MRC Holland

Newborn Screen
Spinal Muscular Atrophy

MC002

Why screen newborns for Spinal Muscular Atrophy (SMA)?

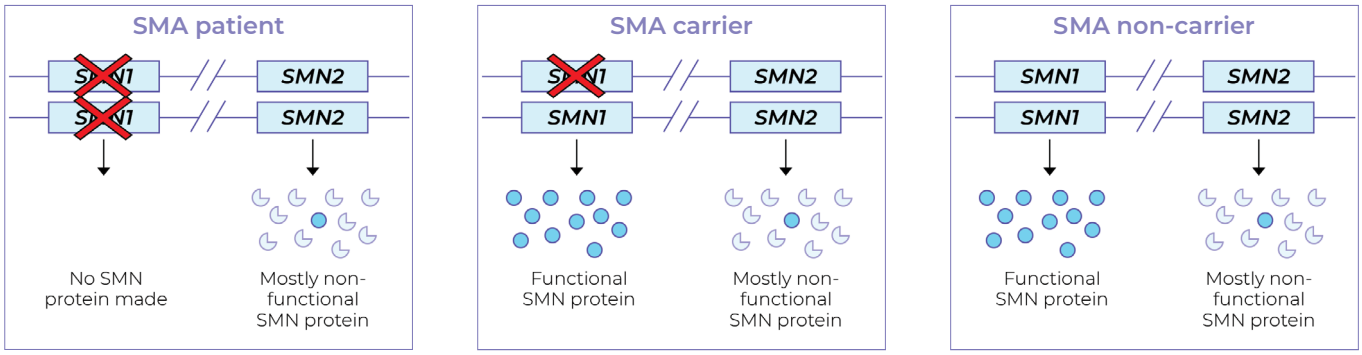
SMA is a life-threatening genetic neuromuscular disorder that affects the nerve cells controlling the muscles. SMA results in neuronal degeneration and muscular atrophy. Worldwide, SMA affects 1 in 6,000-10,000 live births. There are four types of SMA, distinguished by age of disease onset and severity. The most severe type, type 1, has an onset before 6 months of age and those affected, if left untreated, typically do not survive past two years of age.

The increased availability of SMA treatments has led to a corresponding interest in the addition of SMA to national newborn screening programs. This is especially important as new research suggests that earlier treatment is more effective, even in pre-symptomatic patients.

What causes SMA?

SMA is caused by an insufficient amount of survival motor neuron (SMN) protein in cells. The **SMN protein** ensures that motor neurons, responsible for transferring signals from brain to muscle cells, remain healthy. If the SMN protein is lacking or present at a very low level, motor neurons die and muscles wither away.

The SMN protein is primarily produced from the ***SMN1* gene**. SMA patients have no functional copies of the *SMN1* gene from which SMN protein can be produced. People with one *SMN1* copy are SMA carriers: they do not have SMA symptoms. However, a carrier couple may have children with SMA.



The highly similar ***SMN2* gene** also plays a role in SMA. *SMN2* mostly generates non-functional SMN protein, and a tiny amount of functional SMN protein. This difference in *SMN1* and *SMN2* protein stability is the result of a single nucleotide difference (a C>T change in exon 7), resulting in different splicing.

SMN2 copy numbers vary between individuals in the population. For SMA patients, the more *SMN2* copies they have, the less severe their symptoms tend to be. Quantifying *SMN2* copies is therefore important for disease prognosis. In addition, a patient's *SMN2* copy number can affect treatment options.

SALSA® MC002 SMA Newborn Screen: MRC Holland's solution for SMA neonatal screening

MRC Holland's SALSA® MC002 SMA Newborn Screen is based on melt curve analysis: a simple and affordable technique that utilises the fact that different genetic sequences have different DNA melting temperatures. Making use of an amplification and probe binding-based approach, peaks specific for *SMN1* and *SMN2* are generated. The method is highly sequence specific, sensitive, and easy to perform. The assay accurately determines the presence or absence of the *SMN1* and *SMN2* gene, reliably identifying SMA patients (0 *SMN1* copies) - but not carriers (1 *SMN1* copy).

Confirmation of MC002 SMA Newborn Screen results on patients' dried blood spot (DBS) extracts can be done using the SALSA® MLPA® Probemix P021 SMA. With P021 SMA, *SMN2* copy number is also directly determined for quick prognosis and treatment.

MRC Holland's MLPA assays are the worldwide market leader in diagnostic tests for SMA. MRC Holland's latest arrival, MC002 SMA Newborn Screen, was developed in close collaboration with top institutes involved in neonatal screening and SMA diagnosis.

SALSA® MC002 SMA Newborn Screen: meeting your SMA newborn screening needs

- ✓ **Reliable:** sensitivity (95-100%) and specificity (100%) for SMA patient detection
- ✓ **Fast and simple:** from DBS punch to results in 4 hrs, no DNA purification needed
- ✓ **No carrier detection:** added advantage for newborn screening programs
- ✓ **Low cost:** high volume discounts available
- ✓ **CE-marked** for IVD use in newborn screening
- ✓ **Minimal start-up costs:** only a thermocycler with melt curve capability needed
- ✓ **Robust:** less sensitive to sample carry-over contamination than qPCR tests
- ✓ **Controls:** threshold controls supplied in every kit and built-in DNA quantity control

