EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Eonis SCID-SMA Kit
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN200044

B Applicant

PerkinElmer Inc.

C Proprietary and Established Names

Eonis SCID-SMA Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QUE	Spinal Muscular Atrophy newborn screening test system	21 CFR 866.5980	Immunology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the Eonis SCID-SMA Kit

B Measurand:

Survival Motor Neuron 1 (SMN1)

C Type of Test:

Multiplex polymerase chain reaction (PCR)

III Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Eonis™ SCID-SMA kit is intended for the qualitative detection of the SMN1 gene exon 7 as an aid in screening newborns for Spinal Muscular Atrophy (SMA). The test is intended for DNA from blood specimens dried on a filter paper and for use on the QuantStudio™ Dx Real-Time PCR instrument.

This test is only intended for use for screening of SMA that bear the homozygous deletion of SMN1 exon 7.

This test is not intended for use as a diagnostic test and a positive screening result should be followed by confirmatory testing.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For in vitro diagnostic use.

D Special Instrument Requirements:

QuantStudio Dx real-time PCR instrument

IV Device/System Characteristics:

A Device Description:

The Eonis SCID-SMA kit contains reagents to detect three biomarkers: TREC, KREC and exon 7 in the SMN1 gene. Detection of TREC and KREC was cleared in K203035.

The newborn screening workflow for the Eonis SCID-SMA kit includes:

- Two liquid handling platforms (one for DNA extraction and one for PCR master mix setup)
- QuantStudio Dx Real-Time PCR instrument
- Eonis Analysis Software

Each Eonis SCID-SMA kit contains reagents for up to 384 reactions or 1152 reactions including kit controls. The kit contents are listed in Table 1. Materials required but not provided include the Eonis DNA Extraction Kit, Eonis Analysis Software and consumables (Table 2).

Table 1. Eonis SCID-SMA Kit content

Component	Quantity
SCID-SMA Kit Controls	2 filter paper cassettes containing 4 sets of dried blood spots for 384 reaction kit 4 filter paper cassettes containing 8 sets of dried blood spots for 1152 reaction kit

C1 Analyte-negative (TREC/KREC/SMN1) control	
C2 Low TREC/KREC and normal SMN1 control	
C3 High TREC/KREC and normal SMN1 control	
PCR Reagent 1	1 vial, 2.7 mL for 384 reaction kit 3 vials, 2.7 mL for 1152 reaction kit
PCR Reagent 2	1 vial, 2.7 mL for 384 reaction kit 3 vials, 2.7 mL for 1152 reaction kit
Lot-specific quality control certificate	1 pc

Table 2: Materials required to be used with the Eonis SCID-SMA Kit

Item	Product number	Note
Eonis™ DNA Extraction kit 3240-001U	3240-001U	
QuantStudio™ Dx Real-Time PCR instrument	2031-0030 or 2031-0040	Appropriately configured to run Eonis SCID-SMA kit
Liquid Handling Equipment	JANUS® extraction Instrument (prod. no. CJM8M01) Or JANUS Extraction Mini instrument (prod. no. CJSM001)	Appropriate manual pipettors, or equivalent equipped with necessary accessories and consumables that are capable of transferring and dispensing 3 µL or more into a 384-well or a 96-well PCR Plate with enough accuracy and precision (CV% ≤ 10%), e.g., JANUS Extraction Instrument (prod. no. CJM8M01) or a JANUS Extraction Mini instrument (prod. no. CJSM001).
	JANUS PCR Mastermix instrument (prod. no. CJS8001)	Appropriate manual pipettors, or equivalent and/or capable of transferring and dispensing 12µL or more into a 384-well PCR Plate with precision (CV%) ≤ 10%, e.g., JANUS PCR Mastermix instrument (prod. no. CJS8001).
Centrifuge(s)	-	Capable of centrifuging the 96-well PCR Plate) or the 384-well PCR Plate at 350 x g or above for 5 minutes.
Vortexer(s)	-	Capable of vortexing plates and tubes/vials.
Eonis Analysis Software	5020-1000	-

For a detailed list of required but not provided reagents and consumables, refer to the product labeling (Eonis SCID-SMA Kit Instructions for use).

B Principle of Operation

The Eonis SCID-SMA Kit is a multiplex real-time PCR-based assay. It uses target sequence-specific primers and TaqMan™ probes to amplify and detect four targets: TREC, KREC, SMN1 exon 7 and RPP30, in the DNA extracted from newborn dried blood spot (DBS) using the Eonis DNA Extraction kit (3240-001U) in a single PCR reaction. Each TaqMan™ probe has a unique dye linked to their terminal 5'-end, allowing the simultaneous detection of the four targets if present. The amount of each target present in the DNA is determined by the intensity of fluorescence emitted by each dye released from the degraded probe during amplification and detected by the QuantStudio™ Dx Real-Time PCR instrument. The instrument measures fluorescence signals and converts them into comparative quantitative readouts which are expressed as a function of cycle threshold (Ct) values. RPP30 is used as an internal amplification control for DNA quality and to relatively quantitate TREC and KREC (copies/10^5 cells) using TREC ΔCt and KREC ΔCt. The complete Eonis SCID-SMA Kit workflow is depicted presented in Figure 1.

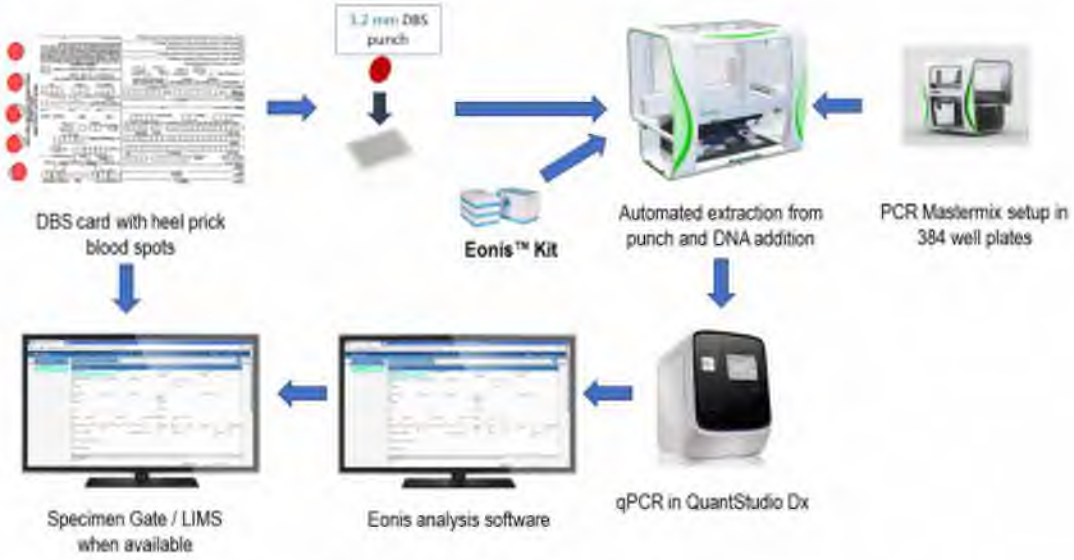

Figure 1: Eonis system workflow from DBS card to results

The Eonis Analysis Software provides algorithms such as automatically flagging quality control (QC) violations and interpreting results according to the cut-offs. SMN1 exon 7 deletion results are presented as: Qualitative result- Reported as "Presumptive Positive" or "Presumptive Normal".

