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Characterization of Reference Materials for Spinal Muscular Atrophy Genetic Testing: A Genetic Testing Reference Materials Coordination Program Collaborative Project

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Abstract

Spinal muscular atrophy (SMA) is an autosomal recessive disorder predominately caused by bi-allelic loss of the *SMN1* gene. Increased copies of *SMN2,* a low functioning nearly identical paralog, is associated with a less severe phenotype. SMA was recently recommended for inclusion in newborn screening. Clinical laboratories must accurately measure *SMN1* and *SMN2* copy number to identify SMA patients, carriers, and to identify individuals likely to benefit from therapeutic interventions. Having publicly available and appropriately characterized reference materials with various combinations of *SMN1* and *SMN2* copy number variants is critical to assure accurate SMA clinical testing. To address this need, the Centers for Disease Control and Prevention based Genetic Testing Reference Material Coordination Program (GeT-RM), in collaboration with members of the genetic testing community and the Coriell Institute for Medical Research, have characterized 15 SMA reference materials derived from publicly available cell lines. DNA samples were distributed to four volunteer testing laboratories for genotyping using 3 different methods. The characterized samples had 0–4 copies of *SMN1* and 0–5 copies *SMN2*. The samples also contained clinically important allele combinations (eg. 0 copies SMN1, 3 copies SMN2), and several had markers indicative of a SMA carrier. These and other reference materials characterized by the GeT-RM will support the quality of clinical laboratory testing and are available from the Coriell Institute.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder predominately caused by bi-allelic deletion of the *survival motor neuron 1* gene (*SMN1).*[1] It is characterized by dysfunction and then loss of the alpha motor neurons in the spinal cord that causes progressive muscle atrophy and weakness.[2–4] A large study found the overall carrier frequency to be 1 in 54, with a calculated incidence of 1 in 11,000.[5] Historically, SMA has been the leading monogenic cause of death in infancy, but there is reason for hope that this will greatly change with widespread early administration of newly approved disease modifying therapies.[6, 7] In 2008, the American College of Medical Genetics and in 2017, the American College of Obstetricians and Gynecologists recommended SMA for inclusion in population-based genetic screening.[8, 9] On July 2, 2018, the Secretary of the US Department of Health and Human Services accepted the Advisory Committee on Heritable Disorders in Newborns and Children's recommendation to add SMA to the Recommended Uniform Screening Panel(Health and Resources and Services Administration https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/previous-nominations/sma-consumer-summary.pdf last accessed 7/62020).

SMA manifests across a continuous gradient of phenotype severity, separated by functional "type" based on age of onset and maximum motor milestones achieved.[3] Individuals with onset of weakness in the first 6 months of infancy who never achieve an ability to sit independently, once known as "Werdnig-Hoffmann disease" but now classified as "SMA type 1," constitute approximately 60% of all individuals with SMA.[10, 11] Approximately

30% of patients are diagnosed with “SMA type 2”. These patients present with weakness recognized in later infancy and achieve the ability to sit, but not walk, independently. Those able to walk are grouped under the “SMA type 3” (Kugelberg-Welander syndrome) and constitute approximately 10% of the patient population. Outlier, “SMA type 0” refers to fetal onset with severe weakness, joint contractures, and respiratory compromise presenting at birth; and “SMA type 4” denotes a small group who first manifest weakness in adult years.[12]

Importance of *SMN1* copy number measurements

Regardless of severity, approximately 95% of SMA patients have a homozygous loss of the *SMN1* gene on chromosome 5q13.2, detection of which serves as the primary diagnostic assay for the disorder. The absence of *SMN1* can occur by deletion, typically a large deletion that includes the whole gene, or by conversion to *SMN2*.[13, 14] The absence of detectable *SMN1* in individuals with SMA is a reliable and powerful diagnostic test for the majority of SMA patients and should be used for an individual suspected to have SMA. The detection of a *SMN1* exon 7 deletion is used for the molecular diagnosis of SMA.

