November 9, 2022

PerkinElmer Inc
Casey Fox, Ph.D.
Sr. Manager, Regulatory Affairs
940 Winter St
Waltham, MA 02451

Re: K203035
Trade/Device Name: Eonis SCID-SMA kit
Regulation Number: 21 CFR 866.5930
Regulation Name: Newborn screening test for severe combined immunodeficiency disorder (SCID)
Regulatory Class: Class II
Product Code: PJI
Dated: April 28, 2021
Received: April 30, 2021

Dear Dr. Fox:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part

801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Pamela S. Gallagher -S

Digitally signed by Pamela S. Gallagher -S
Date: 2022.11.09 09:36:15 -05'00'

Pamela Ebrahimi, Ph.D.
Deputy Branch Chief
Division of Molecular Genetics
and Pathology
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K203035

Device Name
Eonis™ SCID-SMA kit

Indications for Use *(Describe)*
The Eonis™ SCID-SMA kit is intended for the semi-quantitative determination of TREC (T-cell receptor excision circle) as an aid in screening newborns for Severe Combined Immunodeficiency (SCID) and for the semi-quantitative determination of KREC (Kappa-deleting recombination excision circle) as an aid in screening newborns for X-linked agammaglobulinemia (XLA). 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 not intended for screening of SCID-like Syndromes, such as DiGeorge Syndrome, or Omenn Syndrome. It is also not intended to screen for less acute SCID syndromes such as leaky-SCID or variant SCID. The test is not indicated for screening B-cell deficiency disorders other than XLA, such as atypical XLA, or for screening of XLA carriers.

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

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) Summary

This summary of safety and effectiveness information is supplied in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned number is k203035

Submitted by: PerkinElmer, Inc.
940 Winter Street
Waltham MA 02451

Contact Person: Casey Fox
Tel: 408-823-5561

Trade Name: Eonis SCID-SMA kit

Common Name: Eonis SCID-SMA kit

Regulation: 21 CFR 866.5930

Classification: II

Panel: 75 Chemistry

Product Code: PJI

Predicate device: PerkinElmer ENLITE™ Neonatal TREC Kit (DEN140010)

1. Device Description

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 three targets: TREC, KREC, and RPP30, in the DNA extracted from newborn dried blood spot (DBS) using Eonis DNA Extraction kit in a single PCR reaction.

Each Eonis SCID-SMA kit contains reagents for up to 384 reactions (for 3241-001U) or 1152 reactions (for 3242-001U) including kit controls.

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) control	
C2 Low TREC/KREC control	
C3 High TREC/KREC 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

2. Intended Use

The Eonis™ SCID-SMA kit is intended for the semi-quantitative determination of TREC (T-cell receptor excision circle) as an aid in screening newborns for Severe Combined Immunodeficiency (SCID) and for the semi-quantitative determination of KREC (Kappa-deleting recombination excision circle) as an aid in screening newborns for X-linked agammaglobulinemia (XLA). 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 not intended for screening of SCID-like Syndromes, such as DiGeorge Syndrome, or Omenn Syndrome. It is also not intended to screen for less acute SCID syndromes such as leaky-SCID or variant SCID. The test is not indicated for screening B-cell deficiency disorders other than XLA, such as atypical XLA, or for screening of XLA carriers.

TaqMan is a trademark of Roche Molecular Systems, Inc.
QuantStudio is a trademark of Thermo Fisher Scientific.

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

3. Substantial Equivalency

The PerkinElmer Eonis SCID-SMA kit claims substantial equivalency to the PerkinElmer ENLITE™ Neonatal TREC Kit (DEN140010). Both devices are test systems intended for screening newborns for inherited immunodeficiency disorders.

The EnLite™ Neonatal TREC Kit is an in vitro diagnostic device intended for the semi-quantitative determination of TREC (T-cell receptor excision circle) DNA in blood specimens dried on filter paper. The test is indicated for use as an aid in screening newborns for severe combined immunodeficiency disorder (SCID).