Amplification and Detection

The Eonis SCID-SMA Kit uses the mature technologies, real-time PCR and TaqMan™ probe signal generation, for amplification and detection, respectively. Primers and probes sequences have been developed to selectively amplify human SMN1/SMN2 exon 7 sequences. As SMN1 and SMN2 genes are highly homologous, an SMN2 sequence-specific probe that has no terminal

5'-end modification serves as a blocker to prevent binding of the SMN1 probe to the SMN2 sequence. Target probes (excepting the SMN2 probe) have a unique dye linked to their terminal 5'-end, allowing the simultaneous detection of SMN1 exon 7 and RPP30 if present above the pre-determined signal threshold value and calculated using the QuantStudio™ Dx Real-Time PCR instrument software.

Instrument and Software

The Eonis SCID-SMA Kit is run on the QuantStudio Dx Real-Time PCR instrument (K123955). This system is a real-time nucleic acid amplification and detection platform that measures fluorescence signals and converts them into comparative quantitative readouts using fluorescent detection of dual-labeled hydrolysis probes.

The Eonis Analysis Software gives an interpretation of data obtained from the QuantStudio Dx Real-Time PCR instrument. The Eonis Analysis Software imports raw data from the file created by the QuantStudio Dx software. The Eonis Analysis Software checks the validity of the run, based on the run controls and detection of RPP30, and automatically interprets the results by indicating whether SMN1 exon 7 molecule has been detected for each specimen based on the analyte-specific clinical cut-offs. It uses an algorithm to interpret the patient samples results and generates a controlled formatted report.

Interpretation of Results

The Eonis Analysis Software interprets the results automatically. First, the software checks the integrity of the data file and the essential information of the data collection and analysis setting populated in the data file and generates the report or creates an error log entry if an error is detected. The software then checks the validity of the test controls and reports the presence of a valid or invalid result in the generated report. If one or more test control levels are reported as "Invalid", all the clinical specimens processed in the same 96-well DNA extraction plate will be reported as "Invalid" in the report (Table 3). The result validity of each analyte in a clinical specimen is further determined by the internal control, RPP30. The RPP30 Ct value should fall within pre-specified limits to guarantee that the samples contained adequate DNA. After all the validity checks and if no specific amplification is detected for SMN1, "SMN1 Not Detected" will be reported for that clinical specimen.

The SMN1 Ct cut-off value is pre-set in the Eonis Analysis Software and a qualitative result, "SMN1 Presumptive Positive" or "SMN1 Presumptive Normal", will be reported accordingly in the generated PDF report.

Table 3: SMN1 Result interpretation matrix

RPP30 Ct values		**SMN1 Ct values**	**Result interpretation for SMN1**
$Ct < 15.0$	Values not considered when RPP30 $Ct < 15.0$		Invalid*
$15.0 \leq Ct \leq 32.00$	AND	$Ct < 15.0$	Invalid*
	AND	$15.0 \leq Ct \leq 31.24$	Presumptive normal

	AND	Ct > 31.24 or no Ct	Presumptive positive*
Ct > 32.00 or no Ct	Values not considered when RPP30 Ct > 32.00 or no Ct		Invalid*

* Samples with initial invalid results should be repeated in singlicate and Presumptive positive results should be repeated in duplicate to obtain final result interpretation. If both retested samples give the Presumptive normal result, the final result interpretation is Presumptive normal. If one or more result is Presumptive positive, the final result interpretation is Presumptive positive.

When an invalid result is reported, the software will also report the cause of the failure in the generated report. Ct < 15.0 is typically caused by abnormal PCR curve, and the recommended corrective action is to repeat the PCR amplification or the test. RPP30 Ct > 32.00 or no Ct is typically caused by poor DNA extraction process, DBS loss and/or poor quality of the specimen, which results in low DNA yields or PCR inhibitor carryover. The recommended corrective action is to repeat the test and/or request a new specimen.

C Instrument Description Information

1. Instrument Name:
   QuantStudio Dx Real-Time PCR instrument

2. Specimen Identification:
   DNA extracted from newborn dried blood spot (DBS)

3. Specimen Sampling and Handling:
   The required sample type of the assay is a 3.2 mm specimen punch from a dried blood spot (DBS) into a 96-well plate. DNA is extracted and purified using a method developed internally by modifying and re-optimizing the protocols published by Gerstel-Thompson JL et al. and Saavedra-Matiz CA et al. The automated extraction can be completed manually or using a liquid handling platform such as JANUS, for processing up to four 96-well plates simultaneously. Please refer to Section VI.A.7 below for the DNA extraction equivalency study.

4. Calibration:
   N/A

5. Quality Control:
   Controls are used throughout the entire procedure from sample preparation to PCR as quality check for optimal performance of the Eonis test.

   The Eonis SCID-SMA Kit contains three multi-level, dried blood spot (DBS) controls: C1, C2 and C3 (Table 4). The DBS controls are filter paper cassettes with each control level being spotted in duplicate.

   C1 is an analyte-negative control and does not contain TREC, KREC and SMN1 analytes. C2 and C3 are analyte-positive controls that have the same level of SMN1 analyte but different levels of TREC and KREC analytes (lower level in C2 and higher level in C3). All three kit

controls have the same level of RPP30 analyte. Blank reaction wells without sample disks (NTC [no template control]) are used to monitor the absence of contamination.

Table 4: Description of test controls

Name	Type	Composition	Result Mimicked
C1	Analyte-Negative Control	Only RPP30 plasmid is spiked in. No TREC, KREC or SMN1 plasmid are added.	This control mimics an affected newborn (SCID, XLA and SMA screen positive).
C2	Low Level Control	RPP30 and SMN1 plasmids and a lower level of TREC and KREC plasmids are spiked in.	This control has normal levels of RPP30 and SMN1 and a TREC and KREC level below clinical cutoff for SCID and XLA.
C3	High Level Control	RPP30 and SMN1 plasmids and a higher level of TREC and KREC plasmids are spiked in.	This control mimics a normal newborn.
NTC	Blank	Contains no plasmids	Monitors the absence of contamination

Each Eonis run includes all four controls in duplicate in each 96-well DNA extraction plate. The newborn samples and DBS controls are punched into the plate, while the well reserved for the Blank control does not contain any punches. The Blank control undergoes the whole process, from DNA extraction to amplification, and the NTC sample consists of elution solution derived from the DNA extraction process.

The test result validation for each 96-well DNA extraction plate is based on the three kit DBS controls and the Blank reaction well. The Eonis Analysis software will identify each control by their designated locations and check their validity. The possible outcome for each control replicate is “Valid" or "Invalid.” If one or more controls are invalid, the results of the clinical specimens processed in the same 96-well DNA extraction plate are reported as “Invalid.” For the Blank reaction well, both replicates must meet the acceptance criteria for the run to be valid. For C1-C3 DBS controls, the results of both replicates must meet the acceptance criteria for the run to be valid. In the event that one of the replicates yields no result, it is acceptable to validate the run based on the remaining replicate to minimize the number of repeats.

V Standards/Guidance Documents Referenced:

CLSI Guideline EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents

CLSI Guideline EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures

CLSI Guideline EP17-A2: Evaluation of Detection Capability for Clinical Laboratory

Measurement Procedures:

CLSI Guideline EP07-A2: Interference Testing in Clinical Chemistry

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

Precision

The objective of this study was to demonstrate the precision of the Eonis SCID-SMA assay. The variation of the Eonis SCID-SMA assay was determined using 3 kit lots, 3 sets of Eonis test systems (including 3 designated CLS150056 JANUS Extraction Instruments, 3 designated CLS150057 JANUS PCR Mastermix Instruments, and 3 designated 2031-0010 QuantStudio™ Dx Real-Time PCR Instruments), 2 operators, and 54 runs over 23 calendar days with 13 representative SMA positive, carrier, and normal DBS samples. Analytical performance studies, including precision/reproducibility, were conducted using contrived samples consisting of cord blood or adult whole blood with hematocrit adjusted to neonate levels. The SMA positive sample was created by spiking SMN1 negative Coriell cells into leukocyte depleted blood and adjusting the hematocrit to 49.5. Sample characteristics are described in Table 5. Samples consisted of cord blood, adult blood or blends. Each run consisted of 1 plate with 2 replicates per sample in a randomized plate map. The total number of measurements was 108 per sample. All samples had 100% correct calls across all sample replicates, expect sample 9 where there was 1 incorrect call (105/106 replicates), resulting in a 95% lower bound confidence interval (CI) of 96.6% (Table 6). SMN1 Ct and RPP30 Ct precision of each sample by kit lot, instrument, days, and runs are shown in Tables 7 and 8, respectively.