Although the absence of both copies of the *SMN1* gene is a very reliable and sensitive assay for the molecular diagnosis of SMA, about 5% of affected patients have other types of mutations in the *SMN1* gene that will not be detected by homozygous deletion testing.[15] Finally, given that SMA is a common recessive genetic disease, detecting carriers of *SMN1* deletions is crucial to identify couples at risk for offspring affected by SMA. The identification of SMA carriers requires the accurate determination of the *SMN1* copy number.

SMA carrier testing based on detecting the number of copies of *SMN1* is currently available. Individuals with one copy of *SMN1* are at risk of a child with SMA if their partner also carries one copy of the *SMN1* gene. While typically the presence of 2 copies of *SMN1* reduces the SMA carrier risk of an individual, it has been noted that some individuals carry 2 copies of the *SMN1* gene on one chromosome and no copies of the *SMN1* gene on the other (*SMN1* 2+0). The risk of a child with SMA to such individuals is similar to that of *SMN1* 1 copy carriers. A variant, g.27134 T>G (NG_008691.1:g.32134T>G, rs143838139, c.*3+80T>G) in intron 7 of the *SMN1* gene is strongly associated with *SMN1* 2+0 genotype in Ashkenazi Jewish and Asian populations, and occurs to varying extents in other ethnicities. Detection of the presence or absence of this variant is useful in improving the SMA carrier risk assessment.[16]

Importance of *SMN2* Copy Number Measurements

SMN2, a low functioning paralog to the *SMN1* gene, is located near *SMN1* on chromosome 5. The functional loss of *SMN1* results in a deficiency of the SMN protein, however the protein is not completely absent in affected individuals due to the presences of *SMN2.* The copy number of *SMN2* varies from zero to up to five copies in the normal population, and correlates inversely with SMA phenotype severity; greater *SMN2* copy number is associated with milder phenotypic presentation.[17–21] Patients with type 2 or 3 SMA have been shown

to often have more copies of *SMN2* than type 1 patients. The majority of patients with the severe type I form have one or two copies of *SMN2*; most patients with type II have three *SMN2* copies; and most patients with type III have three or four *SMN2* copies. In one study, three unrelated individuals with confirmed homozygous deletions of *SMN1* and 5 copies of *SMN2* were unaffected. [22] These cases not only support the role of *SMN2* in modifying the phenotype, but they also demonstrate that expression levels consistent with five copies of the *SMN2* genes may be enough to compensate for the absence of the *SMN1* gene. Thus, the identification of the homozygous deletion of *SMN1* combined with determination of *SMN2* copy number is a powerful predictor of disease and identifies a group who would benefit substantially from new and emerging therapies.

Clinical laboratories in the United States are required by regulation and guided by professional or best practice standards to use characterized reference materials for test development, validation and verification studies, quality control and proficiency testing [23–26] (American College of Medical Genetics https://www.acmg.net/PDFLibrary/Standards-Guidelines-Clinical-Molecular-Genetics.pdf, last accessed 4/9/2020, Washington State Legislature, http://app.leg.wa.gov/WAC/default.aspx?cite=246–338-090, last accessed 4/9/2020, College of American Pathologists https://www.cap.org/, last accessed 4/9/2020 (registration required), New York State Clinical Laboratory Evaluation Program, http://www.wadsworth.org/clep, last accessed 4/9/2020). Despite these requirements, there are a limited number of well characterized quality control and other reference material samples for many genetic tests, including SMA. This lack of reference material samples hinders the ability of laboratories to develop and validate assays, perform necessary quality control, and complicates comparison of assays and assay standardization. The lack of available materials also affects the ability of proficiency testing programs to provide challenges with a variety of clinically relevant and rare variants.