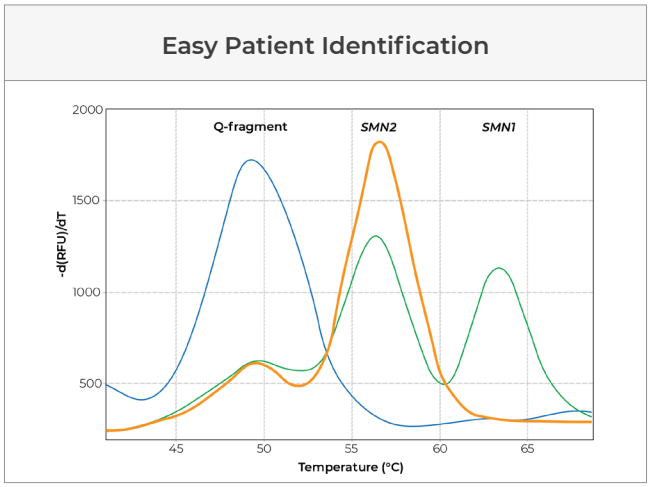


Fig. 1. MC002 SMA Newborn Screen results obtained on a **patient sample**, a **healthy control** and a **no DNA control** reaction. The absence of the *SMN1* exon 7 target in the patient is clearly discernible by the complete absence of a peak for *SMN1*. (Q-fragment: internal control for DNA quantity; high peak indicating insufficient DNA.)

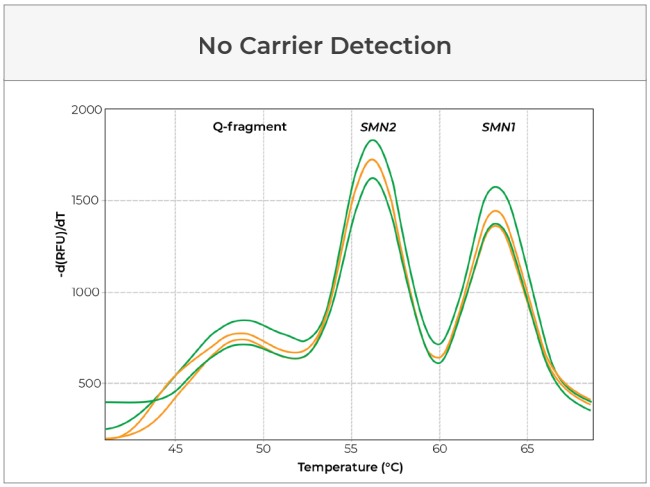


Fig. 2. MC002 SMA Newborn Screen results only show the *SMN1*:*SMN2* ratio, not absolute copy numbers. SMA carriers can therefore not be discerned from other genotypes with the same ratios (*SMN1*:*SMN2* 1:1 = 2:2; etc). This is advantageous as it is undesirable to identify carriers in newborn screening programs.

"The MC002 SMA Newborn Screen showed the feasibility and accuracy of SMA screening in a neonatal screening program"

A clinical performance study by national neonatal screening lab Isala (the Netherlands) using SALSA® MC002 SMA Newborn Screen on anonymised dried blood spot (DBS) cards (47 SMA patients; 375 controls) found 100% diagnostic sensitivity and specificity. MC002 SMA Newborn Screen was able to detect the absence of the *SMN1* exon 7 DNA sequence, thereby reliably discriminating *SMN1* from its genetic homolog *SMN2*. Furthermore, the assay did not detect asymptomatic carriers - an added advantage in newborn screening. The test's concordance with the second-tier 'gold standard' SALSA® MLPA® Probemix P021 SMA was 100%.

Strunk et al. (2019). Validation of a Fast, Robust, Inexpensive, Two-Tiered Neonatal Screening Test algorithm on Dried Blood Spots for Spinal Muscular Atrophy. Int. J. Neonatal Screen 5, 2

SMA newborn screening confirmation by SALSA® MLPA® Probemix P021 SMA

The perfect follow-up for MC002 SMA Newborn Screen

- ✓ **Input:** DBS cards or peripheral blood
- ✓ ***SMN1* + *SMN2* quantification** for rapid confirmation & prognosis
- ✓ **Reliable:** high sensitivity (95-100%) and specificity (100%)
- ✓ **CE-marked** for IVD use in newborn screening

SALSA® MLPA® Probemix P021 SMA is the second tier step in screening neonates for SMA. The ~1:10,000 individuals in which SALSA® MC002 SMA Newborn Screen detects an absence of the *SMN1* exon 7 sequence are subsequently investigated with P021 SMA, widely considered to be the gold standard in SMA patient detection. The P021 SMA assay is able to not only confirm the absence of the clinically relevant *SMN1* gene, but also to quantify the disease-modifying *SMN2* gene. Finally, P021 SMA is CE-marked to be used on DNA extracted from both peripheral blood and DBS cards, making it the perfect follow-up solution.

MRC Holland: market leader in SMA testing

As the market leader in diagnostic SMA tests, MRC Holland offers four different CE-marked assays for SMA that fit the complete range of genetic testing needs.

		MC002 SMA Newborn Screen	P021 SMA	P060 SMA Carrier	P460 SMA (Silent) Carrier
Technique		Melt Assay	MLPA	MLPA	MLPA
Used for	Neonatal Screening	●	○	○	
	Patient		●	○	○
	Carrier		○	●	●
	Silent Carrier[#]				●
Coverage	*SMN1* exon 7	✓[Δ]	✓	✓	✓
	SMN1 exon 8		✓	✓	✓
	SMN2 exon 7	✓[Δ]	✓	✓	✓
	SMN2 exon 8		✓	✓	
	SMN1+2 exon 1-6		✓		
	SMN1+2 exon 7+8		✓		
	Silent Carrier SNP probes				✓

● Primary test
○ Secondary test

[#] Increased detection of Silent Carriers: carriers with 2 *SMN1* copies on one allele + 0 on the other.
[Δ] No absolute copy numbers aside from 0 determined.

Unsure about which test is right for your lab?
We are here to help you, email info@mrcholland.com.

MRC Holland

Confidence in Copy Number Determination

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