Table 5: Summary table of samples used in analytical performance studies. Hematocrits were adjusted to approximately 46-51

Sample	SMN1
Sample 1	Normal
Sample 2	Normal
Sample 3	Normal
Sample 4	Normal
Sample 5	Normal
Sample 6	Normal
Sample 7	Normal
Sample 8	Normal
Sample 9	Normal
Sample 10	Carrier-like (Heterozygosity)
Sample 11	Homozygous deletion of exon 7

Sample	SMN1
Sample 12	Normal
Sample 13	Normal

Table 6: SMN1 precision data

Sample	N	N Below Cut-off	Proportion Below Cut-off (%) (with 95% CI)	N Above Cut-off	Proportion Above Cut-off (%) (with 95% CI)
1	107	107	100% (96.6% - 100%	0	0
2	108	108	100% (96.6% - 100%)	0	0
3	106	106	100% (96.6% - 100%)	0	0
4	108	108	100% (96.6% - 100%)	0	0
5	106	106	100% (96.6% - 100%)	0	0
6	107	107	100% (96.6% - 100%)	0	0
7	107	107	100% (96.6% - 100%)	0	0
8	107	107	100% (96.6% - 100%)	0	0
9	106	105	99.1% (96.6% - 100%)	1	0.9% (0.02% - 5.1%)
10	107	107	100% (96.6% - 100%)	0	0
11	107	0	0	107	100% (96.6% - 100%)
12	107	107	100% (96.6% - 100%)	0	0
13	106	106	100% (96.6% - 100%)	0	0

Table 7: SMN1 precision ANOVA analysis with variation estimates across multiple kit lots, instruments, days, and plates. The variation results are presented on Ct scale (SD). All replicate results for C1 and Sample 11 are "No Ct" and thus not included in the table.

	N	Ct Mean	Within Run		Between Run		Between Instrument		Within Kit lot		Between Kit lot		Total	
Sample			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
C2	107	22.0	0.53	2.4	0.27	1.2	0.33	1.5	0.68	3.1	0.09	0.4	0.69	3.1
C3	107	22.0	0.39	1.8	0.43	2	0.25	1.1	0.64	2.9	0.02	0.1	0.64	2.9
1	107	22.6	0.57	2.5	0.20	0.9	0.30	1.3	0.67	3.0	0.16	0.7	0.69	3.1
2	108	22.6	0.34	1.5	0.17	0.8	0.22	1.0	0.44	1.9	0.14	0.6	0.46	2.0
3	106	22.5	0.54	2.4	0.24	1.0	0.19	0.8	0.62	2.7	0.11	0.5	0.63	2.8
4	108	23.4	0.81	3.5	0.00	0.0	0.38	1.6	0.89	3.8	0.24	1.0	0.92	3.9
5	106	23.6	0.47	2.0	0.23	1.0	0.25	1.1	0.58	2.5	0.17	0.7	0.61	2.6
6	107	24.1	0.27	1.1	0.15	0.6	0.21	0.9	0.37	1.5	0.23	1.0	0.44	1.8
7	107	23.7	0.70	2.9	0.00	0.0	0.33	1.4	0.77	3.2	0.28	1.2	0.82	3.5
8	107	24.0	0.31	1.3	0.15	0.6	0.29	1.2	0.46	1.9	0.19	0.8	0.50	2.1
9	106	26.6	0.61	2.3	0.20	0.7	0.30	1.1	0.71	2.7	0.19	0.7	0.74	2.8
10	107	24.8	0.33	1.3	0.35	1.4	0.24	1.0	0.54	2.2	0.13	0.5	0.55	2.2
12	107	23.1	0.54	2.3	0.05	0.2	0.27	1.2	0.6	2.6	0.22	1.0	0.64	2.8
13	106	24.1	0.34	1.4	0.26	1.1	0.28	1.2	0.51	2.1	0.19	0.8	0.54	2.3

Table 8: RPP30 Precision Analysis

Sample	N	Ct Mean	Within Run		Between Run		Between Instrument		Within Kit lot		Between Kit lot		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	107	23.4	0.70	3.0	0.39	1.7	0.30	1.3	0.42	1.8	0.86	3.7	0.96	4.1
2	108	23.2	0.54	2.3	0.44	1.9	0.23	1.0	0.34	1.5	0.73	3.2	0.81	3.5
3	106	23.2	0.69	3.0	0.25	1.1	0.09	0.4	0.34	1.5	0.74	3.2	0.81	3.5
4	108	24.2	0.99	4.1	0.33	1.4	0.36	1.5	0.45	1.9	1.11	4.6	1.20	4.9
5	106	24.0	0.79	3.3	0.00	0.0	0.22	0.9	0.54	2.2	0.82	3.4	0.98	4.1
6	107	24.4	0.59	2.4	0.24	1.0	0.00	0.0	0.70	2.9	0.64	2.6	0.95	3.9
7	107	24.2	1.17	4.8	0.00	0.0	0.19	0.8	0.64	2.7	1.19	4.9	1.35	5.6
8	107	24.4	0.81	3.3	0.00	0.0	0.20	0.8	0.70	2.9	0.84	3.4	1.09	4.5
9	106	25.6	0.69	2.7	0.00	0.0	0.00	0.0	0.37	1.4	0.69	2.7	0.78	3.0
10	107	23.9	0.62	2.6	0.14	0.6	0.34	1.4	0.56	2.4	0.72	3.0	0.92	3.8
11	107	23.0	0.45	2.0	0.34	1.5	0.00	0.0	0.40	1.8	0.56	2.4	0.69	3.0
12	107	23.7	0.81	3.4	0.28	1.2	0.25	1.1	0.55	2.3	0.89	3.8	1.05	4.4
13	106	24.5	0.81	3.3	0.50	2.1	0.2	0.8	0.72	2.9	0.97	4.0	1.21	5.0

Reproducibility

The objective of this study was to characterize the within-laboratory precision and reproducibility of the Eonis test system across 3 study sites, including 2 external sites. At each of 3 study sites, 2 operators performed 5 runs each during 5 operating days (1 run per day) using 13 samples with 5 sample replicates tested per run. The total number of measurements was 150 per sample (50 replicates per sample in each laboratory). Operating days were not limited, and study runs could occur on consecutive days. All samples showed 100% agreement among replicates for qualitative calls (Table 9). The SMA positive sample replicates resulted in no Ct values. SMN1 Ct and RPP30 Ct precision of each sample by run, operator, and site are shown in Tables 10 and 11, respectively.