For SMA genetic testing, having publicly available and appropriately characterized reference materials with various combinations of *SMN1* and *SMN2* copy number variants is critical to assure that clinical testing for SMA is accurate. This need is especially urgent as more laboratories begin to test for this gene following recommendations for newborn screening and the development of new therapies. To address these needs, the Centers for Disease Control and Prevention's (CDC) Genetic Testing Reference Material Program (GeT-RM), in partnership with four clinical laboratories and the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository and the National Human Genome Research Institute (NHGRI) Sample Repository at the Coriell Institute for Medical Research created a panel of well-characterized genomic DNA reference materials with accurate *SMN1* and *SMN2* exon 7 copy number which clinical laboratories could use for standardization, quality control and assay validation for SMA genetic testing.

Materials and Methods

Cell Line Selection

Fifteen cell lines from the NIGMS and the NHGRI Repositories at the Coriell Institute for Medical Research (Camden NJ) were selected for the study. Fourteen of the samples are lymphoblastoid cell lines and one is a fibroblast line. These samples were selected to create

a panel containing a wide variety of *SMN1* and *SMN2* copy number variants and allele combinations.

DNA Preparation

Approximately 2 mg of DNA was prepared from each of the selected cell lines by the Coriell Institute for Medical Research using Gentra/Qiagen Autopure (Valencia, CA) as per manufacturer's instructions.

Live Cell Culture

Frozen ampoules of requested cell lines were recovered from liquid nitrogen or vapor phase liquid nitrogen storage and placed in culture. The growth medium used for lymphoblastoid cell lines is RPMI-1640 with 15% fetal bovine serum (FBS), and Eagle's MEM (minimal essential medium) with 15% FBS was used for fibroblasts lines. The cultures were inspected for growth and contamination on the following day. Three days later, the cultures were re-inspected, the 25-cm^2 flasks were filled with fresh medium containing only 5% FBS, packaged, and shipped. Cell cultures were shipped when confluency reached about 50–70%. Cells were shipped at ambient temperature in an insulated box in order to keep the cells alive while avoiding overgrowth.

Characterization Protocol

Each of the four testing laboratories received one 10-μg aliquot of DNA and three of the laboratories received one 25 ml flask of live cells from each of the cell lines that they volunteered to test. The cell lines and DNA source (Coriell or DNA extracted by each laboratory) tested by each laboratory is shown in Table 1. The DNA and cell lines provided to the laboratories were labeled with codes, so the recipients were blinded as to the expected copy number of each gene. Each laboratory tested the samples using their standard methods. A variety of different methods were used to test the samples to ensure a robust characterization. If discordances were noted, participating laboratories were asked to re-evaluate their data for the sample(s) in question to determine the cause of the inconsistency. The expected results were not revealed to the laboratory. The consensus genotype for each gene in each sample was determined upon examination of data from all assays.

DNA Extraction from Live Cell Cultures

Lab 2: DNA from cultured cells was extracted on the Qiagen EZ1 (Germantown MD) using the tissue protocol according to manufacturer's instructions.

Lab 3: The Qiagen Gentra Puregene Blood Kit (Germantown MD) was used according to manufacturer's instructions.

Lab 4: DNA from cells was prepared using the QIAamp Mini Kit (Qiagen, Valencia, CA, USA).

Assays used to characterize samples

Multiplex Ligation-dependent Probe Amplification (MLPA) -Labs 1 and 3— Multiplex Ligation-dependent Probe Amplification (MLPA) reactions were performed to detect *SMN1* and *SMN2* copy numbers using MLPA kits, versions P460-A1and P021-B1, (MRC-Holland) according to manufacturer's instructions. The MRC-Holland P460-A1 kit has three probes for the exons 7 or 8 of *SMN1* and *SMN2* genes and one probe for the g.27134 T>G variant; P021-B1 kit contains four probes specific for sequences in exon 7 or 8 of either *SMN1* or *SMN2*. Lab 1 used P460-A1 and Lab 3 used the P021-B1 kit. Amplification products were analyzed on the ABI Prism 3730 automatic sequencing system (Applied Biosystems, Foster City, CA).

