EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR ENLITE™ NEONATAL TREC KIT DECISION SUMMARY

A. DEN Number:

DEN140010

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation of the EnLite™ Neonatal TREC Kit

C. Measurand:

T-Cell Receptor Excision Circles (TRECs)

D. Type of Test:

Semi-quantitative, polymerase chain reaction (PCR) based nucleic acid amplification and time-resolved fluorescence resonance energy transfer (TR-FRET) based detection

E. Applicant:

Wallac Oy, a PerkinElmer subsidiary

F. Proprietary and Established Names:

EnLite™ Neonatal TREC Kit

G. Regulatory Information:

1. Regulation section:

   21CFR 866.5930

2. Classification:

   Class II

3. Product code:

   PJI Severe Combined Immunodeficiency Disorder (SCID) Newborn Screening Test System
   KHO Fluorometer, For Clinical Use

4. Panel:

   Immunology

H. Intended Use:

1. Intended use:

   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.

2. Indication(s) for use:

   Same as intended use above.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   VICTOR™ EnLite instrument and the EnLite™ workstation software.

   Thermal cycler compatible with full-skirted 96-well PCR plates and with heated lid, heating rate of 3ºC - 6ºC /sec, cooling rate of 3ºC - 4.5ºC /sec, heated lid setting using 100ºC - 105ºC or tracking mode of 5ºC above the block temperature.

I. Device Description:

The EnLite™ Neonatal TREC Kit is comprised of the EnLite™ Neonatal TREC Kit, the VICTOR™ EnLite instrument and the EnLite™ workstation software. The EnLite™ Neonatal TREC Kit contains reagents sufficient for 384 reactions or 1152 reactions, and multi-level, dried blood spot (DBS) calibrators and controls. The DBS calibrators and DBS controls have been prepared from porcine whole blood with a hematocrit value of 48–55%, and contain purified salmon-sperm, TREC, and beta-actin DNA. Specific contents of the kit are as follows:

DBS calibrators	Filter paper cassettes containing dried blood spots with three levels (A, B, C) of TREC and beta-actin. The exact TREC and beta-actin concentrations are given on the lot-specific quality control certificate included in the kit but approximate values are targeted as follows: A: TREC 25 copies/µL and beta-actin 18 copies/ µL B: TREC 240 copies/µL and beta-actin 120 copies/ µL C: TREC 770 copies/µL and beta-actin 370 copies/ µL
DBS controls	Filter paper cassettes containing dried blood spots with one negative control and two positive controls (C1, C2, C3) for TREC and two levels of beta-actin. The values for the DBS controls measured by the manufacturer are given on the lot specific quality control certificate included in the kit. Each laboratory should establish its own mean and acceptable range but approximate values are targeted as follows: C1 Low Control: TREC 120 copies/µL and beta-actin 20 copies/ µL C2 No TREC control: TREC 0 copies/µL and beta-actin 600 copies/ µL C3 High Control: TREC 670 copies/µL and beta-actin 600 copies/ µL
Elution Diluent	PCR-grade water
Reagent Concentrate	Ready-for-use Tris-HCl buffered (pH 8) salt solution with EDTA, Tween® 20, deoxyribonucleotide triphosphates, unlabeled oligonucleotides, labeled oligonucleotides, and bovine serum albumin.
5x Reaction Buffer	Ready-for-use buffer with 7.5 mM MgCl2
DNA Polymerase	Ready-for-use solution with 20 mM Tris-HCl (pH 7.4 at +25 °C), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, 200 µg/mL bovine serum albumin, and 50 % glycerol
PCR Diluent	Ready-for-use water solution with MgCl2
Barcode labels for the plate	
Lot-specific quality control certificate	

J. Substantial Equivalence Information:

1. Predicate device name(s) and DEN number(s):

   Not applicable.

2. Comparison with predicate:

   Not applicable.

K. Standard/Guidance Document Referenced:

CLSI NBS01-A6: Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard—Sixth Edition

L. Test Principle:

The EnLite™ Neonatal TREC Kit is a combination of PCR-based nucleic acid amplification and time-resolved fluorescence resonance energy transfer (TR-FRET) based detection. The test detects two targets: TREC (the marker for SCID) and beta-actin (an internal control for the test). Amplification and detection of Beta-actin is used to monitor the PCR amplification of a specimen to aid in detecting samples that do not have a sufficient amount of DNA for analysis. Beta-actin is used as a control for monitoring specimen amplification. The determination of TREC and beta-actin is performed simultaneously for each specimen.

The EnLite™ Neonatal TREC Kit is designed to measure TREC and beta-actin DNA in newborn dried blood spots from filter paper and involves the following steps: (1) A small punch is made from a dried blood spot (DBS) specimen from a filter paper intended for newborn screening; (2) The DBS punch, and reagents are added to a 96 well microtiter plate; calibrators and controls are also added to separate wells in the plate (3) TREC DNA is eluted during incubation in a thermocycler; (3) Target sequence-specific TR-FRET probes, which include donor and acceptor fluorophores coupled to opposite ends of a single probe molecule, are added to the microtiter plate; (4) The microtiter plate is returned to the thermocycler for DNA amplification and hybridization. Upon hybridization with the target sequence, energy transfer towards the acceptor emits a specific fluorescence at a given wavelength based on prolonged decay time of the energy-transfer induced acceptor signal; (5) Signal detection is performed on the Victor™ EnLite Instrument bench top microtiter plate fluorometer. The instrument automatically measures the microtiter plates and outputs quantitative results based on the measured fluorescence signals.

The EnLite™ workstation software interprets test results utilizing fluorescence counts measured at 615 nm, 665 nm, and 780 nm. Corrected fluorescence counts, “TREC Response” and “Beta-actin Response” for all reactions are calculated from the raw fluorescence counts. The result interpretation is based on two separate calibration curves which use blanks and DBS calibrators A-C. The response, corrected counts, are fitted against the ArcSinh transformed concentrations (copies/μL) using the unweighted linear regression. The test quality control is based on three kit control result interpretations. Each test generates results for TREC and beta-actin.

M. Performance Characteristics:

1. Analytical performance:

a. Reproducibility/Precision:

Three reproducibility studies were conducted using the EnLite™ Neonatal TREC Kit. These included a site-to-site reproducibility study, an independent precision study to evaluate the within-lot, between-lot and total variation of the EnLite™ Neonatal TREC Kit and a thermocycler reproducibility study.

Study I. Site-to-Site reproducibility:

The reproducibility of the EnLite™ Neonatal TREC Kit was assessed at three clinical sites: two external sites in the US and one non-US internal site. The reproducibility study at each clinical site was conducted over 5 non-consecutive days, using one lot with two operators per site performing 5 runs each (10 runs per laboratory). A run consisted of one plate with 3 replicates per sample. The total number of measurements was 90 per sample (10 runs x 3 laboratories x 3 replicates/sample). Each run included calibrators in triplicate and controls.

The reportable range of the test is 29-473 TREC copies/µL. Results below 29 are reported as <29 and results above 473 are reported as >473. However for the purpose of demonstrating reproducibility of the test, six levels of TREC specimens ranging from 3 copies/µL to 550 copies/µL were tested by all three sites. Four specimens (TR2-TR5) were within the measuring range of the test (29-473 TREC copies/µL) b(4) . The lowest TREC level (TR1), below the measuring range, was prepared from adult blood only containing low to zero TREC. The highest TREC level specimen (TR6) above the measuring range was prepared by spiking adult blood with TREC plasmid DNA. b(4) . Samples were then processed in accordance with the instructions for use (package insert).

Table 1 summarizes the within-laboratory, between-laboratory, within-run, between-run and total variation of TREC results across three clinical sites. These results demonstrate that within the measuring range of the test (29-473 TREC copies/ µL), the within-laboratory percent coefficient of variation (%CV) of the EnLite™ Neonatal TREC Kit was 41.7 to 52.1%, the between-laboratory variation range was 15.1-24.3%, the within-run variation was 31.8 to 47.4%, the between-run variation was 12.0 to 36.1% and the total variation of the test range was 48.5 to 59.4%. Imprecision was larger when specimens had TREC values outside the measuring range. Table 2 summarizes the variation for the within-laboratory, between-laboratory, within-run, between-run and total variation for the controls.

