Poster # PC88

Dried Blood Spot Testing with AmplideX® SMA Plus* Resolves *SMN1* and *SMN2* Exon 7 Copies

Walairat Laosinchai-Wolf[1], Sarah Edelmon[1], Laura Blasco-Pérez[2], Mar Costa-Roger[2], Marta Codina-Solà[2], Gary J Latham[1], Eduardo F Tizzano[2], and John N Milligan[1]

[1]Asuragen, a Bio-Techne Brand, Austin, TX U.S.A.; [2]Department of Clinical and Molecular Genetics, University Hospital Vall d´Hebron, Barcelona, Spain

SUMMARY

- Newborn screening for SMA comprises testing dried blood spot (DBS) samples using qPCR as a qualitative assessment of *SMN1* presence, and a confirmatory diagnostic test from whole blood to confirm absence of *SMN1* and determine *SMN2* copy number for positive screening results.
- We show the feasibility of a single-reaction test used as both a qualitative *SMN1* screen and as a confirmation test to determine *SMN1* and *SMN2* copy numbers directly from DBS in less than four hours. The assay also identifies disease modifier variants.
- This approach reduces overall turnaround time by simplifying the workflow and providing more information during initial DBS testing. Shorter turnaround may enable earlier treatment and improved patient outcomes.

INTRODUCTION

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder commonly caused by homozygous absence of *SMN1*. *SMN2*, an *SMN1* paralog, modulates SMA severity. Breakthrough therapies rely on rapid quantification of *SMN1* and *SMN2* copies, and newborn screening using dried blood spot (DBS) samples has become a public health priority. However, most screening assays only determine presence/absence of *SMN1* exon 7, excluding copy numbers (CN) for *SMN1* and *SMN2* and disease-modifier variants (*SMN2*(NM_017411):c.859G>C, abbreviated as c.859G>C). Here, we present data from the AmplideX® SMA Plus* Kit from 378 DBS measurements, demonstrating feasibility of a rapid, comprehensive, single-tube method that provides valuable information for both newborn screening and diagnosis directly from DBS samples.

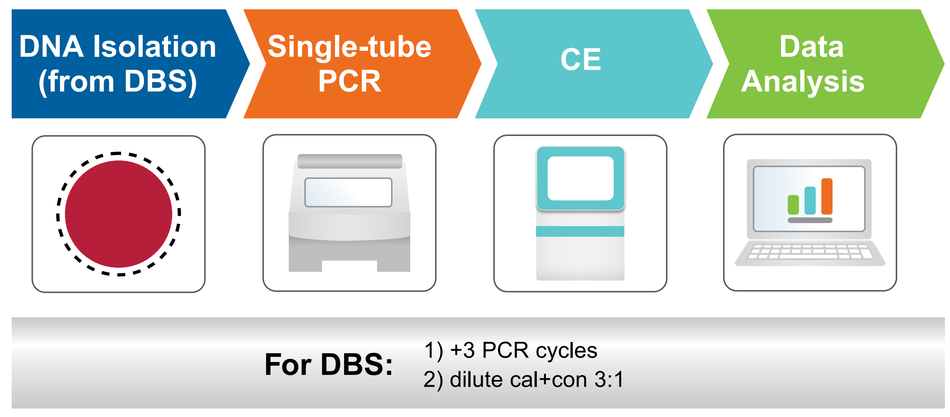

Figure 1. AmplideX SMA Plus DBS Workflow. Same protocol with minor changes.

MATERIALS AND METHODS

We tested 42 DBS samples with three DNA isolation methods on three genetic analyzer models (378 measurements total) using the AmplideX SMA Plus* Kit, which quantifies *SMN1* and *SMN2* exon 7 CN and detects c.859G>C in a single-tube workflow (Figure 1). Samples consisted of blood spotted on Whatman® 903 filter paper (n=20) or Whatman FTA® cards (n=22). Samples were isolated with three methods: Quantabio Extracta DBS, Qiagen Generation DNA purification and elution solutions, and Qiagen QIAamp® DNA micro columns. Samples were tested using 2 ul of extraction eluate per PCR reaction as input following the kit protocol with two modifications: 1) 3 PCR cycles were added, and 2) The Calibrator and Control were diluted 3 to 1 using kit Diluent (See Figure 1). All 126 isolations were analyzed in singleton on ABI 3500, 3730, and SeqStudio Genetic Analyzers. Genotypes were determined using AmplideX PCR/CE Reporter software. Reference values were determined using matched whole blood with the AmplideX SMA Plus Kit (n=20) or MLPA and sequencing (n=22). QC failures were excluded from calculations. For qualitative *SMN1* exon 7 presence/absence determination, *SMN1* CN of 0 were assigned the *SMN1* exon 7 status “absent”, and *SMN1* CN ≥1 were assigned “Present”.