Asauragen AmplideX^{r} PCR/CE *SMN1/2* Plus Kit – Lab 2—Briefly, using reagents from Asuragen (Austin TX) the PCR amplification was performed with 20ng total DNA from each sample, 2µl of internal control sample and 2µl of calibrator in a PCR reaction containing PCR buffer/enzyme mix, and SMN1/2 Plus HEX primer mix. Twenty-five cycles with the following thermal protocol were performed: melting (94°C; 30 seconds), annealing (52°C; 30 seconds) and elongation (72°C; 30 seconds). Subsequently, 4 µl of PCR products were mixed with ROX1000 ladder and Hi-Di formamide and separated by capillary electrophoresis by using Genetic Analyzer 3500XL (ThermoFisher Scientific, Waltham MA). Copy number is calculated by normalizing ratios of *SMN1*, *SMN2*, and hybrid genes peaks by area under the curve using GeneMarker 2.6 and PCR/CE Reporter SMN analysis module Ver 1.0.10.

Quantitative PCR Assay – Lab 4—*SMN1* and *SMN2* copy number was determined using a real-time allele specific PCR (RT-ASPCR) reaction by probing the c.840C>T variation in exon 7 of these genes. The cystic fibrosis conductance regulator (*CFTR*) gene present at two copies per genome was used as an endogenous control in PCR. The allele-specific primers are designed such that the forward primer provides the specificity. TaqMan® minor grove binder (MGB) probes were designed with 6-FAM on the common probe to the *SMN1* and *SMN2* genes, and VIC fluorophore. The gene copy number of *SMN1* and *SMN2* are determined by using the delta, delta (ΔΔ)Ct method. This assay was performed on the ViaA7 Real-Time PCR System (Life Technologies/ThermoFisher Scientific, Waltham MA).

Detection of the g.27134 T>G variant was performed using TaqMan (Applied Biosystems/ ThermoFisher Scientific, Waltham MA) quantitative PCR assay on ViaA7 Real-Time PCR System (Life Technologies) with RNase P gene as internal PCR control. Positive, negative and blank results are defined by Cycle of Threshold (CT) Value and Relative Quantification (RQ) reference ranges established during the assay development.

Results

The goal of this study was to create a comprehensive panel of well-characterized and publicly available human cell line-based genomic DNA reference materials for spinal muscular atrophy genetic testing. A group of clinical laboratory directors experienced with SMA testing were consulted to recommend the composition of an "ideal" SMA reference material panel that would be needed to assure that clinical assays could unambiguously

determine the copy number of the *SMN1* and *SMN2* genes. Together, the group selected 15 cell lines from the NIGMS Human Genetic Cell Repository and the NHGRI Repository at the Coriell Institute for Medical Research that were expected to have a range of clinically relevant S*MN1* and *SMN2* copies and allele combinations[27, 28] The samples included *SMN1* alleles ranging from zero to four copies and *SMN2* alleles ranging from zero to five copies.

The *SMN1* and *SMN2* copy numbers were measured in DNA supplied from the Coriell Institute for each of the 15 samples by the four laboratories who volunteered for the study (Table 1). In addition, three of the laboratories tested DNA samples that they extracted from live cell culture using their normal DNA extraction methods to identify any analytic differences that may be caused by the method used for DNA preparation. Measurement of *SMN1* and *SMN2* copy number by laboratories 3 and 4 was not affected by the method used to extract the DNA that was tested. Laboratory 2 reported that samples extracted from cell lines tend to have lower copy number of the genes than those in the corresponding Coriell DNA samples. The Asuragen AmplideX® PCR/CE *SMN1/2* Plus Assay uses a SMN calibrator that normalizes the area ratio of the peaks of all sample results. When Lab 2 used DNA extracted from cell line GM22807 as a calibrator to recalibrate the peak ratio of all cell line samples, the copy numbers in cell line samples showed more consistency with those in DNA samples. The reason for the trend of a lower peak ratio for both genes with the cell line samples is not fully understood, and it may be possible that there are some residual reagents from the DNA extraction procedure that interfere with PCR amplification.