Table 1. Within-laboratory, between-laboratory, within-run, between-run and total

variation of TREC results across three clinical sites

Sample	n	Mean TREC copies /µl	Within laboratory variation % CV	Between laboratory variation % CV	Within Run Variation %CV	Between Run Variation %CV	Total variation % CV
1 TR1	90	3.16	119.1	20.2	110	29.6	123.0
e TR2	90	56.26	46.2	15.1	39.4	21.2	48.5
TR3	90	68.03	48.5	21.2	47.4	12.0	54.5
2 TR4	90	200.34	41.7	22.3	31.8	26.4	48.5
TR5	90	287.15	52.1	24.3	35.0	36.1	59.4
TR6	90	544.57	69.8	41.7	46.2	47.4	86.9

Table 2. Within-laboratory, between-laboratory, within-run, between-run and total variation for the three test controls.

Sample	n	Mean TREC copies /µl	Within laboratory variation % CV	Between laboratory variation % CV	Within run variation %CV	Between run variation %CV	Total variation % CV
No TREC control	60	1.86	88.4	13.1	62.0	54.5	91.5
Low control	60	63.43	88.4	39.4	86.9	15.1	102.9
High control	60	262.43	104.6	30.7	94.7	35.0	113.5

Study 2. Precision: Within run, Within-Lot, Between-lot and Between Instrument Reproducibility

A study was conducted to determine the precision of the EnLite™ Neonatal TREC Kit among multiple operators, days, lots, instruments, and thermocyclers. The study consisted of 3 kit lots, 3 Victor™ EnLite instruments and 3 thermocyclers in various combinations (4 replicates per sample x 3 lots x 3 instruments = 36 results). One of five different operators was randomized to perform each run. Twenty-seven (27) runs were performed over 20 days. Ten TREC samples spanning the range of TREC values 3-473 copies/µL were assessed. For evaluation of TREC precision, 8 samples were prepared from adult EDTA whole blood spiked with TREC plasmid DNA; one low level TREC sample was prepared from un-spiked adult blood, and one sample consisted of cord blood (containing endogenous TREC). For evaluation of beta-actin precision, 7 beta-actin samples from porcine whole blood were chosen to span the range of 16-608 copies/µL beta-actin. For the beta-actin samples, the blood was spiked with TREC plasmid DNA to obtain constant TREC values in all samples.

Results for the TREC precision within-run, within-lot, between-lot, between-run, between-instrument and total variation for TREC samples 2 through 10 are as follows: Within the measuring range of the test, %CV for within-run ranged from 45% to 88%, the % CV for between-run ranged from 18 to 42%, % CV for within-lot ranged from 67% to 101%, and % CV for between-lot ranged from 11% to 33%, between-instrument variation ranged from 0 to 50% and total %CV ranged from 73% to 106%. Outside of the measuring range, imprecision was greater. Sample 1 had mean TREC copies/µL of 2 b(4)

Testing of sample 4 yielded one outlier.

A summary of the variation (%CV) for the within-run, between-run, within-lot, between-lot, between-instrument and total variation components for beta-actin are as follows: Within the measuring range, the %CV for the precision results for beta-actin within-run were 46-71%, the %CV range for between-run was 7-37%, the %CV range for within-lot was 55-95%, the %CV range for between-lot was 10-24%, the %CV range for between-instrument was 0 to 21% and the range of variation for total beta-actin the %CV range was 56-100%.

The distribution of results of the reproducibility study categorized by the cut-off (36 copies/uL) is shown in Table 3. Results below the cut-off would be deemed SCID positive, while results above the cut-off would be deemed SCID negative.

Table 3: Dichotomized Results for Precision Samples

Sample	N	Mean TREC copies /µl	Below Cut-off (%)	Above Cut-off (%)
1	108	3	108 (100%)	
2	108	19	83 (77%)	25 (23%)
3	108	29	70 (65%)	38 (35%)
4	108	43	41 (38%)	67 (62%)
5	108	59	25 (23%)	83 (77%)
6	108	74	18 (17%)	107 (100%)
7	108	144		108 (100%)
8	108	276		108 (100%
9	108	473		108 (100%)
10	108	1790		108 (100%)

Study 3. Thermocycler Reproducibility

A thermocycler reproducibility study was performed using 3 instruments from different manufacturers testing a panel of samples with varying levels of TREC copies. b(4) . Blood was dispensed onto filter paper from two different paper manufacturers and 12 samples were analyzed in the study b(4) The study acceptance criteria was based on between-thermal cycler comparison for 5 levels of TREC copies within the measuring range, which is evaluated by testing for statistically significant differences between thermocyclers. Test calibrators and controls were included in each run. Table 4 shows the within-run, between-run, between paper manufacturer, between-thermal cycler and total variation. Total variation is variation that has been pooled across two distinct paper manufacturers and across all three thermal cyclers. One well of the run performed on the 2nd day of the study showed no signals for TREC or beta-actin in one thermocycler. Investigation revealed that part of the contents of the well was missing due to incomplete seal of the plate

and this result was excluded from data analysis. Recommendations for specific thermocycler specifications are made in the instructions for use. The total variation within the measuring range of the test is similar (63-74% CV) between samples. Imprecision is higher outside of the measuring range. Analysis using one-way ANOVA showed that there were no differences between the thermocyclers from the different manufacturers at any of the TREC sample levels.

Table 4. TREC within-run, between-run, between-paper manufacturer, between-thermal cycler and total variation

Sample	N	Mean TREC (copies /µl)	Within-run variation % CV (copies/µl)	Between-run variation SD (copies/µl)	Between-paper manufacturer %CV (copies/µl)	Between thermal-cycler variation %CV (copies/µl)	Total variation %CV (copies/µl)
TR1	60	4	123	0.3	5.58	0.04	123
TR2	60	52	73	0.01	12.4	0.01	74
TR3	60	83	51	33.6	0.01	0.01	63
TR4	60	124	64	13.3	0.1	0.03	66
TR5	60	194	61	24.9	0.03	0.02	67
TR6	59	393	59	39.9	0.07	0.01	75

b. *Linearity/test reportable range:*

The objective of the study was to evaluate the TREC linearity of the EnLite™ Neonatal TREC Kit. Human adult EDTA whole blood sample was used to represent the low concentration TREC sample. A high concentration samples was prepared by spiking TREC plasmid DNA into the same adult blood specimen. b(4) Samples with intermediate TREC levels were prepared by pooling the low concentration sample with high concentration sample. A total of 15 samples were prepared, 8 of the samples (sample number 4 through sample number 12) spanned the measuring range of the test (29-473 TREC copies/µL). The dried blood spot (DBS) samples were prepared by spotting the samples using filter paper intended for neonatal screening, b(4)

The TREC levels of the sample blood spots were measured with one kit lot using one thermal cycler and one VICTOR EnLite™ instrument. The different dilutions were scattered in random order and each sample was analyzed in in 5 replicates in a single run. Two separate runs were conducted in the experiment using the same set of samples. To investigate potential outliers of the first run, a second run was conducted. Test calibrators [blank reaction and three DBS calibrators in 3 replicates and Test controls (duplicates)] were included in the run. b(4)

Linearity analysis within the entire range tested was performed using the combined data (run1 + run 2). A polynomial evaluation of linearity was used for the data analysis. b(4)

b(4)

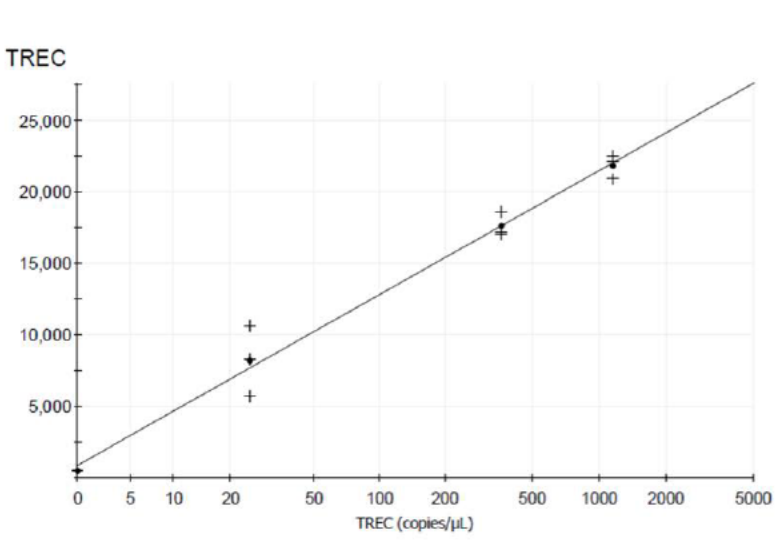
All sample dilutions met the pre-defined criteria for acceptance. The EnLite™ Neonatal TREC Kit is linear within the range of 22-1164 copies/µL. The reportable range of the test is 29-473 Copies/µL for TREC and 16 Copies/µL to 608 Copies/µL for beta actin.