RESULTS

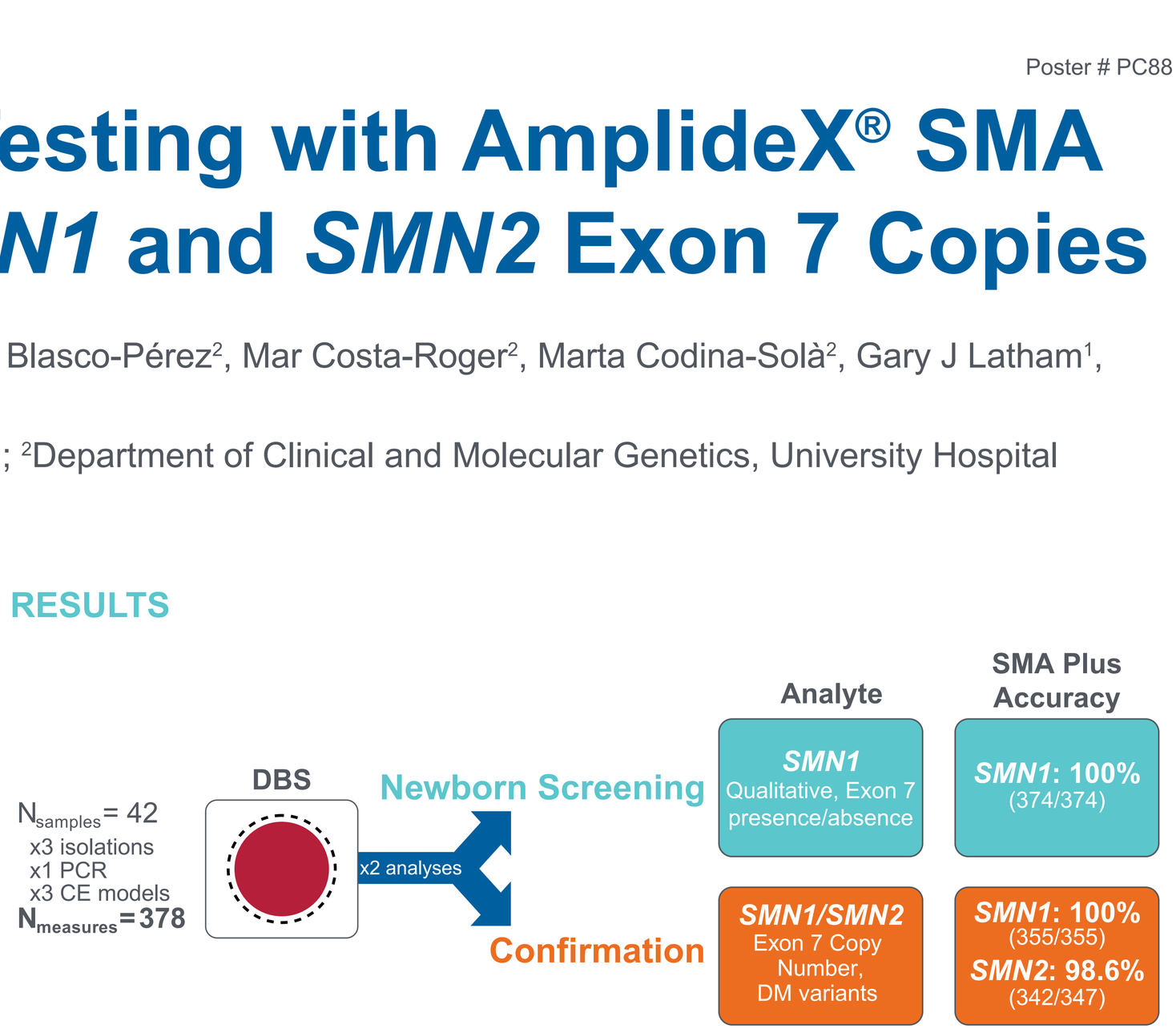

Figure 2. Experiment and Results Summary. 42 Dried blood spot (DBS) samples were tested with three isolation methods across three instrument configurations with the AmplideX SMA Plus assay, generating 378 results. These results were analyzed as both a newborn screening workflow to assess presence/absence of *SMN1* E7 (teal) and as a diagnostic workflow to confirm screening results that provides full *SMN1* and *SMN2* copy number and disease modifier (DM) information directly from DBS (orange). A single assay result was used for both analyses. Measurand and assay results are summarized in boxes.

3A

SMN1 Exon 7 Detected	*SMN1* Exon 7 Expected: Absent	*SMN1* Exon 7 Expected: Present
Absent	90	0
Present	0	284

3B

Isolation Method	*SMN1* Agreement	*SMN2* Agreement
Extracta	100% (120/120)	98.3% (116/118)
Generation	100% (118/118)	98.3% (114/116)
Qiagen Micro	100% (117/117)	99.1% (112/113)
Total:	**100% (355/355)**	**98.6% (342/347)**

3C

SMN1 Cp#	0	1	2	3	4
0	78	0	0	0	0
1	0	71	0	0	0
2	0	0	180	0	0
3	0	0	0	17	0
4	0	0	0	0	9

