# Spinal Muscular Atrophy Carrier Screening and Diagnostic Assay

## Background

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder, with an incidence of approximately 1 in 10,000 births. The condition has variable severity and age of onset, and has been categorized into clinical types 0-IV (OMIM# 253300, 253550, 253400, 271150). SMA I accounts for 60% of all SMA and has onset of symptoms in infancy. SMA types 0 and IV are rare, with SMA 0 at the most severe end of the spectrum, correlating with prenatal onset of muscular weakness and neonatal respiratory failure, while SMA IV has the mildest presentation and correlates with adult onset of muscle symptoms. Treatment was previously limited to supportive care but is now available to prevent or slow the progression of SMA.

In all types, the genetic cause maps to an inverted duplication on chromosome 5q13.2. The complexity of the 5q13 region increases the likelihood of errors during DNA replication, resulting in a relatively high risk of deletions, gene conversions, and new mutations. *SMN1* and *SMN2* are two highly homologous genes located in this region, sharing more than 99% nucleotide identity. One difference between these genes affects protein coding, with alternative splicing of exon 7 in *SMN2* that results in decreased production of the full length, functional SMN protein compared to *SMN1*. Both *SMN1* and *SMN2* produce the survival motor neuron (SMN) protein, with *SMN2* producing just a small percentage of this protein, due to this alternate splicing. Therefore, with *SMN1* accounting for the majority of SMN production, mutations in or deletions of *SMN1* are the cause of the SMA phenotype.

The majority (95%) of patients with SMA have homozygous deletions of the *SMN1* gene (noted as 0+0 genotype). Of the remaining 5%, most are heterozygous for a deletion of *SMN1* on one chromosome and a small pathogenic, or disease-causing, variant in the *SMN1* copy on the other chromosome (1+0 genotype). Pathogenic sequence variants are not detected by this test. There is no correlation between *SMN1* copy number or presence of pathogenic variants and disease severity. Disease severity is inversely correlated with the *SMN2* gene copy number. In addition, the presence of the disease modifier variant c.859G>C is associated with reduced disease severity due to improved *SMN2* splicing.

*SMN1* copy number varies among healthy non-carrier individuals (1+1 or 2+1 genotypes). The SMA carrier rate is about 1 in 50, and varies with ethnicity (see table below). SMA carriers are also healthy. Most SMA carriers have a single copy of *SMN1* on one chromosome, while the other copy is deleted (1+0 genotype). At least 4% of the population has two *SMN1* copies on one chromosome (2+0 genotype), although the 2+0 genotype is more frequent in some ethnic groups, such as up to 27.5% in African-Americans. There are also reports of some carrier individuals having three copies of *SMN1* on the same chromosome (3+0 genotype). Thus, the presence of three or more copies of *SMN1* reduces but does not entirely eliminate the residual carrier risk.

Carrier risk estimates for individuals with 2 copies of *SMN1* (2+0 genotype) may be further refined using the presence or absence of two common benign variants associated with *SMN1* duplication, c.*3+80T>G in intron 7 and c.*211_*212del in exon 8. Presence of either variant indicates an increased risk of being a silent carrier (2+0 genotype) in some ethnicities. The presence or absence of these variants does not adjust the silent carrier risk for individuals with three copies of *SMN1* (3+0 genotype). Neither *SMN2* copy number nor presence or absence of the disease modifier variant c.859G>C impact carrier status.

## Carrier Screening

Both the American College of Medical Genetics and Genomics and the American College of Obstetricians and Gynecologists recommend that SMA carrier screening be offered to all women/couples who are planning a pregnancy or currently pregnant. In individuals with a family history of SMA, it is best to obtain genetic test reports from family members before testing, to confirm the diagnosis and type