Table 9: SMN1 reproducibility data

Sample	N	N Below Cut-off	Proportion Below Cut-off (%) (with 95% CI)	N Above Cut-off	Proportion Above Cut-off (%) (with 95% CI)
1	150	150	100% (97.6% - 100%)	0	0
2	150	150	100% (97.6% - 100%)	0	0
3	150	150	100% (97.6% - 100%)	0	0
4	150	150	100% (97.6% - 100%)	0	0
5	150	150	100% (97.6% - 100%)	0	0
6	150	150	100% (97.6% - 100%)	0	0
7	150	150	100% (97.6% - 100%)	0	0
8	150	150	100% (97.6% - 100%)	0	0
9	150	150	100% (97.6% - 100%)	0	0

Sample	N	N Below Cut-off	Proportion Below Cut-off (%) (with 95% CI)	N Above Cut-off	Proportion Above Cut-off (%) (with 95% CI)
10	150	150	100% (97.6% - 100%)	0	0
11	150	0	0	150	100% (97.6% - 100%)
12	150	150	100% (97.6% - 100%)	0	0
13	150	150	100% (97.6% - 100%)	0	0

Table 10: Reproducibility study SMN1 precision analysis with between variation estimates across run, operator and site. All replicate results for Sample 11 (SMA positive) are "No Ct" and thus not included in table.

Sample	N	Ct Mean	Within Run		Between Run		Between Operator		Between Site		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	150	23.2	0.66	2.87	0.21	0.93	0.00	0.00	0.10	0.43	0.71	3.04
2	150	23.3	0.71	3.07	0.37	1.60	0.00	0.00	0.10	0.44	0.81	3.49
3	150	24.4	0.51	2.10	0.06	0.25	0.00	0.00	0.17	0.69	0.54	2.22
4	150	23.5	0.75	3.21	0.00	0.00	0.00	0.00	0.26	1.10	0.80	3.39
5	150	24.1	0.23	0.95	0.05	0.20	0.10	0.42	0.22	0.91	0.34	1.40
6	150	24.4	0.21	0.87	0.06	0.24	0.00	0.00	0.21	0.85	0.30	1.23
7	150	24.3	0.44	1.81	0.00	0.00	0.01	0.02	0.10	0.40	0.45	1.86
8	150	24.4	0.24	0.97	0.12	0.47	0.00	0.00	0.15	0.62	0.30	1.25
9	150	26.1	0.36	1.39	0.00	0.00	0.05	0.20	0.17	0.64	0.40	1.54
10	150	24.9	0.29	1.15	0.00	0.00	0.08	0.32	0.18	0.71	0.35	1.39
12	150	23.4	0.44	1.87	0.01	0.03	0.00	0.00	0.19	0.82	0.48	2.04
13	150	24.1	0.19	0.78	0.08	0.33	0.05	0.21	0.19	0.80	0.29	1.19

Table 11: Reproducibility study RPP30 precision analysis with between variation estimates across run, operator and site.

Sample	N	Ct Mean	Within Run		Between Run		Between Operator		Between Site		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	150	23.6	0.70	2.98	0.25	1.07	0.00	0.00	0.21	0.89	0.78	3.29
2	150	24.2	0.78	3.21	0.39	1.61	0.00	0.00	0.24	0.99	0.90	3.72
3	150	24.2	0.54	2.24	0.00	0.00	0.00	0.00	0.07	0.31	0.55	2.26
4	150	24.1	0.89	3.70	0.00	0.00	0.00	0.00	0.07	0.30	0.89	3.71
5	150	24.7	0.42	1.71	0.00	0.00	0.00	0.00	0.21	0.83	0.47	1.91
6	150	24.1	0.33	1.37	0.06	0.23	0.00	0.00	0.06	0.26	0.34	1.41

Sample	N	Ct Mean	Within Run		Between Run		Between Operator		Between Site		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
7	150	25.1	0.69	2.77	0.00	0.00	0.00	0.00	0.20	0.79	0.72	2.88
8	150	23.9	0.37	1.53	0.00	0.00	0.00	0.00	0.07	0.28	0.37	1.56
9	150	24.8	0.51	2.07	0.00	0.00	0.00	0.00	0.07	0.27	0.52	2.09
10	150	23.5	0.41	1.75	0.00	0.00	0.00	0.00	0.14	0.59	0.43	1.85
11	150	22.6	0.56	2.49	0.08	0.35	0.08	0.35	0.12	0.55	0.59	2.60
12	150	23.7	0.60	2.52	0.00	0.00	0.00	0.00	0.14	0.59	0.61	2.59
13	150	23.7	0.32	1.34	0.00	0.00	0.00	0.00	0.14	0.59	0.35	1.46

Filter Paper Reproducibility

This study was performed to demonstrate the performance equivalency and reproducibility of the Eonis SCID-SMA assay with DBS samples prepared on different filter papers used in neonatal screening. Between filter paper manufacturer and paper lots, differences were evaluated using samples spotted on 3 lots of 2 brands of filter paper each (filter paper #1 and filter paper #2). This study was performed by a single operator using 1 kit lot and manually spotting samples in 85 µl per spot.

One operator performed 1 run per day for 5 days, totaling 5 runs, and using 1 kit lot and 1 Eonis test system. Six samples (samples 1, 2, 3, 11, 12, 13 in Table 5) were prepared on 3 lots of each filter paper brand, totaling 36 conditions (6 samples x 3 lots x 2 filter paper brands). Each run consisted of 3 96-well DNA extraction plates (2 randomized sample sets per plate plus 2 sets of kit controls) and 1 384-well PCR plate on each testing day. To assess the homogeneity of the analytes across the DBS, 5 replicates of each condition were punched from 5 different locations of each spot in the order: top, left, center, right, bottom. The study generated a total of 900 results (25 replicates x 6 samples x 3 filter paper lots x 2 filter paper brands) with 150 results per sample and 450 results per filter paper brand.

The data presented show that the filter paper from both the manufacturers (filter paper #1 and filter paper #2) have equivalent performance (Table 12). Similarly, the different lots of the filter paper from both manufacturers have equivalent performance. 95% confidence intervals showed that there are no statistically significant differences in detection between manufacturers or between paper lots for any of the samples with respect to the qualitative calls. The SMA positive sample replicates resulted in no Ct values. Results show acceptable precision for both SMN1 (Table 13) and RPP30 (Table 14) across run, paper lot, and manufacturer.

Table 12: The SMN1 proportion of positive and negative results along with 95% confidence intervals

Filter Paper Brand #	Sample	N	Presumptive Normal	Presumptive Positive	Proportion Below Cut-off (%) (with 95% CI)	Proportion Above Cut-off (%) (with 95% CI)
1	1	75	75	0	100% (95.2% - 100%)	0
2		75	75	0	100% (95.2% - 100%)	0
1	2	73	73	0	100% (95.1% - 100%)	0
2		74	74	0	100% (95.1% - 100%)	0
1	3	75	75	0	100% (95.2% - 100%)	0
2		75	75	0	100% (95.2% - 100%)	0
1	11	75	0	75	0	100% (95.2% - 100%)
2		75	0	75	0	100% (95.2% - 100%)
1	12	75	75	0	100% (95.2% - 100%)	0
2		75	75	0	100% (95.2% - 100%)	0
1	13	75	75	0	100% (95.2% - 100%)	0
2		75	75	0	100% (95.2% - 100%)	0

Table 13: Filter paper study: SMN1 precision analysis with between variation estimates across papers and paper lots. All replicate results for Sample 11 (SMA positive) are "No Ct" and thus not included in table.