Both devices are test systems intended for the semi-quantitative determination of TREC (T-cell receptor excision circle) DNA in blood specimens dried on filter paper on PCR instruments. In addition, Eonis SCID-SMA kit is intended for the semi-quantitative determination of KREC (Kappa-deleting recombination excision circle) as an aid in screening newborns for X-linked agammaglobulinemia (XLA).

The EnLite™ Neonatal TREC Kit uses a polymerase chain reaction (PCR) based nucleic acid amplification and time-resolved fluorescent-based detection to semi-quantify concentration. The PerkinElmer Eonis SCID-SMA Kit also uses fluorescent-based PCR nucleic acid amplification and detection to semi-quantify the same concentration. The use of PCR in newborn screening is well-established, and introduction of well-established real-time detection (qPCR) does not raise new questions of safety or effectiveness due to its use in this device.

Both devices are not intended for use as a diagnostic test or for screening of SCID-like Syndromes, such as DiGeorge Syndrome, or Omenn Syndrome. It is also not intended to screen for less acute SCID syndromes such as leaky-SCID or variant SCID.

In a study of 3018 clinical newborn samples with known medical status, 17 of which were confirmed positive for SCID and 6 were confirmed positive for XLA, the PerkinElmer Eonis SCID-SMA kit demonstrated the following sensitivity and specificity:

Analyte		Sensitivity	False-negative rate	Specificity	False-positive rate
TREC	Percent	100 %	0 %	99.7 %	0.3 %
	Confidence Limits	80.5 % - NA	NA - 19.5 %	99.4 % - 99.9 %	0.1 % - 0.6 %
KREC	Percent	100 %	0 %	99.7 %	0.3 %
	Confidence Limits	54.1 % - NA	NA – 45.9 %	99.4 % - 99.9 %	0.1 % - 0.6 %

In a study of 5437 clinical newborn samples with known medical status, 17 of which were confirmed positive for SCID, the PerkinElmer EnLite Neonatal TREC kit (excluding invalid results) demonstrated the following

sensitivity and specificity (DEN140010):

Analyte		Sensitivity	False-negative rate	Specificity	False-positive rate
TREC	Percent	100 %	0 %	99.7 %	0.3 %
	Confidence Limits	79.4 % - NA	NA – 20.6 %	99.4 % - 99.8 %	0.2 % - 0.6 %

Table below provides the similarities and differences of the proposed device and the predicate. The analytical and clinical tests performed with the EONIS SCID-SMA kit have demonstrated that the two products are substantially equivalent.

Characteristics	**Proposed Device** EONIS SCID-SMA kit	**Predicate** ENLITE™ Neonatal TREC Kit (DEN140010).
Intended Use/ Indications for Use	The Eonis™ SCID-SMA kit is intended for the semi-quantitative determination of TREC (T-cell receptor excision circle) as an aid in screening newborns for Severe Combined Immunodeficiency (SCID) and for the semi-quantitative determination of KREC (Kappa-deleting recombination excision circle) as an aid in screening newborns for X-linked agammaglobulinemia (XLA). 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 not intended for screening of SCID-like Syndromes, such as DiGeorge Syndrome, or Omenn Syndrome. It is also not intended to screen for less acute SCID syndromes such as leaky-SCID or variant SCID. The test is not indicated for screening B-cell deficiency disorders other than XLA, such as atypical XLA, or for screening of XLA carriers. This test is not intended for use as a diagnostic test and a positive screening result should be followed by confirmatory testing.	The EnLite™ Neonatal TREC Kit is an in vitro diagnostic device intended for the semi-quantitative determination of TREC (T-cell receptor excision circle) DNA in blood specimens dried on filter paper. The test is for use on the VICTOR™ EnLite instrument. The test is indicated for use as an aid in screening newborns for severe combined immunodeficiency disorder (SCID). This test is not intended for use as a diagnostic test or for screening of SCID-like Syndromes, such as DiGeorge Syndrome, or Omenn Syndrome. It is also not intended to screen for less acute SCID syndromes such as leaky-SCID or variant SCID.
Test Methodology	Semi-quantitative, multiplex real-time fluorescent-based polymerase chain reaction (PCR) based nucleic acid amplification and detection	Semi-quantitative, polymerase chain reaction (PCR) based nucleic acid amplification and time-resolved fluorescence resonance energy transfer (TR-FRET) based detection

