DETECTION OF *SMN1* LOSS WITH PCR-BASED SCREENING TEST

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Spinal muscular atrophy (SMA) is an inherited neuromuscular disease characterized by progressive skeletal muscular weakness and atrophy. The newborn screening for spinal muscular atrophy should define all molecular forms of SMA. The aim of this study is to compare a PCR-based test for detection of homozygous SMN1 loss with multiple ligation probe amplification (MPLA) in patients with spinal muscular atrophy and other numerical changes of the *SMN1* gene. PCR-based test was used to detect exon 7 of *SMN1* gene homozygous loss. The study included 341 samples of patients with clinical suspicion of SMA from Biobank of Centre of Molecular Medicine of Pavlov State Medical University (Saint-Petersburg, Russia). Group 1 included 206 whole blood samples and Group 2 included 135 dried blood spot (DBS) samples. Copy number of the *SMN1* and *SMN2* genes had been evaluated with MLPA as a reference method. The results showed that kit was able to detect homozygous *SMN1* loss in all samples from group 1 and 2 (Group 1: n = 67; 33%; Group 2: n = 19; 14%). At the same time in all samples with 1–3 copies of the *SMN1* gene, the results of the kit were negative for homozygous loss of *SMN1* gene (Group 1: n = 139; 67%; Group 2: n = 116; 86%). Kit showed high effectiveness in the detection of homozygous loss *SMN1* gene. The kit detects all possible molecular forms of homozygous *SMN1* gene loss in both DNA samples extracted from the whole blood and DBS.

Keywords: Spinal muscular atrophy, *SMN1* gene, newborn screening, real-time PCR

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ДЕТЕКЦИЯ РАЗЛИЧНЫХ ФОРМ ПОТЕРИ ГЕНА *SMN1* С ПОМОЩЬЮ НАБОРА ДЛЯ ПЦР-РВ

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Проксимальная спинальная мышечная атрофия 5q (5q-СМА) — аутосомно-рецессивное нервно-мышечное заболевание, характеризующееся потерей двигательных нейронов в передних рогах спинного мозга. С 2023 г. СМА включена в обязательный неонатальный скрининг на территории Российской Федерации. Неонатальный скрининговый тест на 5q-СМА должен выявлять все типы гомозиготной потери гена *SMN1*. Целью исследования была сравнительная оценка возможности определения гомозиготной потери экзона 7 гена SMN1 с помощью теста на основе ПЦР-РВ с методом MLPA у пациентов с 5q-СМА, а также с различными изменениями числа копий гена *SMN1*. С помощью набора было проанализировано 206 образцов ДНК (группа 1), выделенных и очищенных из цельной крови, и 135 образцов ДНК (группа 2), выделенных и очищенных из сухих пятен крови, с известным количеством копий генов *SMN1* и *SMN2*. Количество копий генов *SMN1* и *SMN2* определяли методом MLPA, который был выбран в качестве референсного метода. Показано, что набор обнаруживает гомозиготную потерю гена *SMN1* во всех образцах, у которых подтверждена гомозиготная потеря гена *SMN1* с помощью MLPA (группа 1: n = 67; 33%; группа 2: n = 19; 14%). В то же время во всех образцах с 1–3 копиями гена *SMN1* результаты набора были отрицательными (группа 1: n = 139; 67%; группа 2: n = 116; 86%). Набор демонстрирует высокую эффективность и позволяет обнаруживать все возможные молекулярные формы гомозиготной потери гена *SMN1* как в образцах ДНК, выделенных из цельной крови, так и из сухих пятен крови.

Ключевые слова: спинальная мышечная атрофия, ген *SMN1*, неонатальный скрининг, ПЦР-РВ

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Proximal spinal muscular atrophy 5q (5q-SMA) is autosomal recessive neuromuscular disease, characterized by the loss of motor neurons in the anterior horns of the spinal cord, that leads to progressive muscle weakness and skeletal muscle atrophy. 5q-SMA is caused by the mutation in the survival motor neuron 1 gene (*SMN1*), situated on the chromosome 5q13. The incidence of SMA is 1 per 6000–10,000 newborns [1]. Rapid and irreversible loss of the motor neurons in SMA begins during the first 3 months of life, and 95% of motor neurons are affected in the patients under the age of 6 months [2]. Delayed molecular genetic diagnosis of SMA leads to untimely initiation of the treatment, which causes a limited clinical effectiveness of therapeutical intervention.