Sample	N	Mean Ct	Within Run		Between Run		Between Paper Lot		Between Manufacturer		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	150	24.5	0.56	2.29	0.00	0.00	0.09	0.37	0.01	0.05	0.57	2.32
2	147	24.4	0.91	3.71	0.01	0.03	0.00	0.00	0.01	0.05	0.91	3.71
3	150	24.5	0.54	2.23	0.00	0.00	0.00	0.00	0.10	0.39	0.55	2.26
12	150	23.8	0.61	2.56	0.18	0.78	0.00	0.00	0.01	0.04	0.64	2.68
13	150	24.4	0.37	1.51	0.00	0.00	0.09	0.35	0.06	0.26	0.38	1.57

Table 14: Filter paper study RPP30 precision analysis with between variation estimates across papers and paper lots

Sample	N	Mean Ct	Within Run		Between Run		Between Paper Lot		Between Manufacturer		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	150	24.2	0.84	3.46	0.00	0.00	0.16	0.65	0.01	0.04	0.85	3.52
2	147	24.2	1.35	5.58	0.00	0.00	0.14	0.56	0.14	0.57	1.37	5.63
3	150	24.3	0.67	2.78	0.00	0.00	0.00	0.00	0.21	0.87	0.71	2.91
11	150	23.6	0.57	2.41	0.00	0.00	0.21	0.90	0.03	0.13	0.61	2.58

Sample	N	Mean Ct	Within Run		Between Run		Between Paper Lot		Between Manufacturer		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
12	150	23.6	0.76	3.22	0.00	0.00	0.00	0.00	0.09	0.37	0.77	3.24
13	150	24.2	0.52	2.17	0.00	0.00	0.24	0.98	0.26	1.10	0.63	2.62

2. Detection Limits:

Limit of Blank

The SMN1 limit of blank (LoB) study was performed with contrived analyte-negative samples that were created by spiking SMN1-negative cells obtained from Coriell Institute for Medical Research (Camden, NJ) into leukocyte-depleted human blood. The Coriell cell line was added at a target of 30,000 genome-copies/µL.

The study was performed by 1 operator using 2 sets of Eonis systems, 2 kit lots, and run in 5 days, totaling 10 runs.

A summary of the measurement results of all the LoB study samples for SMN1 is shown in Table 15. All the replicates had no Ct value and, thus, false positive rates were 0% with both kit lots. For RPP30, the percentage of false positive results was also 0% for both kit lots.

Table 15: SMN1 Limit of Blank results

Kit lot	N	False Positive	Negative	False Positive %
Lot 1	150	0	150	0.00
Lot 2	150	0	150	0.00

3. Analytical Specificity:

Interference

Endogenous and exogenous interfering substances were tested using a paired-difference method (low interferent level compared to high interferent level). The highest concentration tested was equal to or higher than that recommended by the CLSI Guideline EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition (2005) and is described in Table 16 below. Three SMN1 presumptive normal contrived samples were tested, and each sample was run at 13 replicates per level in a single run using 1 kit lot and 1 Eonis test system. Three operators performed 7 runs over 3 testing days. The study generated a total of 544 sample results not including controls (7 interfering substances x 2 interferent levels x 3 target DNA levels x 13 replicates).

Table 16: Interfering substances tested and their concentrations

Tested substance	Added concentration of tested substance

Conjugated bilirubin	16.6 mg/dL in blood
Hemoglobin	200 g/L in blood
Unconjugated bilirubin	10 mg/dL in blood
Intralipid®	1500 mg/dL in blood
Li-heparin	7500 USP /dL in blood
EDTA	9.8 mg/mL in blood
Na-citrate	0.0645 mol/L in blood

The results indicated there was no interference observed for any of the tested interferents, as all sample replicates were Presumptive normal, and results showed 100% concordance between samples with and without interferents. Hemoglobin at up to 200 g/L, conjugated bilirubin at up to 16.6 mg/dL (=166 mg/L), unconjugated bilirubin at up to 10 mg/dL (=100 mg/L), Intralipid at up to 1500 mg/dL (15 mg/mL), Li-heparin at up to 7500 USP/dL, Na-citrate at up to 0.0645 mol/L, and EDTA at up to 9.8 mg/mL did not significantly interfere with the Eonis SCID-SMA test.

Primer and Probe Specificity

The primer and probe specificity study was performed for SMN1 and RPP30 *in silico* using online Standard Nucleotide BLAST tool published by the National Center for Biotechnology Information (NCBI). The primers for SMN1/SMN2 were designed and published by Maranda et al. and anneal to both SMN1 and SMN2 sequences, so they amplify both genes. The specificity of the assay is achieved by using two probes with a single nucleotide difference in exon 7.

The SMN1 and SMN2 genes are coded on chromosome 5q13. Normally, this region contains one telomeric SMN1 gene (MIM600354) and one centromeric paralogue SMN2 (MIM601627). The two genes differ in their coding sequences only by a synonymous point mutation in exon 7, at cDNA residue 840. At this nucleotide position, SMN1 bears a cytosine (C) while SMN2 bears a thymine (T). This single nucleotide difference does not affect the protein sequence, but the T residue at this position in SMN2 reduces the splicing efficiency of exon 7, so most SMN2 transcripts are inactive, lacking exon 7.

BLAST analysis confirmed only hits with 100% homology to the RPP30 locus on chromosome 10 along the full length of the primer and probe sequences. BLAST analysis confirmed the SMN primers only hit with 100% homology to the Survival Motor Neuron (SMN) gene on chromosome 5 along the full length of the primer sequences. The SMN1 probe has a quencher at the 3' end and it thereby will not be able to be extended by the DNA polymerase; thus, other non-SMN sequences of the genome will not be amplified.

4. Stability:

Specimen Stability

The specimen stability study was performed to determine the short-term (0-28 days) stability of DBS specimens for use with the Eonis SCID-SMA Kit. Four blood samples representing 1

SMA positive, 2 normal near cutoffs, and 1 high normal were spotted on filter paper (85 µL/spot) and dried overnight at room temperature in a vertical laminar flow cabinet. After drying, the samples were packed in aluminum foil bags. For analysis, 3.2 mm diameter blood spot discs were punched. The zero time-point samples were punched immediately after drying, and the punches were stored in a 96-well extraction plate covered with an adhesive foil seal overnight at +2 to +8°C before extracting and analyzing. The samples for time-points 1, 7, 14, 21 and 28 days were stored after drying overnight under different temperature and humidity storage conditions, ranging from -20°C to 35°C and up to relative humidity of 80%. At each time-point, the test samples were taken from the sample stability storage conditions, let to stand at room temperature for 1 hour, and then punched. The punches at each time-point were also stored in 96-well extraction plates covered with an adhesive foil seal overnight at +2 to +8°C before extracting and analyzing. Results showed no differences in sample stability based on the qualitative calls or SMN1 Ct values at day 28 compared to day 0. Thus, sample stability is not significantly affected under different temperature and humidity conditions.

Eonis DNA Extraction Kit In-Use and On-Board Stability

To demonstrate the in-use and on-board stability of the Eonis DNA Extraction Kit components after the bottles are opened for the first time and the solutions are loaded into the extraction instrument, this study was performed after a transport simulation treatment that simulated the worst-case conditions that can occur during packaging and shipping. Eonis DNA Extraction Kit components (packed and shipped at +19 - +25°C) were exposed to the following conditions to simulate the transport treatment before the stability study: 1 day at -20°C (-30 to -16°C), 5 days at +25°C (+23 to +27°C), and 1 day at +35°C (+33 to +37°C). Kit controls were used in this stability study.

After the simulated shipping treatment, the components were stored at +19 to +25°C. The testing was performed at 7 months of the components' shelf life. In the in-use stability and on-board stability testing, the "worst case scenario" was analyzed. Day 0 was the day when the bottles were opened for the first time after they were capped in production. On day 0, one half of each solution was removed from the bottle and used for stability testing. The bottles with left-over solutions were recapped and stored at +19 - +25°C. The left-over solutions in the bottles were used for stability testing on day 15. The removed portion of the solutions as well as the left-over solution were analyzed.

The testing on day 15 was combined with on-board stability testing: Wash Solution and Elution Solution were placed in the JANUS G3 Standard module for 2.5 hours at +19 - +25°C. Controls were tested in replicates of 9 for each of the above stress conditions.