The *SMN1* and *SMN2* copy number measurement from each laboratory and the consensus *SMN1* and *SMN2* genotypes determined by this study for each sample are shown in Table 2. The copy numbers measured in each sample was consistent across laboratories and assays used, with one exception. Sample GM03814, which was expected to have five copies of *SMN2*[27], was shown to have five copies using a MLPA assay (Lab 3). The other laboratories that tested this sample identified either 4 or 4+ copies as these assays were not designed or validated to detect five copies.

Three of the laboratories (Labs 1, 2 and 4) tested for the presence of the g.27134T>G variant in intron 7 of *SMN1*, which is highly associated with the presence of two copies of *SMN1* on the same chromosome in the Ashkenazi Jewish and other populations.[16] All three laboratories detected the presence of the variant in the same samples (Table 2), although the tests were not designed to determine whether the sample had 1 or 2 copies of the variant. Based on the presence of the variant and two copies of *SMN1*, it is possible that GM22807 has both copies of *SMN1* on one chromosome, and 0 copies on the other (the 2+0 *SMN1* haplotype). Sample GM19123, which has 3 copies of *SMN1*, likely has 2 copies and the variant on one chromosome and 1 copy without the variant on the other. Other samples that had 4 copies of *SMN1* in the presence of the variant, such as GM19235, may have 2 copies on each chromosome, but it is possible that there may be 3 or even 4 copies on a single chromosome.

Discussion

This study describes the characterization by four clinical laboratories of 15 publicly available and renewable genomic DNA reference materials for *SMN1* and *SMN2* genetic testing. The samples had a wide variety of copy numbers for each of the genes, ranging from zero to four copies of *SMN1* and zero to five copies of *SMN2*. The samples also contained a variety of allele combinations, ranging from GM22807 that has two copies of each gene, to those with one or no copies of *SMN1* and three, four, or five copies of *SMN2* (eg. GM23255 (0–3), HG01773 (1–4), and GM03814 (1–5) respectively). Also included were several samples that tested positive for the g.27134T>G variant and contained at least two copies of *SMN1*, suggesting that the cell lines may have both copies on the same chromosome, indicative of a SMA carrier. The ability of SMA assays to accurately measure *SMN1* and *SMN2* copy number is essential to ensure the quality of testing.

Reference materials, such as those characterized as part of this study, play an important role in assuring the quality of these tests. The samples characterized as part of this study were selected to cover important clinical aspects of SMA testing which include diagnostic testing, carrier testing, and prognosis. SMA fits the criteria and is recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists for inclusion in population-based genetic screening.[8, 9]

There are known limitations of the carrier test. First, approximately 2% of SMA cases arise as the result of de novo rearrangement events which will not be detected by most assays.[29] Second, the copy number of *SMN1* can vary on a chromosome; it has previously been observed that about 4% of the normal population possess three copies of *SMN1*.[30] Thus, carriers possessing one chromosome with two copies and the other chromosome with zero copies are relatively common.[31–34] This is referred to as the '2 + 0' genotype. The finding of two *SMN1* genes on a single chromosome has serious genetic counseling implications, because a carrier with two *SMN1* genes on one chromosome and a *SMN1* deletion on the other chromosome will have the same dosage result as a noncarrier with one *SMN1* gene on each chromosome 5. In most populations, approximately 3–4% of carriers have been shown to have the "2+0 genotype.[16] However, the estimated frequency of alleles with two or more copies of *SMN1* is 3–8 times more prevalent in African Americans when compared to other ethnic groups.[35] This translates to a much higher frequency of individuals with the SMA carrier [2+0] genotype amongst African Americans compared to other races. The presence of the g.27134T>G variant which is associated with chromosomes carrying 2 *SMN1* in *cis*, has been shown to be highly significant in the Ashkenazi Jewish population and can be informative in other populations. In the Spanish population, it was reported that 19.35% of the *cis* carriers were positive for the g.27134T>G variant.[34] Thus, the absence of the variant does not preclude one from being a *cis* carrier. Family studies can also be extremely helpful in identifying *cis* chromosomes. Lastly, the dosage testing does not identify carriers of other types of intragenic mutations in the *SMN1* gene. Thus, the finding of two *SMN1* copies significantly reduces the risk of being a carrier, however there is still a small residual risk of future affected offspring for individuals with 2 *SMN1* gene copies. Risk assessment calculations using Bayesian analysis are essential for the proper genetic counseling of SMA families.[33, 34]