High incidence of the disease is related to the innate genetic instability of 5q13 region due to the presence of an inverted sequence of functional genes *SMN1*, *SERF1A*, *NAIP*, *GTF2H2A* and their centromeric pseudogenes *SMN2*, *SERF1B*, *NAIPΔ5*, *GTF2H2B* [1]. In 95% of cases, 5q-SMA results from the homozygous loss of the *SMN1* gene. The remaining cases of the disease occur in compound heterozygotes when the loss of the *SMN1* gene on one allele is combined with point mutations in the *SMN1* gene on another allele [3]. Today these forms of SMA are not detected in neonatal testing programs in any country of the world [4]. The prevalence of heterozygous loss of the *SMN1* gene in the Russian population is 1 : 36 [5]. Besides, the duplication of the *SMN1* is a frequent finding in general population, but it is not associated with SMA [1]. The pseudogene of the *SMN1* is the *SMN2* gene, which also consists of 9 exons and encodes the same SMN protein. The genes differ in 5 nucleotide in region from the 6-th intron to the 8-th exon. The key difference is exchange of cytosine to thymine in the 7-th exon of the *SMN2* gene (c. 840 C > T), which leads to the loss of the binding site for the exon splicing enhancer and the formation of the binding site for the exon silencer. As a result of this nucleotide change, the 7-th exon is excluded in 90% of *SMN2* transcripts. The resultant *SMNΔ7* protein becomes functionally defective and unstable, which causes its rapid degradation by the ubiquitin-proteasome system [1]. Nevertheless 10% of SMN protein, produced from *SMN2* gene, retains 7-th exon and can perform its function [6]. Therefore, copy number variation of *SMN2* can modify the severity of the disease in persons with *SMN1* deletion. SMA type 0 is characterized by absence of SMN1 gene and 1 copy of *SMN2* gene. This SMA form presents antenatally with severely decreased fetal movements. SMA type I (Werdnig–Hoffmann disease) is the most frequent type of SMA, accounting for approximately half of patients (*SMN2* gene — 2–3 copies) [3,7]. Affected infants appear normal at birth, but subsequently develop hypotonia, delayed motor milestones, and feeding difficulties within the first 6 months of life. Infants with SMA type I never sit independently. In the less severe SMA type II (Dubowitz disease), children present between 6 and 18 months of age and are able to sit independently, but never walk. The majority of these patients survive into adulthood (*SMN2* gene — 2-4 copes). SMA type III (Kugelberg–Welander disease) includes patients who walk at some point (and for any period of time) during childhood (*SMN2* gene — 3–5 copies). SMA type IV is the mildest and least common form of SMA with onset in adulthood [3,7].

The loss of functional *SMN1* genes plays the main role in the pathogenesis of this disease. There are several types of the *SMN1* gene loss: complete deletion, conversion of the *SMN1* gene into *SMN2*, the formation of hybrid *SMN1/SMN2* structures, and loss of only a few exons of the *SMN1* gene (partial deletion). The complete deletion of the *SMN1* gene occurs without an increase in the number of copies of the *SMN2* gene. Partial deletion is characterized by the isolated loss of several exons, including 7 exon, of the *SMN1* gene without changes of the number of copies of these exons in the *SMN2* gene [8]. During the conversion, loss of *SMN1* gene is accompanied by an increase in the number of copies of the *SMN2* gene [9]. The formation of chimeric *SMN1/SMN2* genes, in which there are sites belonging to both the *SMN1* gene and the *SMN2* pseudogene and which are characterized by an homozygous loss *SMN1*'s 7 exons and heterozygous loss *SMN1*'s 8 exon and unequal ratio of 7 and 8 exons in the *SMN2* gene, is another mechanism for the loss of the *SMN1* gene [10]. It should be noted that in patients with SMA, homozygous loss of the *SMN1* gene can be associated with both the individual types of "deletion" described, and with combined cases.