Results indicate that Eonis DNA Extraction Kit components (Wash Solution and Elution Solution) are stable for 14 days at +19 - +25 °C after first use based on no change in SMN1 Ct values up to day 15.

Eonis DNA Extraction Kit Real-Time and Transport Simulation Interim Stability

To demonstrate that the Eonis DNA Extraction Kit components can tolerate the currently defined product shipment conditions before placing the kit at the recommended storage conditions for the duration of the real-time study, a real-time and transport simulation stability study was performed using 3 lots of the Eonis DNA Extraction kits. SCID-SMA Kit Controls were used in this study.

For the stability testing, 3.2 mm discs were punched from the QA Control or Kit Control samples. Ten replicates of each of the C1, C2 and C3 spots were used to extract DNA with the tested Extraction Solutions and tested at 0, 3, 5, and 7 months. The DNA extraction was followed by multiplex real-time PCR-based nucleic acid amplification using target specific primers and probes.

To demonstrate that the components of the Eonis DNA Extraction Kit tolerate possible variations in transport conditions, Wash Solution and Elution Solution of one kit lot were subjected to a transport simulation treatment. The shipping stress cycle consisted of the following phases: 1 day at -20°C (-30 to -16°C), 5 days at +25°C (+23 to +27°C), and 1 day at +35°C (+33 to +37°C). After the 'shipping treatment', the components were stored at +19 to +25°C.

Results show there was no difference in SMN1 Ct values up to day 7 across all 3 extraction kit lots (Table 17). At all tested time points the results passed the data validity check. Based on the transport simulation study, the Eonis DNA Extraction Kit can be shipped at room temperature. The results of the real-time stability studies support a shelf life of 6 months for Wash Solution and Elution Solution components of the Eonis DNA Extraction Kit. The real-time stability studies for 3 lots of the Eonis DNA Extraction Kit will continue up to 25 months.

Table 17: Eonis DNA Extraction Kit Real-time Stability testing for SMN1

Lot	Time (Months)	Sample	Mean Ct	SD
1	0	C1	N/A	
		C2	22.3	0.59
			21.8	0.35
		C3	21.8	0.58
			21.5	0.42
	3	C1	N/A	
		C2	21.8	0.46
		C3	21.9	0.54
	5	C1	N/A	
		C2	23.7	0.66
		C3	23.7	0.42
	7	C1	N/A	
		C2	21.5	0.27
		C3	21.5	0.55
2	0	C1	N/A	
		C2	22.6	0.33
			22.3	0.38
		C3	22.5	0.57
			22.2	0.48
	2	C1	N/A	
		C2	22.6	0.61
		C3	22.6	0.55
	4	C1	N/A	

		C2	21.4	0.46
		C3	23	0.45
	7	C1	NA	
		C2	22.5	0.8
		C3	22.7	0.83
3**	0	C1	N/A	
		C2	21.7	0.59
			21.6	0.26
		C3	21.7	0.41
			21.7	0.44
	3	C1	N/A	
		C2	21.9	0.48
		C3	21.6	0.32
	5	C1	NA	
		C2	21.8	0.17
		C3	21.8	0.58
	7	C1	N/A	
		C2	22.5	0.20
		C3	22.6	0.41

Eonis SCID-SMA Kit Interim In-Use and On-Board Stability Report

To demonstrate the stability of the Eonis SCID-SMA Kit components when they are taken into use and loaded into the instruments, a stability study was performed in two parts: 1) stability testing of kit controls; and 2) stability testing of PCR Reagents 1 and 2.

The tests were performed after a transport simulation treatment that simulated the worst-case conditions that can occur during packaging and shipping. To simulate transport treatment, Eonis SCID-SMA Kit components (packed and shipped with ice) were exposed to the following conditions before the stability study: 1 hour at +25°C (+23 to +27°C), 6 hours at -20°C (-30 to -16°C), 8 days at +4°C (+2 to +8°C), 1 day at -20°C (-30 to -16°C), 4 days at +4 °C (+2 to +8°C), ≥ 1 days at -20°C (-30 to -16°C). After the shipping treatment, the reagents were stored at -30 to -16°C until starting the in-use study.

For PCR Reagents 1 and 2 in-use and on-board stability, reagents were tested on day 0 and day 15. On day 0, PCR Reagents 1 and 2 were used right away, mixed together, and dispensed into a 384-well plate for testing, then tubes were placed at +2 - + 8°C. The testing was repeated at 15 days. On day 15, the reagents were removed from +2 - +8°C storage, placed on the JANUS PCR Mastermix module, and left uncapped for 2.5 hours. The testing was combined with the on-board stability test where the reagents were left in the trough for 16 minutes before being dispensed into a 384-well plate. The plates with PCR reagents mixture were sealed and placed into a light-protected place. The testing was resumed after 2.5 hours.

For SCID-SMA Kit Controls in-use and on-board stability, they were tested on day 0 and day 15. On day 0, the original package was opened, and cassettes were punched. Then the cassettes were placed in their original bags inside a re-sealable plastic bag with a desiccant

added and stored at -30 - -16°C. The testing was repeated on day 15. On day 15, the testing was combined with on-board stability test for which the Kit controls after they were punched into 96-well extraction plates, were placed in the JANUS Extraction module. After 4 hours DNA was extracted and analyzed.

The in-use/on-board testing was performed at the time point of 7 months for Kit Controls and 10 months for PCR Reagents 1 and 2 with 10 replicates.

For each target of PCR Reagents 1 and 2 and SCID-SMA Kit Controls, a mean Ct for at least 9 replicates of the tested samples and standard deviation (SD) were evaluated on day 15 and compared to day 0. There was no difference in SMN1 Ct values of the assay controls or PCR Reagents 1 and 2 up to day 15. Based on these data, PCR Reagents 1 and 2, once thawed from -30°C to -16°C, are stable for 14 days at +2°C to +8°C. The SCID-SMA Kit Controls are stable for 14 days after first use when stored at -30°C to -16°C in a sealed bag with desiccant. The in-use/on-board stability of the Eonis SCID-SMA kit will be confirmed at time points 13, 19 and 25 months.

Eonis SCID-SMA Kit Real -Time and Transport Simulation Interim Stability
Before the start of the stability testing, 4 lots of Kit Controls and 3 lots of PCR Reagents 1 and 2 were tested using their corresponding QC test methods and acceptance limits. During the real-time stability testing, all the materials were stored under their labeled storage conditions at -30°C to -16°C.

To demonstrate that the components of Eonis SCID-SMA Kit tolerate possible variations in transport conditions, stability kit lot 3 of PCR Reagents and stability lot 2 of Kit Controls were subjected to a transport simulation treatment. To simulate transport treatment, Eonis SCID-SMA Kit components were exposed to the following conditions: 1 hour at +25°C (+23 to +27°C), 6 hours at -20°C (-30 to -16°C), 8 days at +4°C (+2 to +8°C), 1 day at -20°C (-30 to -16°C), 4 days at +4 °C (+2 to +8 °C), and ≥ 1 days at -20 °C (-30 to -16 °C). After the shipping treatment, the Eonis SCID-SMA Kit components were stored at -30 to - 16°C. Kit components were tested at time points from 0 to 7 months in replicates of 10.

There was no change in SMN1 Ct values for assay kit controls or PCR Reagents 1 or 2 measured up to 10 months. Based on the results of the real-time and transport simulation stability study, a shelf life 180 days (6 months) is claimed for PCR Reagent 1, PCR Reagent 2 and SCID-SMA Kit Control when stored at the labelled storage temperature of -30°C to -16°C. The real-time stability studies for 3 lots of PCR Reagents 1 and 2 and for 3 lots of SCID-SMA Kit Controls will continue up to 25 months.

