% (168/168) |

4B

| | User-Defined Calibration (UDC) | | | | |
|---|---|---|---|---|---|
| | 3500xL | 3730xl | 3130xl | SeqStudio | Total |
| *SMN1* Exon 7 | 100% (40/40) | 100% (42/42) | 100% (39/39) | 100% (41/41) | 100% (162/162) |
| *SMN2* Exon 7 | 100% (38/38) | 100% (42/42) | 97.2% (35/36) | 100% (40/40) | 99.4% (155/156) |
| c.*3+80T>G | 100% (42/42) | 100% (42/42) | 100% (39/39) | 100% (41/41) | 100% (164/164) |
| c.*211_*212del | 100% (42/42) | 100% (42/42) | 100% (39/39) | 100% (41/41) | 100% (164/164) |
| c.859G>C | 100% (42/42) | 100% (42/42) | 100% (39/39) | 100% (41/41) | 100% (164/164) |

**Figure 4. Concordance of Buccal Swab *SMN1/2* Copy Number with Matched Blood Results.** Reference results were established in house using a validated version of the *SMN1/2* Plus Kit with DNA isolated from 43 blood samples matched to buccal samples from the same donor using a 3500xL genetic analyzer. Results are shown for **A)** default calibration, and **B)** user-defined calibration (UDC). QC failures were excluded from analysis. The UDC was excluded from UDC analysis.

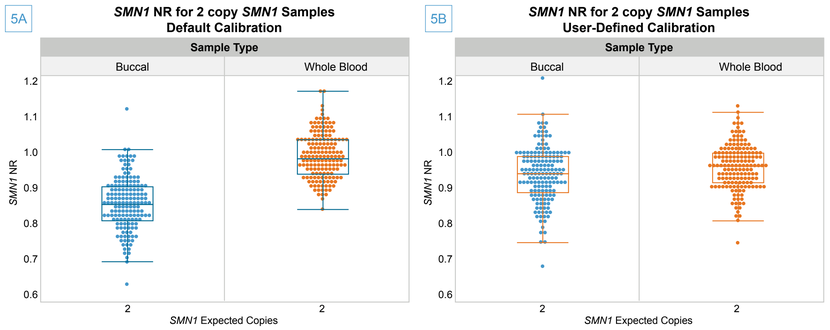


**Figure 5. Comparison of Blood and Buccal Results with Default and User-Defined Calibration (UDC).** *SMN1* NR values for 2 copy *SMN1* buccal (blue) and whole blood (orange) samples across all genetic analyzers analyzed with **A)** default calibration or **B)** UDC.

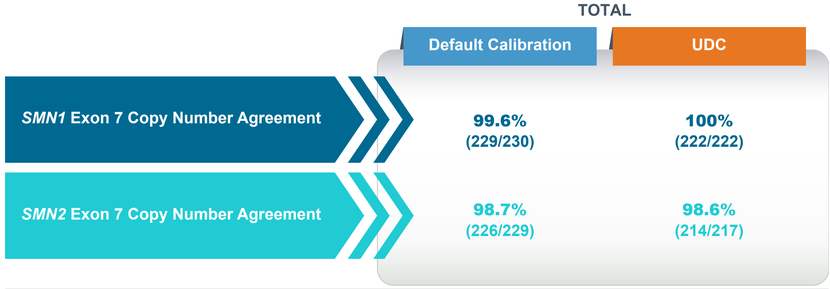


**Figure 6. Cumulative Buccal Swab Results Compared to Reference Results.** Percent agreement for *SMN1* and *SMN2* copy number results for all 60 buccal swab specimens tested in both cohorts across 4 genetic analyzers. Hard QC Failures (*) include Ladder (LD) QC Failures and Low Signal (LS) QC Failures.

## Conclusions

- *SMN1* and *SMN2* copy number calls for buccal samples agreed with reference values for >98% of measurements across 4 genetic analyzers. Variant detection results for c.*3+80T>G, c.*211_*212del, and c.859G>C were in perfect agreement with reference values for all measurements.
- While *SMN1* and *SMN2* exon 7 copy numbers were accurately determined in buccal samples using default calibration, a sample type bias compared to blood was observed. This bias was reduced with UDC, while maintaining accurate quantification of *SMN1/2* copy numbers.
- Signal intensities on CE were generally lower for buccal swab samples than blood samples, especially on the 3130*xl* genetic analyzer. Increased DNA input or injection time or voltage may improve signals.
- These results demonstrate that DNA from buccal swab samples may be analyzed with comparable results to blood samples using the AmplideX PCR/CE *SMN1/2* Plus Kit and AmplideX Reporter software.

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