Calibrator Linearity

An unweighted linear regression algorithm is used for the calibration curve. b(4)

An example calibration graph for TREC from a reproducibility study run is shown in Figure 1 below.

Figure 1. Calibration graph for TREC from a reproducibility study run



b(4)

c. Traceability, Stability, Expected values (controls, calibrators, or methods)

i. Traceability

There is no recognized standard or reference material for TREC DNA. The EnLite Neonatal TREC Kit contains calibrators and controls employed in test calibration and quality control. Several in-house reference materials b(4) b(4)) are used in the calibration process and in the value assignment of the test calibrators.

The EnLite™ Neonatal TREC Kit contains calibrators and controls that are provided on a filter paper cassette and are prepared from porcine whole blood dispensed onto filter paper intended for neonatal screening. The blood used to prepare the calibrators and controls is spiked with plasmid DNA containing the TREC target sequence to cover the range of 0-576 copies/µL blood.

Controls

The test consists of the following set of controls that are used to monitor test performance: Blank reactions serve as no template control reactions and there are 3 control DBS specimens, including low control (C1: low TREC, low beta-actin), no TREC control (C2: no TREC, normal beta-actin) and high control (C3: normal TREC, normal beta-actin).

Control	TREC	Beta-actin
C1 Low control	120	20
C2 No TREC control	0	600
C3 High control	670	600

Calibrators

The EnLite™ Neonatal TREC calibrators consist of blank reactions and three DBS calibrators, A-C, with known quantities of TREC and beta-actin DNA.

Calibrator	TREC	Beta-Actin
A	25	18
B	240	120
C	770	370

ii. Stability:

Specimen Stability

The objective of the sample stability testing was to define the short term stability (1-28 days) of TREC in dried blood spot samples collected using filter paper based sample collection devices. The study was conducted using various storage conditions. Adult whole blood and cord blood were mixed in various ratios to obtain samples within the desired TREC levels, such that the samples (n=4) would cover the expected measuring range of the EnLite™ Neonatal TREC Kit.

The test blood samples were spotted using two filter paper brands intended for newborn screening 55 µL/spot and dried overnight at room temperature. Analysis of sample stability was performed using 5 different timepoints: 0, 7, 14, 21 and 28 days. Analysis of the zero timepoint was performed immediately after the drying step using 4 plates containing 5 replicates of the sample stability study samples on each plate. Analysis of the remaining 4 timepoints used 4 plates/timepoint (2 replicates of the sample stability study samples on each plate). Each of these sample plates also included calibrators in triplicate and controls in duplicate.

TREC concentration was measured at the following conditions: storage at -20°C with no packing, storage at -20°C in a sealed bag with desiccant, storage at 4°C with no packing, storage at 4°C in a sealed bag with desiccant, storage at 22°C, room humidity (RH) at 22-33% with no packing and storage at 35°C, RH 80% with no packing. b(4) . Figures below shows TREC concentrations measured at different storage conditions and timepoints using 3 kit samples, including 5 TREC copies/µL (Figure 2), 56 TREC copies/µL (Figure 3 and 5) and 273 TREC copies/µL (Figure 4).

As shown in these figures, the decline in the measured TREC concentration was the steepest for samples stored at 35°C, RH 80% with no packing; this was the least optimal storage condition. The decline in the measured TREC concentration was the smallest for samples stored at -20°C. b(4) .

Storage of specimens in an environment with elevated temperatures and humidity increases the risk of false positive TREC screening results. TREC concentration may drop over 30% after 7 days at room temperature with low humidity (RH 22-33%). When stored at high temperature (+35 °C) and humidity (RH 80%), the concentration may drop over 50% during the first 7 days, and after 7 days approximately 80%. The results are shown in Figures 2, 3, 4, and 5 for three values of TREC and two filter papers. For long-term storage (up to 19 months), specimens should be placed into plastic bags together with a desiccant and stored at -30 to -16 °C. The recommended storage condition in the package insert for storage is -30°C to -16°C.

Figure 2. Sample 1 (5 TREC copies/µL) measured at different storage conditions and timepoints on Filter paper brand 1.

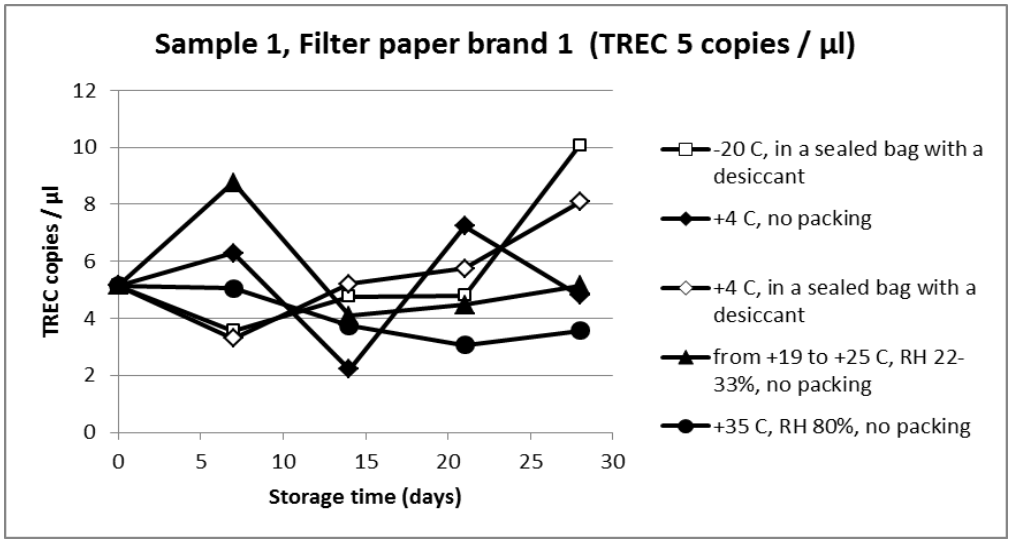


Figure 3. Sample 2 (56 TREC copies/µL) measured at different storage conditions and timepoints on Filter paper brand 1.

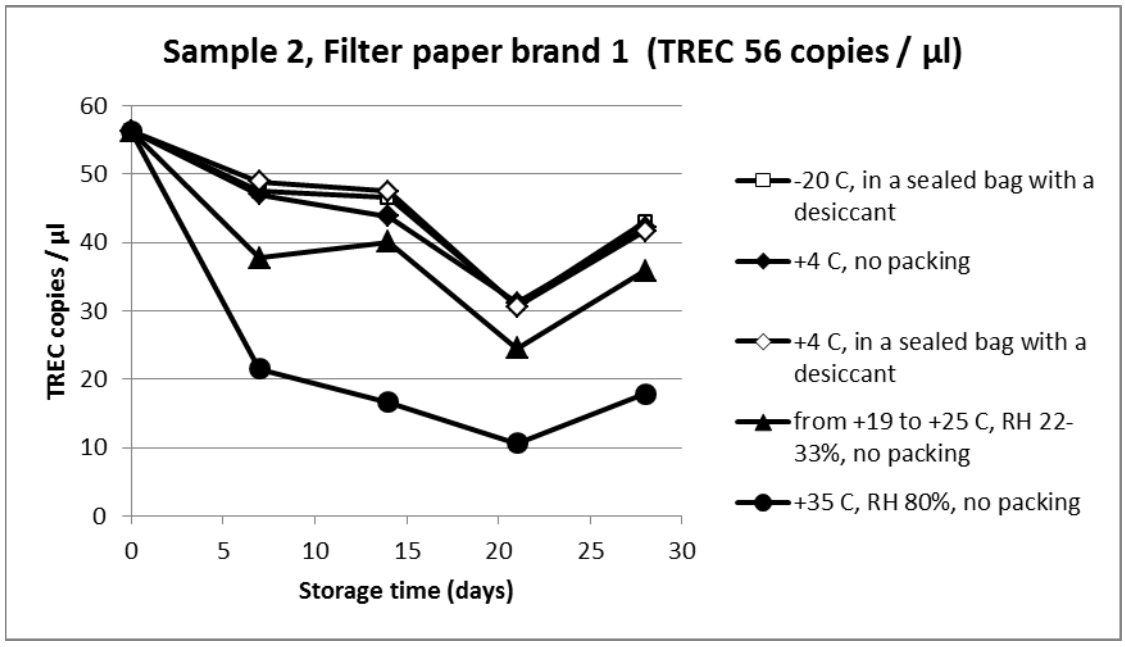


Figure 4. Sample 3 (273 TREC copies/µL) measured at different storage conditions and timepoints on Filter paper brand 1