<table>
<tr><td>Instrument / Software Platform</td><td>QuantStudio™ Dx Real-Time PCR instrument (K123955) and Eonis Analysis Software</td><td>VICTOR™ EnLite instrument and the EnLite™ workstation software.</td></tr>
<tr><td>Sample Type</td><td>Punch from dried blood spot (DBS) specimen</td><td>Same</td></tr>
<tr><td>Reportable Range</td><td>TREC 242 – 4320 copies/10^5 cells
KREC 459 - 24300 copies/10^5 cells</td><td>TREC 29-473 copies/µL blood</td></tr>
<tr><td>Lower Limits of Measure</td><td>TREC LoB=0 copies/10^5 cells, LoD=LoQ=242 copies/10^5 cells
KREC LoB=0 copies/10^5 cells, LoD=LoQ=459 copies/10^5 cells</td><td>TREC LoB=3 copies/ µL blood, LoD=20 copies/ µL blood, LoQ=29 copies/ µL blood</td></tr>
<tr><td>Calibrators / Standards</td><td>Calibration is based on internal reference (RPP30) in each well and manufacturer calibration for each kit lot.</td><td>3 levels of DBS calibrators prepared from porcine whole blood spiked with TREC and beta-actin (reference) plasmids, and a no-template blank</td></tr>
<tr><td>Controls</td><td>3 levels of DBS controls prepared from leucocyte-depleted human red blood cells and TREC, KREC, SMN1 and RPP30 plasmids spiked in, plus No template control (NTC)</td><td>3 levels of DBS controls prepared from porcine whole blood, TREC and beta-actin (reference) plasmids spiked in.</td></tr>
<tr><td>Expected Values</td><td>
<table>
<tr><th>Analyte copies/10^5 cells</th><th>N</th><th>Median</th><th>Min</th><th>Max</th><th>0.3%</th><th>0.5%</th><th>1.0%</th></tr>
<tr><td>TREC</td><td>3341</td><td>2520</td><td>117</td><td>9990</td><td>262</td><td>413</td><td>563</td></tr>
<tr><td>KREC</td><td>3341</td><td>3590</td><td>12</td><td>20100</td><td>129</td><td>261</td><td>484</td></tr>
</table>
</td><td>
<table>
<tr><th>Analyte copies/µL blood</th><th>N</th><th>Median</th><th>2.0%</th><th>2.5%</th><th>5.0%</th></tr>
<tr><td>TREC</td><td>2846</td><td>150</td><td>34</td><td>36</td><td>46</td></tr>
</table>
</td></tr>
<tr><td>Reproducibility</td><td>
<table>
<tr><th></th><th>Sample Range (copies/10^5 cells)</th><th>Lognormal %CV Range</th></tr>
<tr><td>TREC</td><td>299 - 3867</td><td>27%-65%</td></tr>
<tr><td>KREC</td><td>763 - 9648</td><td>26%-50%</td></tr>
</table>
</td><td>
<table>
<tr><th></th><th>Sample Range (copies/µL blood)</th><th>Lognormal %CV Range</th></tr>
<tr><td>TREC</td><td>56 - 545</td><td>49%-87%</td></tr>
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</td></tr>
</table>

QuantStudio is a trademark of Thermo Fisher Scientific.

4. Summary of the Studies

SITE-TO-SITE REPRODUCIBILITY

The reproducibility of the Eonis SCID-SMA assay was determined using a panel of dried blood spots at different TREC, KREC levels, 1 reagent kit lot, 2 external newborn screening laboratories and 1 internal site. In each laboratory, 2 operators performed 5 runs each during 5 operating days. Each run consisted of 1 plate with 5 replicates per sample. Total number of measurements was 150 per sample (50 replicates per sample in each laboratory). The analysis of variance approach was used to calculate the following:

The values in the mean TREC and KREC copies/10^5 cells column are transformed from logarithmic (Ln) mean values, and therefore they represent geometric means in the copies/10^5 cells scale. The analysis of variance approach was used to calculate the results presented as SDs in the logarithmic (Ln) scale complemented with total %CV in lognormal scale. Summary mean, min and max copies/10^5 cells and SD and %CV results without log transformation for total imprecision are also shown.