To detect carriers, it is imperative for clinical testing laboratories to do an accurate determination of the *SMN1* copy number. This requires the availability of reference materials with variable copies of *SMN1.* Laboratories must also be able to detect the presence of two *SMN1* copies on the same chromosome, which requires access to samples with the g.27134 T>G variant linked to the 2 *SMN1* in *cis* chromosome. The reference materials characterized in this study provide the appropriate alleles and allele combinations needed for laboratories to design and validate assays and conduct accurate carrier studies.

Newborn screening allows patients to be treated at the earliest time period and to obtain proactive intervention earlier in the disease progression. In infants with type 1 SMA, rapid loss of motor units occurs in the first three months and severe denervation with loss of more than 95% of motor units within six months of age.[36] Therefore a very small window for beneficial therapeutic intervention exists in infants with type 1 SMA. Therapies need to be administered within the newborn period for maximum benefit which could potentially be accomplished through a newborn screening program for SMA. Furthermore, identifying SMA-affected individuals at birth eliminates the pain and cost of unnecessary testing that often takes place in attempting to diagnose an affected individual. The results from newborn screening are also important for the child's family because of the possibility for the prevention of additional cases through genetic counseling and carrier testing of at-risk family members. The first disease-modifying therapy, Nusinersen, was approved by the US Food and Drug Administration in 2016, and early treatment has been shown to lead to improved outcomes. The most robust response has been shown to occur in presymptomatic treated children.[37, 38]

Within the setting of newborn screening, *SMN2* copy number analysis is of extreme value in stratifying patients who are more likely to respond to therapeutic strategies designed to upregulate the expression levels of full-length SMN protein from the *SMN2* gene and gene therapy.[39] Biogen's NURTURE clinical trial demonstrates the dramatic impact from early treatment with Nusinersen, with data showing that treatment of patients under six weeks of age who have two or three copies of *SMN2* had significantly better outcomes than treatment after six weeks of age.[38] In the SMA gene replacement therapy, 100% (n=15) patients with two *SMN2* copies were alive and event-free at 20 months of age, as compared with a survival rate of 8% in a historical cohort.[40] In early 2018, Cure SMA convened a group of expert clinicians and scientists to develop a treatment algorithm for infants diagnosed with SMA via newborn screening using a reiterative surveying modified Delphi technique.[41] The working group unanimously recommended immediate treatment for individuals predicted to manifest SMA with the qualifying genotypes of two or three copies of *SMN2*, as supported by the strong positive results arising from pre-symptomatic infants in the NURTURE trial.[41] This study facilitates the implementation of the recommendation by providing reference materials needed for laboratories to accurately determine the *SMN2* copy number so that the affected children identified during newborn screening can be enrolled in appropriate therapies. Furthermore, *SMN2* copy number has recently been adopted as a College of American Pathologists (CAP) proficiency testing challenge, thus the availability of well characterized refence materials can support both CAP and clinical laboratories to assure the quality of SMA testing.