Restitution of SMN protein levels in patients with proximal SMA is valuable treatment option. Experimental models have shown that the time of switching off the SMN protein determined the life expectancy — the earlier the shutdown occurs, the worse observable survival [11]. Moreover, early reconstitution of SMN protein in SMA-models leaded to almost complete recovery [12]. In accordance with this data, presymptomatic therapy in newborns who have a pathological aberration in the *SMN1* gene has been investigated. The phase 2 NURTURE study demonstrated substantial clinical benefit of presymptomatic nusinersen therapy in infants with two or three copies of the *SMN2* gene [13]. In a multicenter phase III clinical trial for gene replacement therapy (SPR1NT), which was aimed at studying the efficiency and the safety of onasemnogene abeparvovec treatment in children with biallelic deletion of the *SMN1* gene and 3 copies of the *SMN2* gene (SMA type 2, respectively), recently published results also confirmed high clinical efficiency of presymptomatic treatment of children with SMA [14]. The available therapy and the possibility of effective usage of disease-modifying treatment in presymptomatic stage of disorder currently justify the screening of newborns for 5q-SMA. Australia, Belgium, Canada, Germany, Italy, Japan, Norway, the Netherlands, Poland and Taiwan, as well as 46 states in the United States have introduced mandatory screening of newborns for SMA. Mandatory screening for SMA for all newborns was launched in 2023 in the Russian Federation [15].

Multiplex ligation probes amplification (MLPA) is the "gold standard" for the diagnosis of SMA, which is able to detect not only homozygous deletions of the *SMN1* gene, but also other types of numerical changes in the *SMN1* and *SMN2* genes. However, this method is not applicable as a screening method due to the complexity and high cost [16, 17]. The most acceptable screening method for the homozygous loss of the *SMN1* gene is various modifications of the polymerase chain reaction method with real-time signal detection (RT-PCR), detecting the homozygous loss of the 7-th exon of the *SMN1* gene. The benefits of this approach have been confirmed by a number of studies and meta-analyses [18, 19, 20]. Taking into account the high clinical relevance of early diagnosis of SMA, as well as the beginning of mandatory screening for SMA of all newborns in the Russian Federation from 2023, there is a need for diagnostic molecular genetic tests that would allow identifying all possible forms of homozygous loss of the *SMN1* gene with high accuracy. It is also should be noted, that one of the most important requirement for neonatal screening test for SMA is possibility to detect homozygous loss of *SMN1* gene in dried blood spots (DBS) samples.

Russian company «DNA-Technology» recently issued new PCR kit for molecular screening for SMA in newborn, but