TREC Reproducibility data pooled across three laboratories.

	Calculations with logarithmic transformation							
Sample	**Geometric Mean (Copies 10^5/cells)**	**Mean Ln (Copies 10^5/cells)**	**Repeata-bility Ln SD**	**Between Run Ln SD**	**Between Operator Ln SD**	**Between Site Ln SD**	**Total Ln SD**	**Log-normal CV%**
6	23	3.14	0.73	0.60	0.01	0.53	1.09	151
13	34	3.53	1.12	0.31	0.00	0.32	1.20	180
10	180	5.19	0.77	0.01	0.00	0.03	0.77	90
5	299	5.70	0.55	0.16	0.01	0.15	0.59	65
3	912	6.82	0.29	0.06	0.00	0.10	0.31	32
12	936	6.84	0.34	0.00	0.00	0.18	0.38	40
4	1026	6.93	0.28	0.10	0.01	0.13	0.33	34
2	1478	7.30	0.26	0.01	0.04	0.04	0.27	27
8	3013	8.01	0.48	0.20	0.01	0.16	0.55	59
11	3290	8.10	0.25	0.05	0.00	0.09	0.27	28
7	3867	8.26	0.29	0.08	0.00	0.11	0.32	33
9	8225	9.01	0.24	0.08	0.02	0.18	0.31	32
1	9000	9.11	0.21	0.08	0.00	0.14	0.26	27

TREC total variation results without logarithmic transformation.

		Calculations without logarithmic transformation				
Sample	**N**	**Mean (Copies/ 10^5 cells)**	**Min (Copies/ 10^5 cells)**	**Max (Copies/ 10^5 cells)**	**SD (Copies/ 10^5 cells)**	**CV%**
6	54	40	4	308	55.3	138
13	71	58	3	239	56	97

		Calculations without logarithmic transformation				
Sample	**N**	**Mean (Copies/ 10^5 cells)**	**Min (Copies/ 10^5 cells)**	**Max (Copies/ 10^5 cells)**	**SD (Copies/ 10^5 cells)**	**CV%**
10	148	223	5	796	126	56
5	150	346	41	971	174	50
3	150	952	220	1792	272	29
12	150	997	239	2563	349	35
4	150	1076	497	2490	335	31
2	150	1526	654	2411	370	24
11	150	3388	513	6401	767	23
7	150	4076	2043	16934	1590	39
8	150	4601	797	218936	17700	384*
9	150	8571	3233	14974	2420	28
1	150	9277	4236	16270	2320	25

*Dataset for sample 8 has one high outlier (max value) affecting the variability estimate. Without the outlier, the estimated CV is 41% and similar to other samples within the measuring range.

KREC Reproducibility data pooled across three laboratories.

	Calculations with logarithmic transformation							
Sample	**Geometric Mean (Copies 10^5/cells)**	**Mean Ln (Copies 10^5/cells)**	**Repeata-bility Ln SD**	**Between Run Ln SD**	**Between Operator Ln SD**	**Between Site Ln SD**	**Total Ln SD**	**Log-normal CV%**
10	348	5.85	0.72	0.01	0.13	0.22	0.76	89
8	763	6.64	0.46	0.05	0.00	0.13	0.48	50
13	792	6.67	0.43	0.09	0.06	0.09	0.45	47
12	2770	7.93	0.24	0.00	0.00	0.17	0.30	31
3	3849	8.26	0.28	0.07	0.00	0.09	0.31	31
7	4037	8.30	0.23	0.02	0.05	0.11	0.26	27
1	4359	8.38	0.19	0.08	0.03	0.06	0.22	22
6	5007	8.52	0.38	0.01	0.00	0.06	0.38	40
4	9313	9.14	0.20	0.05	0.00	0.02	0.21	21
11	9648	9.17	0.25	0.05	0.00	0.05	0.26	26
5	16531	9.71	0.19	0.07	0.06	0.13	0.24	25
2	34982	10.5	0.27	0.00	0.01	0.01	0.27	28
10	348	5.85	0.72	0.01	0.13	0.22	0.76	89

KREC total variation results without logarithmic transformation.