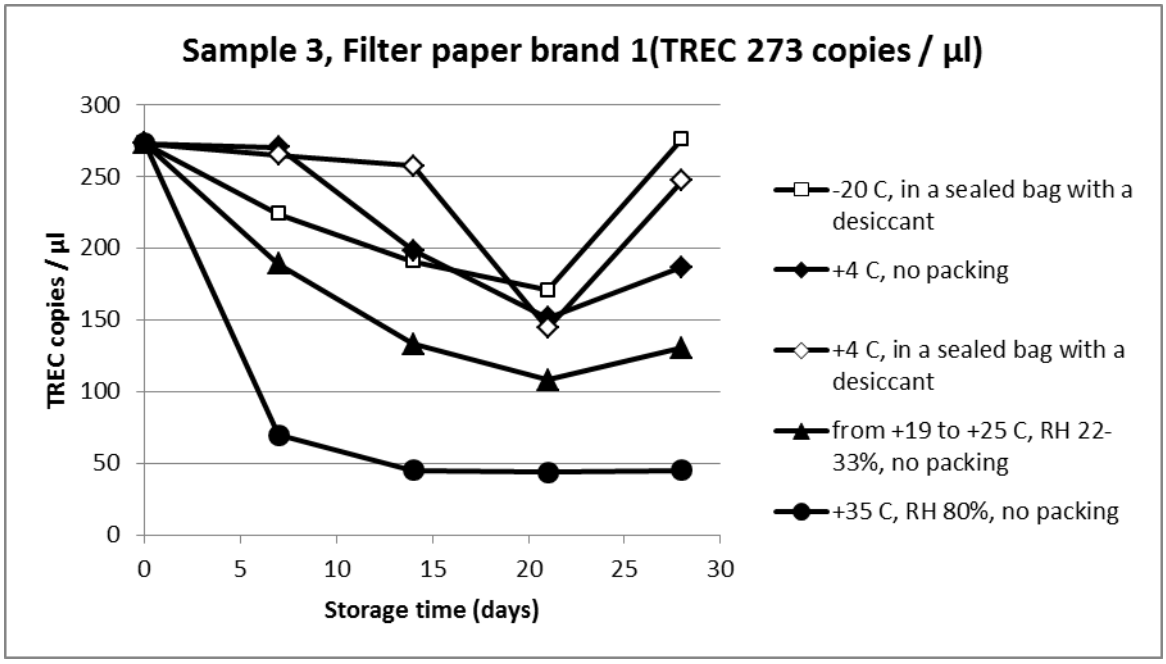
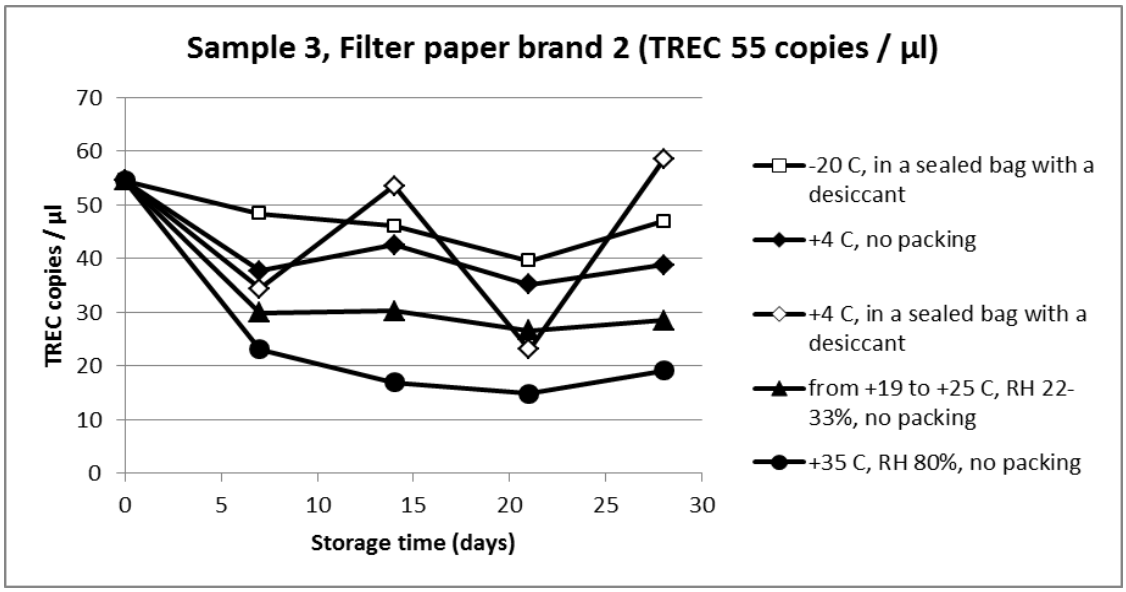


Figure 5. Sample 1 (55 TREC copies/µL) measured at different storage conditions and timepoints on Filter paper brand 2.



Reagent Stability

The objective of this study was to demonstrate the in-use and on-board stability of the EnLite Neonatal TREC Kit components. These studies were performed with or without an additional transport simulation treatment that simulates the worst case conditions that can occur during packaging and shipping.

In Use Reagent Stability Study

For the in use reagent study, elution diluent and reaction buffer were thawed, diluted as appropriate and stored at 2 to 8° for 15 days before use. All other kit components were thawed and frozen for 4 cycles over 4 days (stored at room temperature for one

hour per day and then re-frozen). Calibrators and control cassettes were also opened during thawing and then packed in a re-sealable bag with desiccant. The kit components were otherwise stored at their normal storage conditions (-30 to -16°C). Fourteen days from the first freeze-thaw cycle, reagents were thawed for a fifth time and tested as follows. Calibrators, controls and DBS samples were punched into microtiter plates, which were stored at room temperature (19 to 25°C) and tested after 23 hours. Before starting the test, the reagent mixture was prepared and the mixture was stored at 2 to 8° for 24 hours.

On Board Study

For the on board study, the test plates from study 1 were removed from the VICTOR EnLite™ instrument after the first measurement. These test plates were then stored in the thermal cycler (23°C) for 70 minutes and the plates were then measured again with the VICTOR EnLite™ instrument.

Transport Simulation

A transport simulation treatment was performed for all the kit components, and exposed to the transport simulation treatment before the study. The stress condition sequence for the kit components (packed and shipped with blue ice) was: 1 hour at 25°C, 6 hours at -20°C, 6 days at 4°C, 1 day at -20°C, 2 days at 4°C and ≥ 1 day at -20°C. After the transport simulation, all reagents were stored at their normal storage conditions (-30 to -16°C) until starting the in-use study.

The acceptance criteria for the stability tests are based on product performance specifications for precision. Test-specific acceptance limits corresponding to α=0.5% significance level (2SD) and α=0.3% significance level (3SD) are statistically calculated from the precision requirements (population SD). In addition there are acceptance limits for within and between plates variations to assure that all the individual test results contributing to the mean are acceptable.

The in-use and on-board stability testing was performed in a manner such that the EnLite™ Neonatal TREC Kits were first performed with the in-use treated kit components and the plates were measured without delay. The same plates were re-measured when 70 minutes had elapsed from the end of the PCR program. Table 5 below summarizes the mean TREC results of the in-use study (before on-board testing) followed by results measured 70 minutes after completion of the PCR program (after on-board testing). Table 6 below summarizes the mean beta-actin results on the on-board testing study.

These studies demonstrate that the elution buffer can be stored at 2 to 8° for 14 days, once it is diluted, and used in the EnLite™ Neonatal TREC Kit. The reagent mixture can be stored at 2 to 8°C for 23 hours, once it is prepared. For the calibrators and controls, once they are opened, they can be thawed and re-frozen four times, stored at -30 to -16°C for 14 days in a resealable plastic bag with desiccant (protected from light) and used in the EnLite™ Neonatal TREC Kit. The reagent concentrate, reaction buffer, DNA Polymerase and PCR diluent can

also be thawed and re-frozen four times, stored at -30 to -16°C for 14 days and then used in the EnLite™ Neonatal TREC Kit. Microplates with punched calibrators and controls in wells can be stored at room temperature (19 to 25°C) after PCR and hybridization for 60 minutes before measurement.