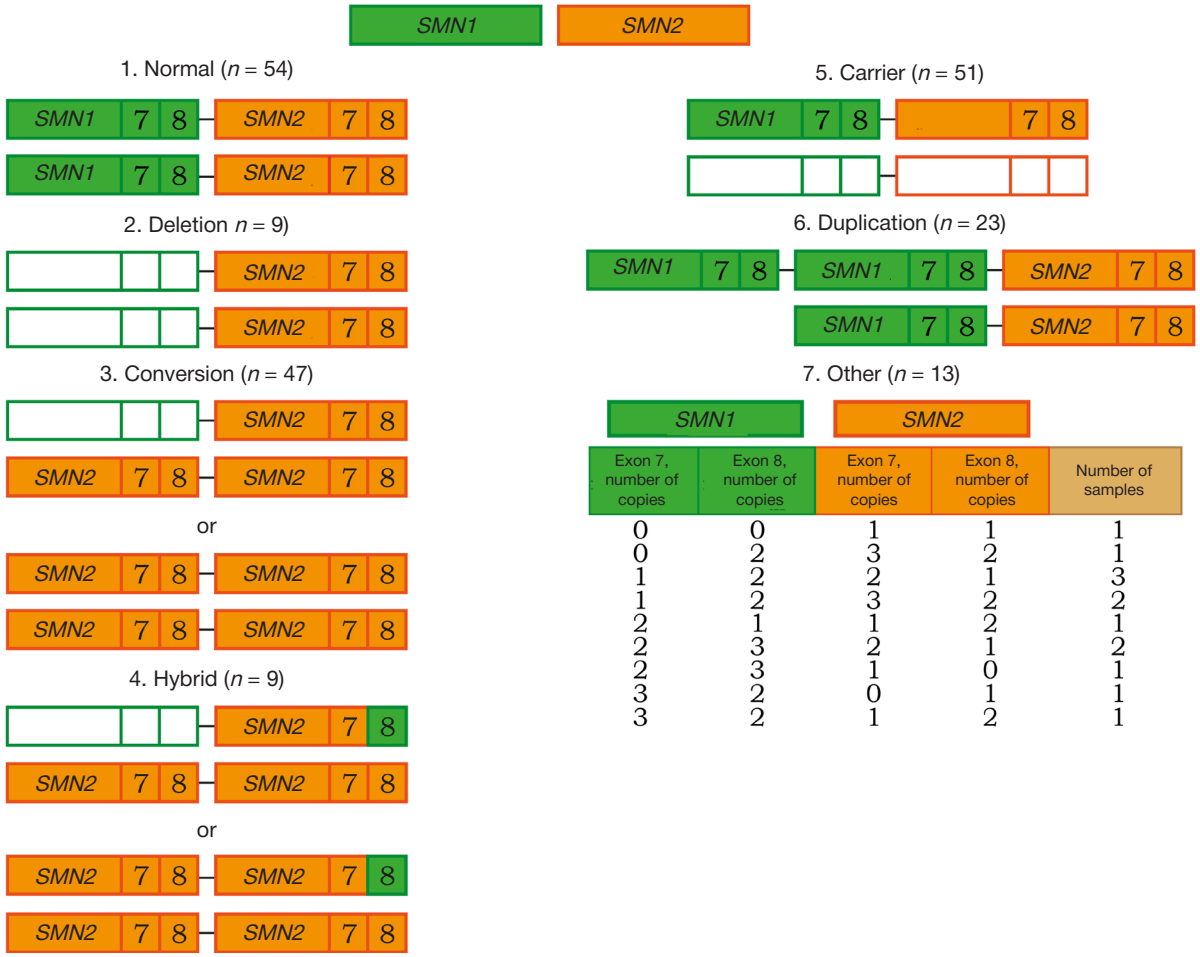

Fig. Schematic representation of *SMN1* and *SMN2* genotypes in each subgroup

extended approbation of this kit for detection of all form of *SMN1* gene loss, detected by MLPA test, is highly needed.

The aim of this study is to compare a PCR-based screening test for detection of homozygous *SMN1* loss with multiple ligation pro be amplification in patients with spinal muscular atrophy and other numerical changes of *SMN1* gene.

METHODS

Sample collection and DNA extraction and purification

DNA samples extracted and purified from the whole blood (Group 1)

To evaluate possibility of detection of different types of *SMN1* copy number variation the cohort consisted of 206 samples from Biobank of Centre of Molecular Medicine of Pavlov State Medical University (Saint-Petersburg, Russia) was collected. Patients with clinical suspicion of SMA were referred to laboratories of Pavlov Medical University from 2019 to 2021. Genomic DNA was extracted from peripheral blood using ExtractDNA Blood & Cells (Evrogen; Russia) according to the manufacturer's instructions, and adjusted to a final concentration of 50 ng/μl. Copy number of *SMN1* and *SMN2* genes had been routinely evaluated with multiplex ligation-dependent probe amplification (MLPA) assay using SALSA® MLPA® Probemix P060-B2 SMA Carrier (MRC-Holland®; The Netherlands) in accordance with manufacturer's instructions.

All the samples were divided into 7 subgroups according to *SMN1* and *SMN2* genotype status. Subgroup 1 «Normal» (54 samples): 2 copies of SMN1 and *SMN2* genes — so called «Reference genotype». Subgroup 2 «Deletion» (9 samples): absence of *SMN1* gene without increase of SMN2 copy number. Subgroup 3 «Conversion» (47 samples): absence of *SMN1* gene with increase of *SMN2* copy number. Subgroup 4 «Hybrid» (9 samples): homozygous deletion of exon 7 and heterozygous deletion of exon 8 SMN1 gene with increase of exon 7 *SMN2* copy number. Subgroup 5 «Carrier» (51 samples): heterozygous deletion of *SMN1* gene. Subgroup 6 «Duplication» (23 samples): 3 copies of *SMN1* gene. Subgroup 7 «Other» (13 samples): different combinations of *SMN1* and *SMN2* copy number that did not meet the criteria of other subgroups. Confidence interval for diagnostic sensitivity and specificity was chosen as 95% according to MedCalc® instrument (https://www.medcalc.org/calc/diagnostic_test.php) (Figure).