		Calculations without logarithmic transformation				
Sample	**N**	**Mean (Copies/ 10^5 cells)**	**Min (Copies/ 10^5 cells)**	**Max (Copies/ 10^5 cells)**	**SD (Copies/ 10^5 cells)**	**CV%**
10	148	431	40	1071	242	56
8	150	841	186	1925	349	41
13	150	862	183	2003	330	38
12	150	2877	1097	5232	792	28
3	150	4025	1075	13447	1300	32
7	150	4162	2241	6788	1030	25
1	150	4457	2104	7746	960	22
6	150	5457	1567	36630	3230	59
4	150	9508	4735	15856	1910	20
11	150	9915	1564	16967	2130	21
5	150	16955	8813	29034	3880	23
2	150	36370	16841	118413	11200	31

PRECISION

The quantitative precision was determined in accordance with CLSI document EP05-A3.

The variation of the Eonis SCID-SMA assay was determined using dried blood spot samples, 3 kit lots, 3 sets of Eonis test systems (including three JANUS Extraction Instruments, three JANUS PCR Mastermix Instruments and three QuantStudio™ Dx Real-Time PCR Instruments), 2 operators, and 54 runs over 23 calendar days. Each run consisted of 1 plate with 2 replicates per sample in a randomized plate map. Total number of measurements was 108 per sample. The analysis of variance approach was used to calculate the following:

The values in the mean TREC and KREC copies/10^5 cells column are transformed from logarithmic (Ln) mean values, and therefore they represent geometric means in the copies/10^5 cells scale. The analysis of variance approach was used to calculate the results presented as SDs in the logarithmic (Ln) scale complemented with total %CVs in lognormal scale. Summary mean, min and max copies/10^5 cells and SD and %CV results without log transformation for total imprecision are also shown.

TREC Precision data across three kit lots and three instruments.

	Calculations with logarithmic transformation							
Sample	**Geometric Mean (Copies 10^5/cells)**	**Mean Ln (Copies 10^5/cells)**	**Repeata-bility Ln SD**	**Between Run Ln SD**	**Between Instrument Ln SD**	**Between Lot Ln SD**	**Total Ln SD**	**Log-normal CV%**
6	20	3.00	0.92	0.00	0.00	0.33	0.97	125
13	34	3.54	0.89	0.00	0.00	0.23	0.92	116
10	159	5.07	0.76	0.17	0.00	0.14	0.79	92
5	464	6.14	0.42	0.06	0.14	0.13	0.47	49

12	1022	6.93	0.34	0.16	0.00	0.08	0.39	40
2	1130	7.03	0.35	0.20	0.00	0.02	0.40	42
4	1176	7.07	0.33	0.13	0.13	0.15	0.41	42
3	2165	7.68	0.37	0.00	0.00	0.05	0.37	38
11	4105	8.32	0.24	0.08	0.16	0.04	0.30	30
7	4146	8.33	0.32	0.15	0.10	0.01	0.37	38
8	4866	8.49	0.35	0.31	0.00	0.07	0.47	50
9	8604	9.06	0.30	0.35	0.19	0.18	0.54	58
1	11048	9.31	0.21	0.14	0.16	0.03	0.30	31

TREC total variation results without logarithmic transformation.