### Residual SMA Carrier Risk after Negative Carrier Screen and Negative Family History

*Residual risk for unlisted ethnicities is unknown.*

| Ethnicity | Carrier Rate | 2 copies *SMN1* exon 7 | 3 copies *SMN1* exon 7 | 2 copies *SMN1* No variant detected | 2 copies *SMN1* At least one variant detected |
|---|---|---|---|---|---|
| **African American/Black** | 1:71 | 1:132 | 1:6997 | 1:375 | 1:39 |
| **Ashkenazi Jewish** | 1:56 | 1:514 | 1:5899 | 1:580 | SMA carrier |
| **Asian** | 1:50 | 1:719 | 1:5185 | 1:779 | 1:57 |
| **Asian Indian** | 1:50 | 1:428 | 1:5252 | Not reported | Not reported |
| **Caucasian/ European** | 1:45 | 1:604 | 1:4719 | 1:814 | 1:12 |
| **Hispanic** | 1:83 | 1:641 | 1:7574 | 1:906 | 1:99 |
| **Iranian** | 1:16 | 1:96 | 1:1604 | Not reported | Not reported |
| **Israeli Jewish** | 1:38 | 1:450 | 1:4004 | Not reported | Not reported |
| **Spanish** | 1:40 | 1:781 | Not reported | 1:888 | SMA carrier |

of mutation. In a pan-ethnic U.S. population studied by Sugarman et al., the carrier detection rate through *SMN1* dosage analysis is estimated at an average of 91%, though it ranges from 70.5% to 94.8% with ethnicity. The addition of the c.*3+80T>G and c.*211_*212del variants increases the detection of silent carriers (2+0 genotype). Ethnicity-specific carrier and detection rates compiled from multiple studies are provided in the Table listed above.

### Methodology

Multiplex Fluorescent Polymerase Chain Reaction (PCR) followed by Capillary Electrophoresis is used to detect *SMN1* (NM_000344.3) and *SMN2* (NM_017411.3) based on fragment size. This test detects copy number of *SMN1* exon 7, which is homozygously deleted in 95% of SMA patients, and copy number of *SMN2* exon 7, which influences severity of disease in affected patients. The test also detects three variants; c.*3+80T>G and c.*211_*212del associated with *SMN1* gene duplication and c.859G>C associated with reduced disease severity due to improved *SMN2* splicing. The c.*3+80T>G and c.*211_*212del variants are reported only for individuals with 2 copies of *SMN1* as presence of either variant indicates an increased risk of being a silent carrier (2+0 genotype). The disease modifier variant c.859G>C is reported only when zero copies of *SMN1* are noted.

Variants interrogated using assembly GRCh38 hg38 (legacy name):

*SMN1* NM_000344.3; rs143838139, c.*3+80T>G, g.70952074T>G (g.27134T>G)

*SMN1* NM_000344.3; rs200800214, c.*211_*212del, g.70952646_70952647del

*SMN2* NM_017411.3; rs121909192, c.859G>C, p.Gly287Arg, g.70076545G>C

### Interpretation

Among patients with a clinical presentation suggestive of SMA, detection of zero *SMN1* copies confirms the diagnosis. In symptomatic patients with one *SMN1* copy, *SMN1* gene sequencing should be considered to identify the small percentage of patients with heterozygous sequence variants or small deletions. Symptomatic patients with two *SMN1* copies are unlikely to have SMA, though very rare cases of homozygous sequence variants have been reported.

Use of this test to predict the likelihood of disease in offspring must also take into consideration that 2% of *SMN1* disease-causing variants occur de novo rather than being inherited. Due to the complex inheritance of SMA by *SMN1* copy number, de novo variant, and/or pathogenic variant, SMA carrier testing will never provide 100% reassurance

that carrier status is eliminated or zero. SMA carrier testing of both reproductive partners will provide the best estimate of reproductive risk and is most useful for individuals with an increased residual carrier risk following this test. Genetic counseling may be appropriate based on clinical or family history.

## References


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15. For more information about SMA, consult GeneReviews.org.

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## Test Overview

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|---|---|
| **Test Name** | Spinal Muscular Atrophy Carrier Screening and Diagnostic Assay |
| **Mnemonic** | SMAGEN |
| **Methodology** | Multiplex Fluorescent Polymerase Chain Reaction (PCR) |
| **Specimen Requirements** | 4 mL peripheral blood, EDTA (lavender) transported and stored ambient up to 48 hours.<br>*Due to difficulties associated with newborn and infant draws, smaller volumes may be submitted (a minimum of 0.5 mL is required).* |
| **CPT Codes** | 81329 |


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