5. Carry-Over:

To demonstrate control of contamination during testing of samples with the Eonis reagent kits, the carry-over test was performed using 1 kit lot. The high-analyte positive sample was prepared by spiking plasmid DNAs of each analyte into leukocyte-depleted human blood at levels lower than 22.31 for SMN1 Ct and 22.71 for RPP30 Ct. The samples, including kit controls, were punched directly into 96 well plates. The high analyte-positive sample and the analyte-negative sample were placed in alternating rows and columns to produce a checkerboard configuration. The punching was performed according to the order of samples

in the plate map. A total of four 96 well plates (384 samples) were punched, corresponding to 8 replicates of each kit control, 176 replicates of the high analyte-positive sample, and 176 replicates of the analyte-negative sample. The carry-over test included 176 replicate results for the analyte-negative sample, and 7 out of those were below the clinical cutoff of 31.24 Ct. This corresponds to a 4% false negative rate in a fraction of the newborn screening population (<0.1%).

Because the carry-over study was performed with artificially high analyte-positive samples, data from the clinical validation (please refer to Section VI.B) were analyzed to further assess the potential for contamination events resulting in false negatives. These data include a large number of normal clinical specimens and clinically confirmed SMA positive specimens. Positive specimens were punched on the plates in random order surrounded by normal newborn screening samples, and when a sample was repeated in duplicate per the assay's algorithm, the two replicates were punched in consecutive wells. The clinical study results showed that all SMA positive specimens were detected. A total of 155 replicates had acceptable well results tested in the study, and all replicates had a 'No Ct' result, indicating no carry-over was observed. All SMA positive samples were tested on plates filled with clinically normal specimens, TREC or KREC positives, or age-matched controls. The clinical validation data support that no clinically significant carry-over was observed in the newborn screening laboratory setting, and the false negative rate based on the clinical data was 0%.

6. qPCR Method Equivalency:

To demonstrate whether the Eonis SCID-SMA Kit has equivalent performance when the qPCR is performed with 96-well fast heating block and 384-well heating block, this study was conducted using samples from the precision study (Table 5).

Two operators performed 1 Eonis extraction and 2 PCR consolidations per day for 6 days using 1 kit lot, totaling 12 runs. Each day an operator ran 1 extraction on a JANUS Extraction Instrument. The PCR set up and the addition of 3 µL of DNA into the PCR plate (consolidation) was done automatically with a JANUS Mini PCR Mastermix Instrument for the 384-well PCR reaction and manually for the 96-well PCR reaction. The operators ran the PCR reactions on 2 QuantStudio Dx instruments: QS1 for 384-well processing and QS4 for 96-well processing. Each extraction run consisted of 1 punched 96-well DNA extraction plate with 5 replicates for the 13 samples per plate totaling 65 samples per plate plus 2 sets of kit controls. The study generated a total of 1560 results (5 replicates x 13 samples x 2 PCR methods x 2 operators x 6 days) with 60 results per sample and 780 results per PCR method.

The data presented in Table 18 show 100% agreement between the 2 heating blocks, demonstrating method equivalency. These data demonstrate that the assay can be performed using either a 96-well heating block or 384-well heating block to amplify the samples for SMN1 and RPP30.

Table 18: The SMN1 proportion of positive and negative results along with 95% confidence intervals

Method	Sample	N	Presumptive Normal	Presumptive Positive	Proportion Below Cut-off (%) (with 95% CI)	Proportion Above Cut-off (%) (with 95% CI)
384-Well	1	60	60	0	100% (94.0% - 100%)	0
96-Well	1	60	60	0	100% (94.0% - 100%)	0
384-Well	2	60	60	0	100% (94.0% - 100%)	0
96-Well	2	60	60	0	100% (94.0% - 100%)	0
384-Well	3	60	60	0	100% (94.0% - 100%)	0
96-Well	3	60	60	0	100% (94.0% - 100%)	0
384-Well	4	60	60	0	100% (94.0% - 100%)	0
96-Well	4	60	60	0	100% (94.0% - 100%)	0
384-Well	5	59	59	0	100% (93.9% - 100%)	0
96-Well	5	59	59	0	100% (93.9% - 100%)	0
384-Well	6	60	60	0	100% (94.0% - 100%)	0
96-Well	6	60	60	0	100% (94.0% - 100%)	0
384-Well	7	60	60	0	100% (94.0% - 100%)	0
96-Well	7	60	60	0	100% (94.0% - 100%)	0
384-Well	8	60	60	0	100% (94.0% - 100%)	0
96-Well	8	60	60	0	100% (94.0% - 100%)	0
384-Well	9	60	60	0	100% (94.0% - 100%)	0
96-Well	9	60	60	0	100% (94.0% - 100%)	0
384-Well	10	60	60	0	100% (94.0% - 100%)	0
96-Well	10	60	60	0	100% (94.0% - 100%)	0
384-Well	11	60	0	60	0	100% (94.0% - 100%)
96-Well	11	60	0	60	0	100% (94.0% - 100%)
384-Well	12	60	60	0	100% (94.0% - 100%)	0
96-Well	12	60	60	0	100% (94.0% - 100%)	0
384-Well	13	59	59	0	100% (93.9% - 100%)	0
96-Well	13	59	59	0	100% (93.9% - 100%)	0

7. DNA Extraction Equivalency:

This study was conducted to demonstrate that the Eonis test system has equivalent performance when the DNA extraction is performed either manually or by a different automated liquid handler and is designed based on the recommendation of CLSI Guideline EP05-A3.

Two operators performed 3 Eonis extractions and PCR consolidations per day for 5 days using 1 kit lot, totaling 30 runs using samples 1, 2, 3, 4, 11, 12, and 13 (Table 5). Each day, an operator would run 1 extraction/PCR consolidation on the same JANUS Extraction/ JANUS PCR Mastermix, 1 extraction/PCR consolidation on a second commercial liquid handler, and 1 manual extraction manual PCR setup/consolidation. The operators ran the PCRs on 2 QuantStudio Dx instruments which were alternated between operator by day. Each extraction run consisted of 1 punched 96-well DNA extraction plate with 5 replicates for the 7 samples per plate, totaling 35 sample replicates per plate plus 2 sets of kit controls. The study generated a total of 1050 results (5 replicates x 7 samples x 3 extraction/PCR

methods x 2 operators x 5 days) with 150 results per sample and 350 results per extraction/PCR method.

There were 45 replicates performed with the JANUS handler, 50 replicates for the second commercial liquid handler, and 50 replicates for the manual extraction process. Results showed 100% concordance for the qualitative calls among extraction techniques. The lower 95% confidence intervals exceeded 92% for all samples and indicate there are no statistically significant differences in detection between extraction methods with any of the SMN1 samples.

B Clinical Studies:

1. Clinical Screening:

The screening performance of the Eonis SCID-SMA Kit was determined in a clinical study conducted in Denmark. Retrospective archived DBS specimens (collected from the US and Denmark) from subjects confirmed positive for SMA were included to enrich the cohort of routine newborn screening specimens obtained from the Danish Newborn Screening Biobank (NBS-Biobank).

Confirmatory test results were used as the comparator for the confirmed positive SMA cases. The clinical status of the routine subjects was determined through a retrospective review by clinical experts to confirm the routine subject cohort samples were from unaffected individuals.