		Calculations without logarithmic transformation				
Sample	**N**	**Mean (Copies/ 10^5 cells)**	**Min (Copies/ 10^5 cells)**	**Max (Copies/ 10^5 cells)**	**SD (Copies/ 10^5 cells)**	**CV%**
6	29	32	5	150	34.9	109
13	43	49	4	173	41.4	84
10	105	200	11	479	114	57
5	107	508	99	1183	216	43
12	106	1094	189	2247	383	35
2	107	1206	230	2576	414	34
4	108	1259	422	3011	479	38
3	107	2263	107	3084	510	23
11	106	4263	2031	7418	1220	29
7	107	4407	1014	8206	1530	35
8	107	5754	2011	74368	7120	124
9	107	9540	471	19530	3760	39
1	107	11501	3218	19982	3170	28

KREC Precision data across three kit lots and three instruments.

	Calculations with logarithmic transformation							
Sample	**Geometric Mean (Copies 10^5/cells)**	**Mean Ln (Copies 10^5/cells)**	**Repeata-bility Ln SD**	**Between Run Ln SD**	**Between Instrument Ln SD**	**Between Lot Ln SD**	**Total Ln SD**	**Log-normal CV%**
9	85	4.44	1.14	0.00	0.00	0.37	1.20	178
10	478	6.17	0.53	0.36	0.00	0.16	0.66	74
13	1033	6.94	0.40	0.13	0.15	0.09	0.45	48
8	1075	6.98	0.47	0.00	0.00	0.13	0.49	52
7	2416	7.79	0.43	0.00	0.08	0.01	0.44	46
12	3361	8.12	0.36	0.10	0.00	0.07	0.38	39
1	5271	8.57	0.35	0.00	0.09	0.04	0.37	38
3	5541	8.62	0.30	0.05	0.00	0.03	0.31	31
6	8691	9.07	0.26	0.26	0.08	0.10	0.39	40
4	11499	9.35	0.29	0.18	0.12	0.06	0.36	38
11	13494	9.51	0.24	0.08	0.17	0.09	0.32	32
5	13767	9.53	0.22	0.21	0.13	0.07	0.34	35
2	39735	10.6	0.20	0.21	0.12	0.07	0.32	33

KREC total variation results without logarithmic transformation.

		Calculations without logarithmic transformation				
Sample	**N**	**Mean (Copies/ 10^5 cells)**	**Min (Copies/ 10^5 cells)**	**Max (Copies/ 10^5 cells)**	**SD (Copies/ 10^5 cells)**	**CV%**
9	10	146	17	435	139	95
10	104	570	43	1396	306	54
13	106	1139	212	4188	546	48
8	107	1195	356	3180	538	45
7	107	2605	203	5513	898	34
12	107	3568	686	7096	1150	32
1	107	5560	474	10461	1620	29
3	106	5774	863	10031	1500	26
6	107	9308	3333	19079	3420	37
4	108	12212	2707	23846	4180	34
11	107	14103	5330	23372	4030	29
5	106	14492	5673	35070	4960	34
2	108	41717	15231	74089	12400	30

Qualitative imprecision was determined in accordance with CLSI document EP12-A2 to classify the results, using the screening performance study cut-offs (262 copies/10^5 cells for TREC, 484 copies/10^5 cells for KREC). The C5-C95 interval was determined to be 79–626 copies/10^5 cells for TREC, and 189–1064 copies/10^5 cells for KREC.

Concentrations outside of these intervals were considered to be consistently analyte negative (concentrations <C5, equals to screen positive) or consistently analyte positive (concentrations >C95, equals to screen negative).

LIMIT OF DETECTION

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were determined in accordance with CLSI document EP17-A2. The data for LoB was analyzed using "Assign LoB = Zero and Confirm" approach. The data for LoD was analyzed with probit approach.

Based on 300 determinations of blank samples (150 for each kit lot) the limit of blank (LoB) for TREC, KREC, SMN1 is 0 copies/µL blood and 0 copies/10^5 cells.

Based on total of 960 determinations, 20 replicates per dilution, the limit of detection (LoD) for TREC is 242 copies/10^5 cells with 95% probability. The Limit of Quantitation (LoQ) for TREC is 242 copies/10^5 cells, which is equal to LoD. The limit of detection (LoD) for KREC is 459 copies/10^5 cells) with 95% probability. The Limit of Quantitation (LoQ) for KREC is 459 copies/10^5 cells, which is equal to LoD.