DNA samples extracted and purified from DBS (Group 2)

To confirm the possibility of detecting a homozygous loss of *SMN1* in DNA isolated from DBS samples, a group of 135 patients, whose blood was transported in a dried state on a membrane, was collected from Biobank of Centre of Molecular Medicine of Pavlov State Medical University (Saint-Petersburg, Russia). Patients with clinical suspicion of SMA were referred to laboratories of Pavlov Medical University from 2019 to 2021. Genomic DNA was extracted from DBS using PREP-CITO DBS (DNA-Technology; Russia) according to the manufacturer's instructions. For all patients copy number of *SMN1* and *SMN2* genes was evaluated by SALSA® MLPA® Probemix P060-B2

Table 1. The number of copies *SMN1* and *SMN2* genes according to MLPA assay in Group 2

Subgroup	MLPA assay results				Number of samples
	SMN1 gene		*SMN2* gene		
	Exon 7	Exon 8	Exon 7	Exon 8	
Normal	2	2	4	4	10
	2	2	3	3	35
	2	2	2	2	54
Deletion	0	0	2	2	3
Conversion	0	0	4	4	2
	0	0	3	3	11
Hybrid	0	1	3	2	2
Carrier	1	1	2	2	9
Other	0	1	2	1	1
	1	2	1	1	4
	1	2	3	2	4

SMA Carrier (MRC-Holland®, Netherlands) in accordance with protocol for DBS membrane.

All the samples were divided into 6 subgroups: «Normal», «Deletion», «Conversion», «Hybrid», «Carrier», «Other» (Table 1).

Multiplex ligation-dependent probe amplification (MLPA) assay

The MLPA assay was performed using SALSA® MLPA® Probemix P060-B2 SMA Carrier (MRC-Holland®; The Netherlands) following the manufacturer's instructions for DNA extracted and purified from whole blood (Group 1) and from DBS membrane (Group 2). The commercial kit contains 17 reference probes and 4 specific probes detecting sequences presented in exon 7 and 8 of either SMN1 and *SMN2* genes. MLPA products were analyzed with ABI 3500 genetic analyzer (Thermo Fisher Scientific®; USA). The relative peak height of each sample was calculated and compared with normal controls using MLPA analysis application by GeneMarker® software (SoftGenetics®; USA). The evaluation criteria of deletion/duplication were based on the MLPA kit instructions.

PCR-based screening test

The NeoScreen SMA/TREC/KREC was performed for all patients from Group 1 and Group 2. NeoScreen SMA/TREC/KREC Real-time PCR Detection Kit (DNA-Technology, Russia) is intended to detect exon 7 of *SMN1* gene homozygous deletion and assess levels of T cell receptor excision circles (TREC) and kappa-deleting recombination excision circle (KREC) in newborns' dried blood spots (DBS) of whole blood for spinal muscular atrophy and primary immunodeficiencies screening by real-time PCR. The method is based on amplification of TREC, KREC, exon 7 of *SMN1* gene, and a fragment of the normalizing gene LTC4S (or endogenous internal control (IC), a single-copy genomic locus of Leukotriene C4 Synthase gene) by multiplex polymerase chain reaction (PCR). The use of several fluorescent dyes allows to simultaneously register the results of different amplification reactions taking place in the same tube. Table 2 shows detection channels of amplification products.

NeoScreen SMA/TREC/KREC assay was carried out using DTprime Real-time Detection Thermal Cycler (DNA-Technology;

Table 2. Detection channels of PCR-products

Fam	Hex	Rox	Cy5
TREC	*SMN1* (exon 7 of *SMN1* gene)	KREC	IC (Internal control)

Table 3. PCR cycling conditions

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurements	Type of the step
1	80	2	00	1		Cycle
	94	5	00			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	10	…	…	Holding		Holding

Table 4. Comparison of the results obtained by MLPA assay and PCR-based test assay technology in Group 1