Table 5. Mean TREC on-board stability test results

Control	Target (Copies/µL)	Mean TREC concentration (Copies/µL)	
		Before on-board testing	After on-board time
Kit control 1	117	85	86
Kit control 2	0	0	0
Kit control 3	470	343	344
TREC Reference Control K1	67	46	51
TREC Reference Control K2	118	72	77
TREC Reference Control K3	287	104	120

Table 6. Mean beta-actin on-board testing results

Control	Target (Copies/µL)	Mean beta-actin concentration (Copies/µL)	
		Before on-board testing	After on-board time
Kit control 1	52	46	38
Beta-actin Reference Control K1	56	52	49
Beta-actin Reference Control K2	80	71	66

d. Detection limit:

The objective of the study was to determine the TREC limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) for the EnLite™ Neonatal TREC Kit.

Limit of Blank (LoB)

Five dried blood spot samples prepared from porcine whole blood with no endogenous TREC and low basal level of beta actin were used to assess the LoB. b(4) The blood was dispensed on filter paper intended for neonatal screening b(4) and dried. Repeated measurements of the blank samples were tested against a full calibration curve (blank reaction and three DBS calibrators in 3 replicates). Three kit controls in duplicate were included in each plate. Ten separate runs of six replicates/sample were performed over five operating days (10 runs x 6 replicates = 60 results/sample). Two kit lots and two thermal cyclers/fluorometer instruments were used for the analysis. b(4)

b(4) The observed LoB of TREC was determined to be 3 copies/µL, which met the pre-defined acceptance criteria for the EnLite™ Neonatal TREC Kit.

Limit of Detection (LoD) and Limit of Quantification (LoQ)
Five low-level samples (19, 29, 42, 59 and 74 TREC copies/µL) were used for defining the LoD and the LoQ for TREC. The samples were dried blood spot samples prepared from adult EDTA whole blood that contains a low endogenous level of TREC. b(4) The blood was spiked with TREC plasmid DNA to obtain low TREC samples. As whole blood contains a considerable level of endogenous beta actin, the beta-actin level is constant in all samples. The DBS samples were prepared by spotting the blood with different TREC concentration levels on filter paper intended for newborn screening, b(4)

Repeated measurements were carried out using five low level TREC dried blood spot samples. For the study, 27 runs were performed using 5 samples with 4 replicates/sample (27 runs x 4 replicates = 108 results per sample) over 20 operating days. Three kit lots and three thermal cyclers/fluorometer instruments were used for the analysis. Three kit controls in four replicates were included in each plate. LoD, was defined as the 95th percentile of measurements that exceeds the LoB. The LoQ is defined as the lowest concentration of the analyte that can be measured with acceptable total variation.

An estimate of the standard deviation for LoD was derived from five low level sample result distributions. b(4) The most conservative highest kit lot SD was used and the LoD was reported as 20 copies/ µL, b(4) b(4)

Since there is no reference method for the value assignment of TREC concentration for the LoQ study and no international reference standard, a functional sensitivity study was used to define LoQ. The total SD less than or equal to the specification for total variation was fulfilled for samples with TREC concentrations equal or higher than 29 copies/µL. The observed LoQ of 29 copies/µL met the acceptance criteria.

The reported values for TREC are LoB 3 copies/µL; LoD 20 copies/µL and LoQ 29 copies/µL.

e. Analytical specificity:

Interfering Substances:
The objective of this study was to evaluate the potential for interfering substances

(endogenous) in dried blood spot samples on the measurement of TREC concentrations with the EnLite™ Neonatal TREC Kit. The effect of bilirubin (unconjugated 100mg/L blood) and conjugated (166mg/L blood), lipids (15mg/mL blood), hemoglobin (200g/L blood (this hemoglobin concentration exceeded the recommended interferent test concentration of CLSI EP7-A2), and heparin (0.375 mg/mL blood) on test performance was evaluated. Additionally, because preliminary studies suggested that there was interference by hemoglobin, blood pools with three different TREC concentration levels (unspiked blood and two spiked TREC levels) and intermediate hemoglobin levels (155, 170 and 185 g/L blood = 25% pool, 50% pool and 75% pool, respectively) were also prepared for testing. Interference on TREC was tested using three different TREC levels (low, mid, high) using one kit lot. Dried blood spot samples were prepared by dispensing the prepared samples onto filter paper intended for newborn screening, b(4)

Potential interference was measured using the paired-difference method in which the tested substance is added to the sample. For the study acceptance criteria, a bias of greater than 1SD (calculated from the total variation of the test) at the 5% level of statistical significance (α=0.05) is considered analytically significant.

Unconjugated bilirubin at a concentration of 100 mg/mL blood, conjugated bilirubin at a concentration of 166 mg/L blood, heparin at a concentration of 0.375 mg/mL blood, lipemic samples at lipid concentration of 15 mg/l blood did not interfere with the measurement of TREC using the EnLite™ Neonatal TREC Kit. However, hemoglobin did have an interfering effect and dose-response studies were performed. Only the lowest tested hemoglobin level (25% pool) displayed no interference at any of the tested TREC levels. Since the control pool had 138g/L of hemoglobin, and the 25% pool had 155g/L of hemoglobin, the amount of hemoglobin added to the 25% pool was 17 g/L. Hemoglobin at concentrations lower than 17 g/L did not interfere with TREC measurements.

Primer and Probe Specificity

TREC DNA is formed from sequences from chromosome 14. Beta-actin gene is located in chromosome 7. In silico analysis was conducted to assess for the possibility of cross-reactivity to other sequences. No homology was observed leading to false results.

Specimen Carryover Contamination:

The objective of this study was to evaluate whether analyte can be carried over from one sample reaction to subsequent sample reactions by the puncher instrument used to punch out blood disks from the DBS sample for analysis. The test was performed using one kit lot of the EnLite™ Neonatal TREC Kit and three Wallac DBS puncher instruments used to punch 1.5 mm blood spot disks for the analysis. There kit controls in duplicate were included in each plate. The high TREC sample to be used in the study was kit calibrator C prepared from b(4) The mid TREC sample to be used was cord blood prepared by spotting onto filter paper b(4) . The zero TREC level was prepared from porcine whole blood, which is not spiked with TREC. b(4)

b(4) The carry-over result for each of the three puncher instruments is summarized in the Table 7 below. The carry-over study meets the predefined acceptance criteria for TREC. b(4) The highest observed mean carry-over percentage was 1.5%.

Table 7. Mean carry-over percentages separately for each of three Puncher instruments. N=number of repetitions of the high- low-low cycle.

Puncher	High sample material	Mean carry-over-percentage (%) (N=8)
1	Calibrator C	0.6
	Cord blood sample	0.4
	Neonatal DBS sample	3.7
2	Calibrator C	0.1
	Cord blood sample	0.0
	Neonatal DBS sample	0.6
3	Calibrator C	-0.1
	Cord blood sample	0.1
	Neonatal DBS sample	0.1

f. Assay cut-off:

The test reportable range for TREC is 29 copies/µL to 473 copies/µL blood. Samples that result in values below 29 copies/µL blood are reported as <29 copies/ µL blood. Samples that result in values above 473 copies/µL blood are reported as >473 copies/µL blood. The reportable range for beta-actin is demonstrated to be from 16 copies/ µL blood to 608 copies/ µL blood. The clinical cut-offs are as follows:

TREC positive: ≥36 copies/µL
TREC negative: < 36 copies/µL

Beta-actin: The test is valid when beta-actin copy number is ≥56 copies/µL

2. Comparison studies:

a. Accuracy:
Not applicable.

b. Matrix comparison:

Between Filter Paper Lot-to-Lot Reproducibility
A filter paper lot-to-lot reproducibility study using filter paper from two manufacturers, was conducted in order to evaluate manufacturer differences, and lot differences, as well as to assess homogeneity of the analyte across the dried blood spot. The samples used in the study were prepared from adult whole blood spiked with TREC plasmid DNA to achieve six TREC levels, five of which were within the 29-473 TREC copies/µL measuring range of the test (43, 69, 93, 155, 316 TREC copies/µL). b(4)

b(4) The six levels were dispensed, b(4) onto six different lots of filter paper (3 lots each from two distinct filter paper manufacturers. The total number of samples analyzed in the study, therefore, was 36 (b(4) The study was performed using one lot of the EnLite™ Neonatal TREC Kit and PCR was performed using three different thermocyclers from the same manufacturer.

b(4)

Overall precision for the EnLite™ Neonatal TREC Kit was calculated as the sum of within-run, between-run, between paper lot and between paper-manufacturer variations.

b(4)

Table 8 below summarizes the within-run, between-run, between paper-lot, between paper-manufacturer and total variation across paper manufacturers and lots. Within the measuring range, within-run variation was between 29 to 47%, between-run variation was between 28 to 32%, between paper-lot variation was between 0.02 to 0.05%, between paper-manufacturer was 0.01 to 0.04% and total variation was 44 to 56%. Imprecision was higher outside of the measuring range. Additionally there are no significant differences between paper manufacturers or between paper lots at the six sample levels tested in the study and that homogeneity is equal between the different paper manufacturers and between paper lots.