Due to the recommendations for carrier screening and newborn screening, as well as for stratification for treatment, it is imperative that accurate and well characterized reference materials be available for laboratories involved in SMA testing. Clinical laboratories planning to develop SMA tests for determination of *SMN1* copy number for diagnosis and carrier determinations, and *SMN2* copy number for prognosis and treatment enrollment must establish validated, non-overlapping cut-off values that can accurately and reliably distinguish *SMN1* and *SMN2* copy numbers of 0, 1, 2, 3, and 4. The well characterized and publicly available genomic DNA reference materials described in this study contain a range of *SMN1* and *SMN2* copies, and can be used by laboratories to develop, validate and assure the quality of their tests. Use of these SMA reference materials will also help laboratories to develop and validate NGS assays that can detect *SMN1* and *SMN2* copy number changes.[42] These and other reference materials characterized by the GeT-RM are available from the Coriell Institute for Medical Research. More information about the GeT-RM program is available through the GeT-RM website (https://www.cdc.gov/labquality/get-rm/index.html, last accessed April 13, 2020).

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Table 1.

Samples tested by each laboratory

Coriell ID	Lab 1	Lab 2	Lab 3	Lab 4
GM19122	DNA*	LCL‡/DNA	LCL/DNA	LCL/DNA
GM19123	DNA	LCL/DNA		LCL/DNA
GM19235	DNA	LCL/DNA	LCL/DNA	LCL/DNA
GM19360	DNA	LCL/DNA		LCL/DNA
GM19429	DNA	LCL/DNA		LCL/DNA
GM20760	DNA	LCL/DNA	LCL/DNA	LCL/DNA
GM20775	DNA	LCL/DNA		LCL/DNA
HG01773	DNA	LCL/DNA		LCL/DNA
HG03625	DNA	LCL/DNA	LCL/DNA	LCL/DNA
GM03814	DNA	Fib§/DNA	Fib/DNA	Fib/DNA
GM12552	DNA	LCL/DNA	LCL/DNA	LCL/DNA
GM22807	DNA	LCL/DNA		LCL/DNA
GM23255	DNA	LCL/DNA	LCL/DNA	LCL/DNA
GM23686	DNA	LCL/DNA		LCL/DNA
GM23687	DNA	LCL/DNA		LCL/DNA

*DNA- Sample was DNA supplied by Coriell

‡LCL- Sample was DNA prepared by recipient laboratory from Lymphoblastoid Cell Line (LCL) culture supplied by Coriell

§Fib- Sample was DNA prepared by recipient laboratory from a fibroblast cell line (Fib) culture supplied by Coriell

Table 2.

Results of *SMN1/SMN2* assays from each laboratory and consensus genotypes

Coriell Cell ID	Consensus *SMN1 -SMN2*	Lab 1 (MLPA) *SMN1 -SMN2*	Lab 2 (Asuragen) *SMN1 -SMN2*	Lab 3 (MLPA) *SMN1 -SMN2*	Lab 4 (qPCR) *SMN1 -SMN2*	Labs 1, 2, 4 g.27134T>G (rs143838139) detected
GM19122	2–0	2–0	2–0	2–0	2–0	no
GM19123	3–0	3–0	3–0	ND *	3–0	yes
GM19235	4–0	4+ ‡ –0	4–0	4–0	4–0	yes
GM19360	4–0	4+–0	4–0	ND	4–0	yes
GM19429	4–1	4+–1	4–1	ND	4–1	no
GM20760	1–1	1–1	1–1	1–1	1–1	no
GM20775	3–1	3–1	3–1	ND	3–1	no
HG01773	1–4	1–4+	1–4	ND	1–4	no
HG03625	2–4	2–4+	2–4	2–4	2–4	no
GM03814	1–4	1–4+	1–4	1–5	1–4	no
GM12552	3–3	3–3	3–3	3–3	3–3	no
GM22807	2–2	2–2	2–2	ND	2–2	yes
GM23255	0–3	0–3	0–3	0–3	0–3	no
GM23686	0–2	0–2	0–2	ND	0–2	no
GM23687	1–2	1–2	1–2	ND	1–2	no

* ND- sample not tested

‡ 4+ - four or more gene copies detected

Copy number is reported based on exon 7 probe results.