Subgroup	Number of samples in each subgroup	MLPA assay results (number of samples depending on exon 7 *SMN1* copy number)			Test results (number of samples depending on exon 7 *SMN1* copy number)		TP	FP	TN	FN
		>1	1	0	Normal (≥1)	Deletion (0)				
1. Reference	54	54	0	0	54	0	0	0	54	0
2. Deletion	9	0	0	9	0	9	9	0	0	0
3. Conversion	47	0	0	47	0	47	47	0	0	0
4. Hybrid	9	0	0	9	0	9	9	0	0	0
5. Carrier	51	0	51	0	51	0	0	0	51	0
6. Duplication	23	23	0	0	23	0	0	0	23	0
7. Other	13	6	5	2	11	2	2	0	11	0
Total	206	83	56	67	139	67	67	0	139	0

Note: TP — true positive; FP — false positive; TN — true negative; FN — false negative.

Protvino, Russia) by following the manufacturer's instructions (Table 3.). To assess the performance of the assay a positive control (included in PCR Detection Kit) and a negative control were included in each PCR-run. Positive control C+ № 1, containing DNA plasmids with specified targets — *TREC*, *KREC*, *SMN1*, *IC*, in equal concentration, is intended to evaluate PCR efficiency. Positive control C+ № 2 with plasmid equivalent exon 7 of *SMN2* gene allows to assess blocking of *SMN2* amplification (control of the *SMN1* amplification specificity).

Analysis of SMN1 exon 7 deletion is based on the estimation of the indicator cycle difference (Δ Cp) between the Cp of SMN1 (Hex channel) and IC (Cy5 channel) (ΔCp = Cp (Hex) – Cp (Cy5)).

Evaluation of homozygous deletion of exon 7 of *SMN1* gene was performed according to the PCR Detection Kit instruction for result's analysis algorithm (performed automatically by the thermocycler software).

RESULTS

The PCR-based screening method was used on 341 samples with known *SMN1* and *SMN2* copy numbers (DNA extracted and purified from whole blood — Group 1, 206 samples; DNA extracted and purified from DBS membrane — Group 2, 135 samples). The MLPA assay was used as a reference method (Table 4, Table 5). The PCR-based test results was considered true positive if homozygous deletion of exon 7 *SMN1* gene is detected for samples with 0 copies of exon 7 *SMN1* gene, established by MLPA assay. Presence of at least one exon 7 of *SMN1* gene identified by MLPA assay and detection of exon 7 *SMN1* signal by test was considered true negative.

The results in Group 1 were as follows. In subgroup 1 «Normal» 54 participants (26%) carried 2 copies of exon 7 SMN1 gene and all samples were true negative by PCR-based screening. In subgroup 2 «Deletion», subgroup 3 «Conversion», subgroup 4 «Hybrid» all samples were true positive (n = 65, 32%). In subgroup 5 «Carrier» all 51 participants (25%) had single copy of searched exon and all of the were true negative. In subgroup 6 «Duplication» all 23 samples (11%) carries 3 copies of exon 7 *SMN1* were true negative by PCR protocol. Finally subgroup 7 «Other» consisted of 13 probes with different *SMN1* and *SMN2* exon count. 6 samples (3%) carried more than 1 copy of exon 7 *SMN1* gene and were true negative. 5 samples (2%) in group 8 had single copy of *SMN1* gene and also were true negative. And 2 probes did not contain any of exon 7 *SMN1* and were true positive (1%). See results in Table 4.

The results in Group 2 are presented below. In subgroup «Normal» 99 participants (73%) had 2 copies of exon 7 *SMN1* gene and all samples were true negative by PCR-based screening. In subgroup «Deletion», subgroup «Conversion», subgroup «Hybrid» all samples were true positive by PCR-based screening (n = 18, 13,3%). Group 2 did not contain samples with *SMN1* gene duplication according to MLPA assay. In subgroups «Carrier» all 9 samples (7%) were true negative. In subgroup «Other» 1 probe (0,7%) did not contain any of exon 7 *SMN1* and were true positive. And 8 samples (6%) had single copy of *SMN1* gene and were true negative by PCR protocol. See results in Table 5.