LINEARITY

Linearity was determined in accordance with CLSI document EP06 ED2:2020 with one kit lot. Three (3) sets of contrived samples were used for this evaluation. Two sample sets were diluted to 9 levels and tested with 4 replicates for each level. For KREC the linearity panel was amended with results from LoD-study to reach lower concentrations. The allowable maximum deviation from linearity in the study was 25%.

The Eonis SCID-SMA assay is demonstrated to be linear for TREC from 94 copies/10^5 cells to 4316 copies/10^5 cells with observed maximum deviation of -14.3%. The KREC analyte is demonstrated to be linear from 117 to 24343 copies/10^5 cells with observed maximum deviation of 17.3%.

INTERFERENCE

The Eonis SCID-SMA kit was evaluated for interference from potential endogenous and exogenous sources in accordance with CLSI document EP07-A3.
The following potentially interfering substances were added to whole blood spiked with at three different TREC plasmid and KREC plasmid concentrations and were found not to interfere at the concentration indicated.

Interference 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

SCREENING PERFORMANCE

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

Confirmatory test results were used as the comparator for the confirmed positive SCID, XLA 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.

Using the data collected to establish the expected values, the cut-off values of the Eonis SCID-SMA kit were determined by calculating the TREC and KREC concentrations corresponding to the 0.3^{th} and 1.0^{th} population percentiles (262 copies/10^5 cells for TREC, 484 copies/10^5 cells for KREC) established in a cut-off study with an independent dataset. The specimens having TREC and KREC levels below the cut-off values 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. Summary of the retest rate and final results for routine screening specimens is provided below.

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Summary of the samples tested in the pivotal study.

Routine screening samples	SMN1	TREC	KREC
Screened samples	3018	3018	3018
Initial screen positive	0	10	18
Initial screen negative	3018	3008	3000
Retest rate	0%	0.3%	0.6%
Final screen positive	0	9	9
Final screen negative	3018	3008	3004
False-positive rate	0%	0.3%	0.3%

The screening performance of the Eonis SCID-SMA kit was established by measuring TREC, KREC (and RPP30) in 3090 DBS specimens.

In total, 17 SCID and 6 XLA retrospective case specimens and 3018 normal newborn screening specimens were available for the study. The screening performance of the Eonis SCID-SMA kit was established by measuring TREC, and KREC (and RPP30) and the final results after retesting are presented below.

Screening performance of Eonis SCID-SMA kit.

TREC		Clinical Status		Total (%)
		SCID affected (%)	Normal (%)	
Screening result	Presumptive positive (%)	17 (100 %)	9 (0.3 %)	26 (0.9 %)
	Presumptive normal (%)	0 (0.0 %)	3008 (99.7 %)	3008 (99.1 %)
	Total (%)	17 (100 %)	3017 (100 %)	3034 (100 %)

KREC		Clinical Status		Total (%)
		XLA affected (%)	Normal (%)	
Screening result	Presumptive positive (%)	6 (100 %)	9* (0.3 %)	15 (0.5 %)
	Presumptive normal (%)	0 (0.0 %)	3004 (99.7 %)	3004 (99.5 %)
	Total (%)	6 (100 %)	3013 (100 %)	3019 (100 %)

*Includes one false positive result from a female subject. In female population the false positive rate is estimated to be 0.1 %

KREC (Male only)		Clinical Status		Total (%)
		XLA affected (%)	Normal (%)	
Screening result	Presumptive positive (%)	6 (100 %)	8 (0.5 %)	14 (0.9 %)
	Presumptive normal (%)	0 (0.0 %)	1515 (99.5 %)	1515 (99.1 %)
	Total (%)	6 (100 %)	1523 (100 %)	1529 (100 %)

Conclusion

The Eonis SCID-SMA kit demonstrates analytical and screening performance that supports its substantial equivalency with the predicate device, PerkinElmer ENLITE™ Neonatal TREC Kit (DEN140010).