Table 8. Within-run, between-run, between paper lot, between paper-manufacturer and total variation across paper manufacturers and lots

Sample	n	Mean TREC (copies /µl)	Within-run variation % CV	Between-run variation %CV	Between-paper lot variation % CV	Between paper-manufacturer variation %CV	Total variation CV (copies/µl)
TR1	180	3	122	32	0.11	0.06	132
TR2	180	43	47	28	0.05	0.02	56
TR3	180	69	37	29	0.03	0.01	48
TR4	180	93	37	29	0.02	0.01	48
TR5	180	155	40	28	0.03	0.01	50
TR6	180	316	29	32	0.04	0.04	44

3. Clinical studies:

a. Establishment of the cut-off

A study was conducted to establish a clinical screen cut-off based on the normal newborn population distribution data with the EnLite™ Neonatal TREC Kits and to confirm the TREC and beta-actin cut-off values derived in a separate feasibility study. The cut-off confirmation study utilized a total of 3243 archived, retrospective newborn specimens from the Danish Newborn Screening Biobank (NBS-Biobank). The samples, which originated from the Danish population, were leftover dried blood spot (DBS) samples collected on devices intended for newborn screening. The samples used for the cut-off confirmation study were independent of samples tested in the feasibility study and the pivotal study (samples in the cut-off confirmation study were different than samples in the feasibility study and the pivotal study).

After sample elimination due to failure to meet specimen inclusion/exclusion criteria, 2846 samples were included in the analysis. Table 9 below summarizes the distribution of the TREC and beta-actin copies for the newborn specimens analyzed with the EnLite™ Neonatal TREC Kit. The 2.5th percentile was identified as the cut-off for use in the pivotal clinical validation study. The cut-off is 36 copies/µL for TREC and 56 copies/µL for beta-actin. Figure 6 shows the TREC copies/µL distribution of the samples. The vertical line indicates the 2.5th percentile cut-off. Figure 7 shows the beta-actin copies/µL distribution of samples. The vertical line indicates the 2.5th percentile cut-off.

Table 9. Distribution of TREC and beta-actin in newborn specimens.

	N	Median	0.5%	1.0%	2.0%	2.5%	5.0%
TREC	2846	150	21	25	34	36	46
Beta-Actin	2846	595	25	34	53	56	79

Figure 6. The TREC copies/µL distribution of samples. The vertical line indicates the 2.5th percentile cut-off for TREC at 36 copies/µL.

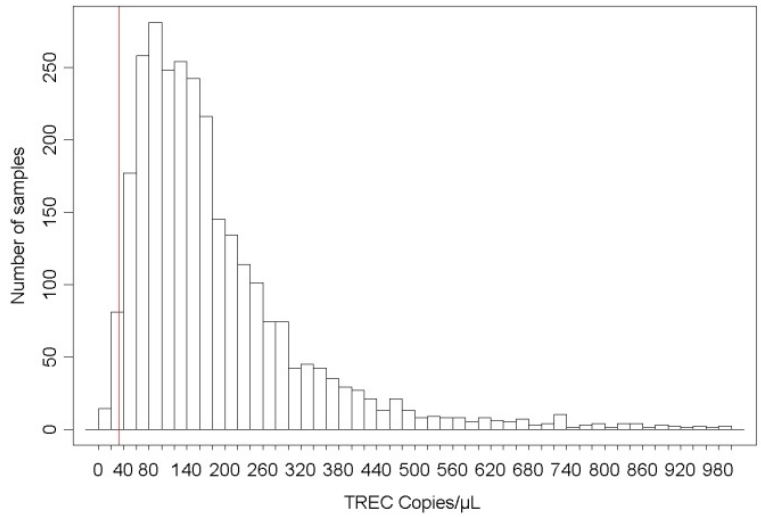
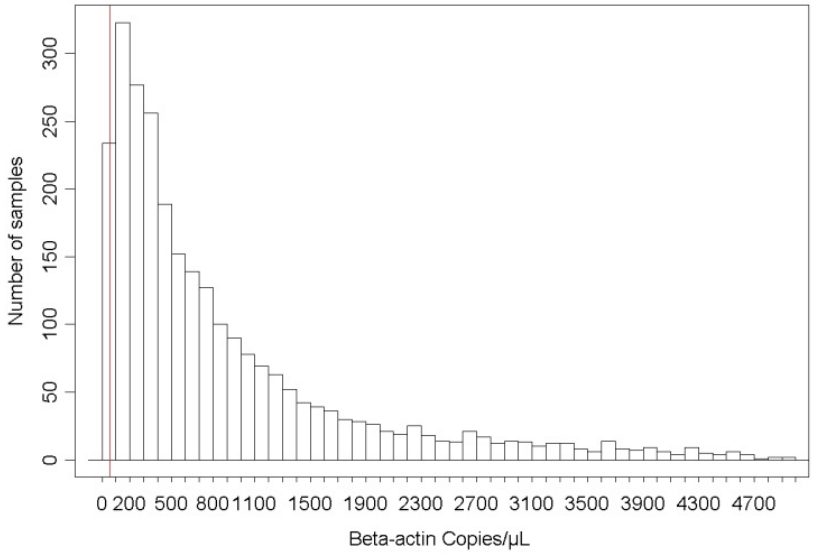


Figure 7. The distribution of beta-actin copies/µL values among samples. The vertical line indicates the 2.5th percentile cut-off for beta actin at 56 copies/µL.



b. Pivotal Study:

The objective of the clinical study was the following: to demonstrate the screening

performance of the EnLite™ Neonatal TREC Kit in the intended use population; to demonstrate that the EnLite™ Neonatal TREC Kit discriminates between normal and SCID cases in the intended use population; to ensure that the EnLite™ Neonatal TREC Kit conforms with defined user needs and intended use; to assess the usability of the VICTOR EnLite and EnLite workstation as part of the EnLite™ Neonatal TREC Kit. The study was conducted at one external newborn screening laboratory located at Statens Serum Institut (SSI) Denmark. The study involved testing of archived, retrospective dried blood spot samples submitted to the laboratory for routine newborn screening and stored in the Danish biobank. A total of 6389 archived, retrospective newborn specimens were tested from the Danish Newborn Screening Biobank (NBS-biobank). To avoid sample selection bias, 6389 routine samples, consecutively received at SSI and archived into the NBS-biobank from newborn patients from June 26, 2012 through August 6, 2012 were sequentially entered into the study according to the inclusion/exclusion criteria for the study. Inclusion criteria for the pivotal study included age of the newborn at time of sample collection ≤31 days. Exclusion criteria included: the DBS not of appropriate quality, insufficient number of punches available from the DBS to complete testing, the parents of the newborn opted out of use of the newborn's sample, the DBS is older than 19 months, or the DBS could not be found in the Biobank. The gestational age of the majority of the subjects was greater than 39-weeks (approximately 72%). Approximately 18% of the subjects were 37-38 gestational weeks, 3.3% were 35-36 weeks, and less than 3% were ≤34 weeks.

Due to the low incidence of SCID, the specimen testing population was enriched with 17 archived confirmed SCID positive DBS specimens obtained from newborn screening laboratories in the US. All of the 17 samples were confirmed for SCID by flow cytometry. In addition to the 17 confirmed positive SCID samples, an additional nine DBS specimens were used from babies with low TREC values ranging from 0 to 20 TREC Copies/µL. Prior to testing all the specimens were stored at -20ºC. An additional 56 normal TREC samples were provided by the laboratories that supplied the confirmed SCID positive samples, in order to mask identification of the true SCID positive samples before they were tested.