The SMN1 Ct cut-off is pre-set at 31.24. The specimens having SMN1 values above cut-off in the initial round of testing were re-tested in duplicate. The final results (presumptive normal, presumptive positive, invalid result) were classified after the second round of testing according to the proposed testing algorithm described in Table 1. Among the presumptive normal specimens, the retest rate was 0% leading to a false positive rate of 0%. The screening performance of the Eonis SCID-SMA Kit was established by measuring SMN1 (and RPP30) in 3069 DBS specimens. In total, 51 SMA retrospective case specimens were available for the study, including 15 SMA type 1, 25 SMA type 2, 8 SMA type 3, and 3 unknown cases with SMN2 copy number ranging from 1-4. The remaining 3018 specimens were normal newborn screening specimens which included subjects who were carriers.

The screening performance of the Eonis SCID-SMA Kit was established with a pre-defined Ct cut-off of 31.24 for SMN1 and showed that the 51 confirmed SMA cases were all detected (100%, 0% false negative rate) (Table 19). The routine screening population returned 3018/3018 presumptive normal results (100%). For calculation of the positive and negative predictive value (PPV and NPV, respectively), the prevalence of 1 in 10,000 was used, resulting in 100% PPV with a 95% lower bound confidence interval of 93.0% and a 100% NPV with a 95% lower bound confidence interval of 99.9%.

Table 19: Screening performance of Eonis SCID-SMA Kit

	Clinical Status	

SMN1		SMA affected (%)	Normal (%)	Total (%)
Screening result	Presumptive positive (%)	51 (100 %)	0 (0.0 %)	51 (1.7 %)
	Presumptive normal (%)	0 (0.0 %)	3018 (100 %)	3018 (98.3 %)
	Total (%)	51 (100 %)	3018 (100 %)	3069 (100 %)

	Positive %-agreement	Negative %-agreement	Overall %-agreement	False positive rate	False negative rate
Percent	100 %	100 %	100 %	0.0 %	0.0 %
Confidence interval	93.0 % - 100%	99.9 % - 100%	99.9 % - 100%	0% - 0.1 %	0% - 7.0 %

2. Accuracy:

Accuracy was established in the clinical validation and was defined using the Eonis SCID-SMA Kit test results of confirmed positive SMA samples (N=51), for which the molecular genetic testing result showing homogenous deletion of SMN1 gene exon 7 was available per study inclusion criteria and presumed negative samples (N=55) assayed in the pivotal phase, which were sent for molecular genetic testing for SMN1.

DNA was extracted from the selected presumed negative (N=55) DBS samples and from 4 confirmed SMA samples as positive controls. The CE-IVD labeled (b)(4) SMA Newborn Screen (manufactured by (b)(4) assay was used for detection of the SMN1 gene-specific exon 7 DNA sequence according to the assay's instructions. Out of the 55 screen negative storage time matched samples, none were found to have the homozygous exon 7 deletion in the SMN1 gene according to the comparator. The accuracy of the Eonis SCID-SMA Kit test system for SMA was 100 % as shown in Table 20.

Table 20: Accuracy of SMA screening determined by comparing to the genetic test result of the SMN1 gene

		Homozygous deletion of *SMN1* exon 7		Total (%)
		Present (%)	Absent (%)	
Screening result	Positive (%)	51 (100)	0 (0)	51(100)
	Negative (%)	0 (0)	55 (100)	**55 (100)**
	Total (%)	**51 (100)**	**55 (100)**	**106 (100)**

	Positive %-agreement (N=51)	Negative %-agreement (N=55)	Overall %-agreement (N=106)
Percent	100 %	100 %	100 %
Confidence interval	94.3 % - 100%	94.7 % - 100%	97.2 % - 100%

Pediatric Extrapolation

In this De Novo request, existing clinical data were leveraged to support the use of the device in a pediatric patient population.

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Risk of false negative results	Certain design verification and validation activities identified in special control (1), including certain device description information and documentation of certain analytical studies and clinical studies Certain labeling information identified in special control (2), including limitations, device descriptions, explanation of procedures, and performance information
Risk of false positive results	Certain design verification and validation activities identified in special control (1), including certain device description information and documentation of certain analytical studies and clinical studies Certain labeling information identified in special control (2), including limitations, device descriptions, explanation of procedures, and performance information

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

There is significant probable benefit for the use of the Eonis SCID-SMA Kit to screen newborns for SMA based on the results of the pivotal clinical study and supportive analytical studies. There is currently no FDA cleared/approved device for SMA screening, and patients with SMA

may benefit from earlier clinical intervention, which can potentially improve clinical outcomes. In the clinical study, the screening performance of the Eonis SCID-SMA Kit was established by measuring SMN1 (and RPP30) in 3069 DBS specimens.

In total, 51 SMA retrospective case specimens were available for the study, out of a total of 3069 specimens retrospectively tested from the intended use population. The remaining 3018 specimens were normal newborn screening specimens and heterozygotic SMN1 exon 7 deletion. Of note, the heterozygote carriers in the intended use population were not misclassified as homozygote SMN1 exon 7 deletions. The pivotal study indicated that the device had robust clinical performance with a 100% sensitivity for the SMA cases (51/51 cases) and 100% specificity (3018/3018 unaffected individuals).

B Summary of the Assessment of Risk:

There is probable risk associated with the use of this device, mainly due to false positive results and false negative results.

The assay is designed to detect the SMN1 gene. In subjects with SMA, the exon 7 in the gene is deleted, resulting in a lack of signal for the detection of the SMN1 gene in the assay. A false negative (false "Presumptive normal") measurement could contribute to failure to detect a possibly affected newborn, which could lead to delayed treatment and clinical benefit from that treatment. However, a negative test result does not exclude newborns from the other medical examinations conducted according to the current diagnostic standard.

A false positive (false "Presumptive positive") measurement can be clarified by a subsequent genetic test; however, there is still the potential for mismanagement of patients due to lack of appropriate confirmatory testing.

C Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D Summary of the Assessment of Benefit-Risk:

The probable benefit of the Eonis SCID-SMA Kit to screen newborns for SMA, outweighs the probable risks of the device, as described above. There is significant probable benefit of this device, based on the pivotal clinical study and supportive analytical studies. Detecting newborns with SMA has significant clinical value, because these children are likely to benefit from earlier clinical intervention, resulting in better clinical outcomes The risks of the test include the false positivity and false negativity. All positives from this test should be confirmed with a subsequent confirmatory test, mitigating the risk of false positives. The risk of false negatives remains, but the patients with SMA may be detected on subsequent medical examination. However, these risks are mitigated by the clinical performance of the device in the pivotal clinical study, which showed 100% sensitivity (51/51) and 100% specificity (3018/3018). In addition, there are a series of risk mitigations outlined below.

The risks of false positive and false negative results during use of this device to screen for SMA are mitigated by design verification and validation activities described in the special controls to ensure reliable results for the intended use population. These include design verification and

validation studies that demonstrate that the test has been appropriately designed to minimize false negative and false positive results. Special controls regarding labeling and risk mitigation ensure that a user receives the information necessary to create the conditions required to generate accurate and reliable results. The risk mitigations in the labeling include clearly stating the intended use of the test as well limiting statements intended to help avoid erroneous use of the test, instructions for correct use and storing of specimens, expiration dating, and storage of reagents and guidelines on conditions that may lead to erroneous results. Given the device's indications for use for the screening of newborn patients for carrying SMN1 homozygous exon 7 deletions and considering the required general controls and special controls established for this device, the probable benefits outweigh the probable risks.

In summation, for the indicated use of screening newborns for SMA, the probable benefit of this device, demonstrated by the pivotal clinical trial with 3069 patients outweighs the probable risks of this device, when taking into account the totality of clinical and analytical performance and the special controls established for this device.

X Conclusion:

The De Novo request is granted, and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): QUE
Device Type: Spinal Muscular Atrophy newborn screening test system
Class: Class II
Regulation: 21 CFR 866.5980