Table 5. Comparison of the results obtained by MLPA assay and PCR-based test assay technology in Group 2

Subgroup	Number of samples in each subgroup	MLPA assay results (number of samples depending on exon 7 *SMN1* copy number)			Test results (number of samples depending on exon 7 *SMN1* copy number)		TP	FP	TN	FN
		>1	1	0	Normal (≥1)	Deletion (0)				
1. Reference	99	99	0	0	99	0	0	0	99	0
2. Deletion	3	0	0	3	0	3	3	0	0	0
3. Conversion	13	0	0	13	0	13	13	0	0	0
4. Hybrid	2	0	0	2	0	2	2	0	0	0
5. Carrier	9	0	9	0	9	0	0	0	9	0
6. Duplication	0	0	0	0	0	0	0	0	0	0
7. Other	9	0	8	1	8	1	1	0	8	0
Total	135	99	17	19	116	19	19	0	116	0

Note: TP — true positive; FP — false positive; TN — true negative; FN — false negative.

DISCUSSION

Spinal muscular atrophy is an autosomal recessive neurodegenerative disease, characterized by progressive skeletal muscle weakness. A laboratory test for neonatal screening of SMA should detect all types of *SMN1* loss in DNA isolated and purified from whole samples and from DBS samples.

There are several methodological approaches for homozygous *SMN1* loss, however, different modifications of real-time PCR proved to be the most suitable for neonatal SMA screening [16, 21]. Approaches, based on PCR, are highly robust, characterized by a very low number of false negative and false positive results, simple in handling, have a low cost and compatible with DBS samples [2, 22]. Screening for homozygous *SMN1* loss with test is based on the detection of homozygous loss of the 7 exon of *SMN1* gene by real-time PCR [6].

SMN1 gene is located in a highly unstable genome region, which is saturated with repeating inverted genes and Alu-sequences. Due to that fact, there is a wide spectrum of genetic aberrations, characterized by variation in copy number of *SMN1*. In order to assess the specificity of detection of homozygous *SMN1* loss with PCR-based kit in Group 1, which consist of DNA samples extracted and purified from the whole blood, 139 samples with 1–3 copies of *SMN1* gene were selected (subgroups «Normal», «Carrier», «Duplication», 11 samples from subgroup «Other»). In all 139 samples screened for homozygous *SMN1* loss with PCR-based test results were negative. Predictably, this emphasizes that a benign change in the copy number of the *SMN1* genes does not affect the effectiveness of detection of homozygous *SMN1* loss.

Detection of different types of homozygous loss of *SMN1* is the main task of neonatal SMA screening. In Group 1 PCR-based kit was able to detect homozygous *SMN1* loss in all samples (n = 67), included in the study. PCR-based test showed high sensitivity and specificity for detection of SMN1 loss. This is in line with a number of studies, dedicated to the methodological problem of SMA screening [2, 23].

To confirm the effectiveness of the kit to detect a homozygous loss of *SMN1* gene in DNA isolated from DBS samples, patients in Group 2 were selected. For all patients DNA was isolated and purified from DBS samples by PREP-CITO DBS kit for subsequent evaluations by PCR-based test. For all patient MLPA analysis was also performed in parallel. In all samples with 1–3 copies of the *SMN1* gene (n = 116), the results of the test were negative for homozygous loss of *SMN1* gene. This highlights that a benign change in the copy number of *SMN1* genes does not affect the specificity of detecting homozygous loss of *SMN1* in DNA isolated from DBS samples with PCR-based test. For samples with 0 copies of exon 7 *SMN1* gene results of screening of homozygous *SMN1* loss by kit were positive for all patients.

A limitation of the PCR-based test is the inability to detect rare forms of 5q-SMA associated with heterozygous loss of one copy of the *SMN1* gene and pathogenic variants on the second copy of the gene (compound heterozygosity). According to research results, the prevalence of this form of 5q-SMA is up to 5% of all cases [3]. However, it should be noted that at the moment this subtype of the disease are not detected in neonatal testing programs in any country of the world [4]. When clinical signs and symptoms of 5q-SMA are identified and screening is negative for homozygous *SMN1* gene loss, an extended study is required to detect SMN1 gene loss at one allele in combination with *SMN1* gene point mutations on another allele.

CONCLUSIONS

This pilot study showed, that PCR-based screening test is able to detect homozygous *SMN1* gene loss in both DNA samples extracted and purified from the whole blood and samples DNA isolated from DBS. Also, according to the results of this study, the test detects all possible molecular forms of homozygous *SMN1* gene loss.

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