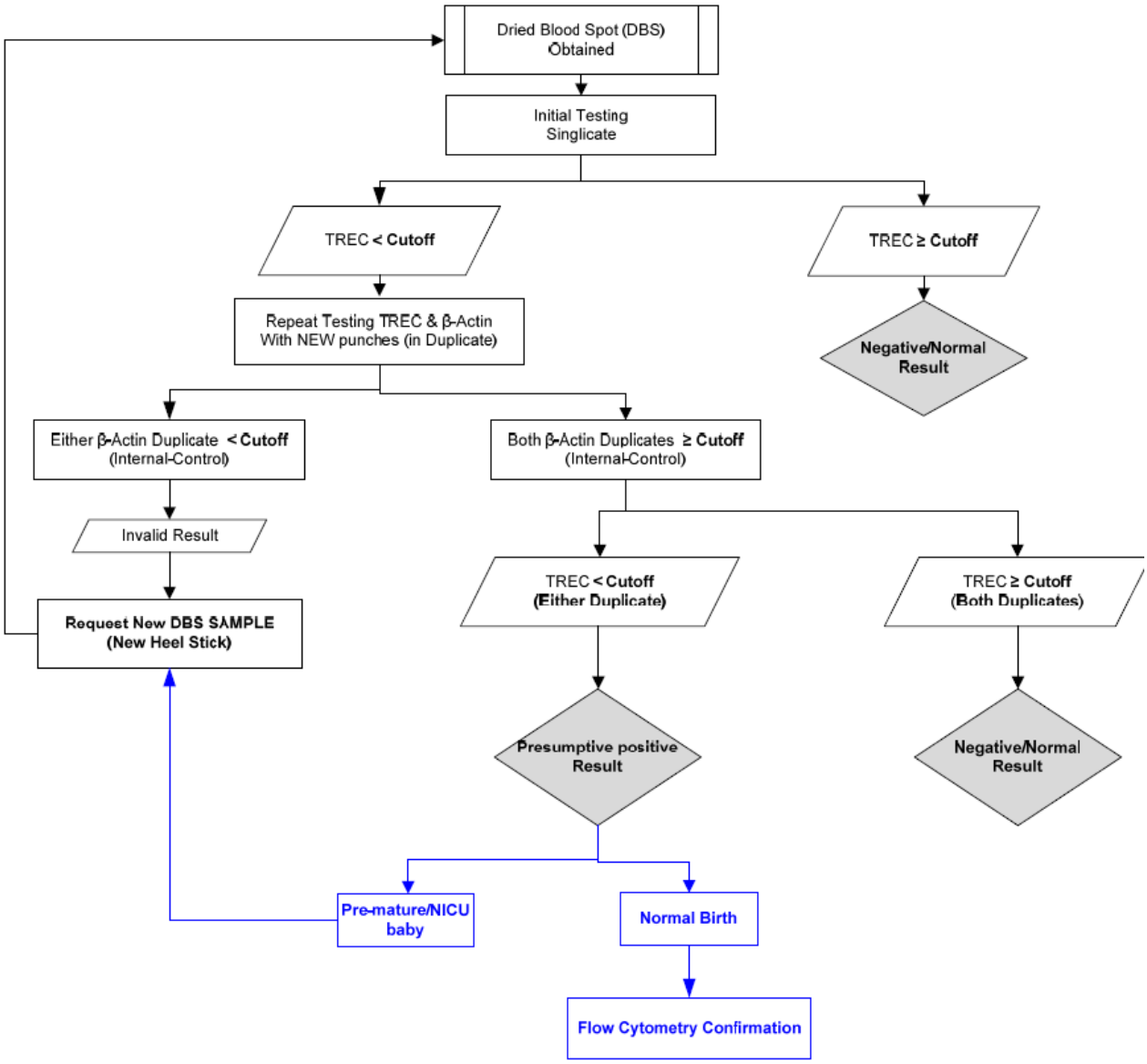
As summarized in Table 10 below, a total of 6471 neonatal specimens were tested in the study. This included 6389 Danish newborn screening biobanked newborn routine DBS samples, and 82 enrichment samples (17 confirmed SCID positive samples, 9 confirmed low-level TREC specimens and 56 samples used for blinding purposes). Of the 6471 neonatal specimens, 30 were removed due to violation of the inclusion/exclusion criteria and 68 specimens were removed for other reasons, leaving 6373 included in the analysis population. Table 10 shows the distribution of specimens tested and reasons for exclusion.

Table 10. Retrospective Dried Blood Spot Samples used in the Pivotal Study

Sample Description	Number of Samples		
	Routine	Enrichment samples	Total
Number of all samples run	**6389**	**82**	**6471**
Samples Removed Due Inclusion/Exclusion Criteria			
Age of newborn ≥ 31 days	29		29
Unacceptable quality sample	1		1
Samples Removed Due to Other Issues			
Sample possessed identifier not traceable in Danish registries; error in identifier	1		1
Specimens used for blinding purposes		56	56
Repeat samples of subjects already in study	2		2
Specimens resulting from protocol deviation #4	9		9
Number of Samples included in the study	**6347**	**26**	**6373**
Confirmed SCID positives		17	
Confirmed low TREC positives		9	

The specimens were analyzed according the EnLite™ Neonatal kit insert. Each run included calibrators in triplicate. The cut-off values for TREC and beta-actin were previously determined to be 36 and 56 copies/µL, respectively, based on the 2.5th percentile of the normal distribution of specimens tested, conducted prior to the pivotal study. Study samples were analyzed in singlicate and repeated in duplicate according to the testing algorithm in the package insert and shown in Figure 8. Final testing round samples that result in values below 16 copies/ µL blood are reported as <16 copies/ µL blood and are considered to have DNA amplification failure. Samples that result in values above 608 copies/ µL blood are considered to be normal in terms of amplification efficiency.

Figure 8. Testing Algorithm for the Pivotal Study using the EnLite™ Neonatal TREC Kit



As the comparator for the over 6347 routine clinical study specimens, the clinical assessment of the study subjects was obtained from their medical records to confirm that the newborn at one year of age (365 days) or older, was not been identified with SCID, or was not deceased from SCID-related complications, and was apparently healthy.

Table 11 summarizes the distribution of TREC results for the EnLite™ Neonatal TREC Kit based on the cut-off determination of 36 TREC copies/µL for specimens in the data analysis. Calculations were performed using the initial testing results. The 2.5th percentile TREC result was observed to be 41-42 copies/µL, which was higher than the pre-defined cut-off of 36 TREC copies/µL determined in the cut-off confirmation study. Table 12 summarizes the distribution of beta-actin results for the EnLite™ Neonatal TREC Kit.

Table 11. Distribution of TREC results

					Mean TREC Copies/uL by bottom percentile				
Population	**N**	**Median**	**Min**	**Max**	**0.5%**	**1.0%**	**2.0%**	**2.5%**	**5.0%**
SCID+low TREC	26	2	0	24					
All Routine Samples	6347	172	6	>473	26	32	39	41	52

Table 12. Distribution of Beta-actin Results

Population	Nobs	Median	Min	Max	0.5%	1.0%	2.0%	2.5%	5.0%
SCID+low TREC	26	1028	77	19900	NA	NA	NA	NA	NA
All Routine Samples	6347	691	2	>608	17	26	42	48	69

Of the available 6347 routine newborn specimens, 910 (14%) cases were loss-to-follow due to the absence of proof-of-life documentation at 12 months of age or older in available vaccination and hospitalization records. Table 13 shows the 5454 specimen results from 5437 routine newborn specimens along with the 17 confirmed positive SCID cases. For the confirmed positive SCID cases, the comparator was the reference tests results. In the initial round of testing the EnLite™ Neonatal TREC Kit identified all of the 17 confirmed SCID specimens as below the cut-off of <36 Copies/µL, which acording to the testing algorithem, requires that the samples be retested. An additional 94 routine newborn screening samples were retested in the first round for a total of 111 samples retested after the first round (17 + 94 =111). Table 14 shows the positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA), along with 95% confidence intervals for the first round of testing.

Table 13. Cross tabulation of newborn specimens (n= 5454) that were categorized as normal or presumptive positive by the EnLite Neonatal TREC Kit.

		Comparator		
		SCID (%)	**Normal (%)**	**Total (%)**
EnLite Neonatal TREC Final test classification	Invalid result (%)	1 (5.9)	11 (0.2)	12 (0.2)
	Presumptive positive (%)	16 (94.1)	16 (0.4)	32 (0.6)
	Normal (%)	0 (0)	5410* (99.4)	5410 (99.2)
	Total (%)	17 (100)	5437 (100)	5454 (100)

* Includes a specimen with known IKK2 mutation but normal T cell level.