3D

SMN2 Cp#	0	1	2	3	4
0	9	0	0	0	0
1	0	124	0	0	0
2	0	0	144	0	0
3	0	0	1	46	3
4	0	0	0	1	19

Figure 3. *SMN1* and *SMN2* Agreement for 42 DBS Samples. A) Qualitative *SMN1* exon 7 agreement, representing performance as an NBS assay. **B)** *SMN1* and *SMN2* copy number agreement by extraction method, representing performance as a confirmation assay. **C)** *SMN1* exon 7 copy number agreement contingency table. **D)** *SMN2* exon 7 copy number agreement contingency table. Data represents 42 samples isolated with 3 methods and tested on 3 instrument configurations (378 measurements total). For copy number assessment, a threshold of 1000 RFU was applied to the endogenous control (EC) peak. Samples with <1000 RFU in the EC peak, indicative of low signal, were less accurate than samples with strong signal (≥1000 RFU). Below this cutoff, agreement for *SMN1* was 82.3% (14/17) and agreement for *SMN2* was 70.6% (12/17). Without EC thresholding, overall agreement for *SMN1* was 99.2% (369/372) and agreement for *SMN2* was 97.2% (353/363).

4A

c.859G>C Measured	c.859G>C Expected: Positive	c.859G>C Expected: Negative
Positive	27	0
Negative	0	347

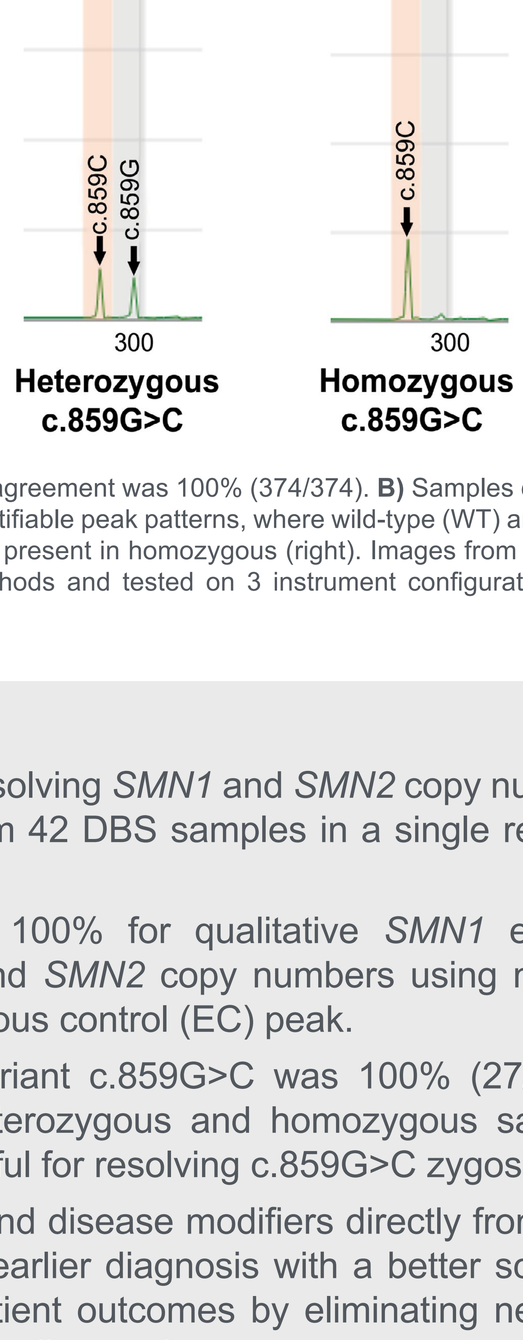

Figure 4. Disease Modifier Agreement and Zygosity. A) c.859G>C agreement was 100% (374/374). **B)** Samples confirmed to be heterozygous and homozygous for c.859G>C showed easily identifiable peak patterns, where wild-type (WT) and variant peaks are both present in heterozygous (left), but only variant peak is present in homozygous (right). Images from AmplideX Reporter software. Data represents 42 samples isolated with 3 methods and tested on 3 instrument configurations (378 measurements total).

CONCLUSIONS

- We demonstrated the feasibility of accurately resolving *SMN1* and *SMN2* copy numbers and disease modifier variant status directly from 42 DBS samples in a single reaction with the AmplideX SMA Plus* Kit.
- Across 378 measurements, agreement was 100% for qualitative *SMN1* exon 7 assessment, and ~99-100% for both *SMN1* and *SMN2* copy numbers using manual application of a signal threshold to the endogenous control (EC) peak.
- Accuracy for the positive disease modifier variant c.859G>C was 100% (27/27) in confirmed patients, and unique profiles in heterozygous and homozygous samples suggest the AmplideX SMA Plus kit may be useful for resolving c.859G>C zygosity.
- Detection of *SMN1* and *SMN2* copy numbers and disease modifiers directly from DBS samples in a single working shift may enable earlier diagnosis with a better scenario to offer immediate treatment and improved patient outcomes by eliminating need for follow-up blood collection or lengthy confirmatory diagnostic assays.

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