Title: A 5-year Study Involving Clinical Cases to Determine Copy Number Distribution of *SMN1* and *SMN2* Genes in Spinal Muscular Atrophy Testing across Medical Facilities in New Hampshire

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Introduction: Spinal Muscular Atrophy (SMA) is an autosomal recessive inherited neuromuscular disorder characterized by muscle weakness and progressive degeneration of motor neurons leading to atrophy. SMA is typically caused by homozygous deletion of the *SMN1* gene with disease severity related to the number of copies of the *SMN2* gene. Both *SMN1* and *SMN2* are located at the *SMN* locus on chromosome 5q13 and share 99% nucleotide sequence identity making it difficult to differentiate the two genes. Genetic counseling recommends preconception carrier screening or carrier screening of all pregnant women in the population.

Methods: Our lab at Dartmouth-Hitchcock Medical Center (DHMC) uses Droplet Digital PCR (ddPCR) to determine *SMN1* and *SMN2* copy numbers. In this study, we analyzed the data from SMA testing (almost exclusively carrier screening) across the health system from April 2019 through July 2024 (5718 patients) with a focus on carrier frequency in our population and most common days of the week for specimen collection for possible turnaround time (TAT) improvement.

Results: The SMA carrier frequency (1 copy of *SMN1*) among all patients tested was 1 in 46 individuals (125/5718; 2.18%), similar to what was observed in previous studies. The average carrier frequency of patients seen locally at DHMC and the immediate vicinity in Lebanon, NH was 2.85%, whereas a carrier frequency of 1.71% was observed among patients seen at other facilities in the network located in the southern part of the state. Results diagnostic for SMA (0 copies of *SMN1*) were found in 4/5718 (1 in 1430) individuals tested. No obvious trends were noted when looking at carrier frequencies or *SMN2* copy number in our population over time (by year or quarter). A review of ordering practices showed specimen collection was most common on Fridays (20.58% of all tests ordered) with only slightly lower numbers of tests ordered on other weekdays.

Conclusions: Monitoring carrier frequency for SMA in our patient population can be a useful tool for quality assurance purposes since allele frequencies are not expected to fluctuate over time in a given population. Changes in carrier frequency can suggest changes in ordering practices or actual changes make-up of the geographical population.