After the second round of testing, the final classification, all but one of the 17 confirmed

SCID positive specimens were classified as presumptive SCID screen positive. One SCID positive specimen was classified as invalid result due to beta-actin amplification failure. Table 14 displays the tabulation of results for the 5454 specimen results with the 12 invalid results specimens removed. In routine newborn screening use, if a subject result is categorized as invalid result, a new blood specimen and retesting should be requested. Overall percent agreement = (16 + 5410) / 5442 * 100% = 99.7 % (95% CI from 99.4% to 99.8%), Positive percent agreement = (16 / 16) * 100% = 100 % (95% CI from 79.4% to 100%), and Negative percent agreement = (5410 / 5426) * 100% = 99.7 % (95% CI from 99.4% to 99.8%).

Table 14. Cross tabulation of newborn specimens (n= 5442) that were categorized as normal or presumptive positive by the EnLite Neonatal TREC Kit (excluding invalid results).

		Comparator		
		SCID (%)	**Normal (%)**	**Total (%)**
EnLite Neonatal TREC Kit Final test classification	Presumptive positive (%)	16 (100)	16 (0.3)	32 (0.6)
	Normal (%)	0 (0)	5410* (99.7)	5410 (99.4)
	Total (%)	16 (100)	5426 (100)	5442 (100)

* Includes a specimen with known IKK2 mutation but normal T cell level.

Follow-up for the remaining 910 patients was performed. Patient outcome for the specimens in the study subject was assessed by evaluating patient records including data available from three different sources: The Danish Vaccination Registry, The Danish National Patient Registry and The Danish Civil Registration System. Clinical information included vaccination records post one year of birth, hospitalization records, and survival status at 17 months. The reason for death and hospitalization history was reviewed to assess the relation to SCID. TREC testing was conducted according to the pre-specified testing algorithm. The retest rate was 1.9%. The false positive rate using the cut-off 36 in the first round of testing was 1.5% and after repeat testing on follow-up cases, was 0.5%.

b. *Clinical specificity:*

See above

c. *Other clinical supportive data (when a. and b. are not applicable):*

N/A

4. Clinical cut-off:

The cut-off is identified as 36 TREC copies/µL
Beta-actin: The test is valid when Beta-actin copy number is ≥56 copies/µL

The following screening algorithm is used in the interpretation of results: The screening algorithm contains two rounds of testing designed to minimize the number of false positive specimens, and also to identify specimens that are deficient in PCR and for which a new blood specimen needs to be requested. Specimens are first tested in singlicate for TREC (i.e, first round). The specimens having low TREC values in the initial round are retested in duplicate to confirm the low TREC result. At the same time, the test results for beta-actin are compared to the cut-off to identify the specimens that are deficient in PCR (DNA amplification failure). Based on the findings in the first and second round of testing, the final interpretation of results can be performed.

Initial EnLite™ Neonatal TREC Kit results:

Initial TREC Result (Copies/µL)	**Interpretation of Result**	**Retest Procedure**
≥ 36 cut-off	Normal	No retest required
< 36 cut-off	Presumptive positive	Retest in duplicate with DBS from same blood sample

Final EnLite™ Neonatal TREC Kit results:

Initial TREC Result	**Beta-actin control Result duplicate retest**	**TREC Result duplicate retest**	**Final result**	**Interpretation of Result**
≥ cut-off	--	No retest required	Normal	Normal
< cut-off	Both ≥ cut-off	Both ≥ cut-off	Normal	Normal
< cut-off	Both ≥ cut-off	Either duplicate < cut-off	Presumptive Positive	If full term newborn proceed to confirmation testing. If premature / NICU* newborn request new sample and retest.
< cut-off	Both < cut-off or no agreement between duplicates	--	Invalid result	Request new sample and retest. If second sample is inconclusive, obtain new sample two weeks later and retest.

5. Expected values/Reference range:

a. The estimated annual incidence of SCID is one case per 40,000--100,000 live births, or a total

of approximately 40--100 new cases among infants in the United States each year. In a screening program implemented 6 states for 126 months of continuous screening, 961,925 newborns have been screened, 364 newborns had a positive screen requiring additional testing and resulting in 60 cases of diagnosed immune deficiency. Table 15 shows the breakdown of the number of SCID positives and SCID negatives observed. (Report by the Secretary's Advisory Committee on Heritable Disorders in Newborn and Children.)

Table 15. Number of Negative and Positive Screens by State:

Screening Result	**State**							**Total Screened**
	WI	**MA**	**Navajo Nation**	**New York**	**California**	**Puerto Rico**	**Louisiana**	
Negative[a]	243,657	161,679	1,296	136,412	357,954	29,107	31,456	961,561
Positive[b]	50	28	1	223	46	8	8	364
Total Screened	243,707	161,707	1,297	136,635	358,000	29,115	31,464	961,925

Negative: TREC copy number above cut-off point. No further analysis needed. Positive: TREC copy number below cut-off point. Case referred for confirmatory diagnostic studies.

b. A study of 5766 de-identified dried blood spot samples submitted to the Wisconsin newborn screening program determined that 1.6% of newborns have <75 TREC copies copies/3µL, or the equivalent of 25 TREC copies/µL. The cut-off of <25 cleared 98.94% of all initial specimens in the initial test. (Baker et al., Implementing routine testing for severe combined immunodeficiency within Wisconsin's newborn screening program, 2010, Public Health Reports, Volume 125 (supplement 2), 88-95).

N. Instrument Name:

The VICTOR EnLite Fluorometer

O. System Descriptions:

1. Modes of Operation:
   The VICTOR EnLite Fluorometer is designed to accept 96-well microtiter plates, which can be loaded manually or with a stacker for automatic operation. The instrument automatically measures the microtiter plates and outputs quantitative results based on the measured fluorescence signals.
2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes √

3. Specimen Identification:
   Specimens are transferred to a barcode-labeled 96-well microtiter plate. Users create a plate map that identifies the specimen in each well using the VICTOR EnLite Workstation Software. The barcode and the plate map are used to track the specimens.

4. Specimen Sampling and Handling:
   Specimens are processed according to EnLite™ Neonatal TREC Kit instructions.

5. Calibration:
   The VICTOR EnLite Fluorometer does not require to be calibrated by end-users.

6. Quality Control

   Run Acceptance

   Before analyzing the EnLite™ Neonatal TREC Kit results, the following steps are performed:

   The blank reaction replicates (= no template control) are monitored to detect contamination. In a successful experiment, blank reaction replicates should have the median corrected 665 nm and 780 nm fluorescence signals below 7500 counts and below 1500 counts for beta-actin and TREC, respectively. If blank reaction replicates have the median corrected fluorescence signals above the acceptable limits, the run for the samples in that test plate should be repeated. The calibrator C replicates (high beta-actin / high TREC) are monitored to confirm successful PCR amplification. After successful amplification, calibrator C replicates should have the median corrected 665 nm and 780 nm fluorescence signals above 22000 counts and above 9500 counts for beta-actin and TREC, respectively. If calibrator C replicates have the median corrected fluorescence signals below the acceptable limits, the run for the samples in that test plate should be repeated.

   The following acceptance criteria were used for the kit control results:

   - C1 (Low control): The mean value of TREC result duplicates (Ln copies) must be within ±2SD from the target. The mean value of beta-actin duplicates (Ln copies) must be below target+2SD.
   - C2 (No TREC control): Both of the C2 control replicates must be equal or below the used TREC cut-off (40 copies/µl). The mean value of beta-actin result duplicates (Ln copies) must be above target-2SD.
   - C3 (High control): The mean value of TREC and beta-actin results (Ln copies) must be above target-2SD.

   Calibrator C results are monitored for the presence of TREC and beta-actin amplification. After successful amplification, calibrator C wells should have ‘TREC Response’ and

'Beta-actin response' above predefined levels. If calibrator C wells have median corrected fluorescence counts below the acceptable limits, an error message 'Low amplification' would be reported and the run would be rejected.

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section above:

A study was conducted to support the concordance between adult blood spiked with synthetic TREC plasmid and cord blood using specimens that spanned the measuring range.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, including adequately identifying the device as a prescription device, and the special controls for this type of device.

R. Identified potential Risks and Required Mitigation Measures:

Identified Potential Risk	Required Mitigations
False negative results due to device or user error	Special Controls (1) and (2)
False positive results due to device or user error	Special Controls (1) and (2)

S. Benefit/Risk Analysis

Summary	
Summary of Benefit(s)	Newborns may potentially benefit from the use of the device as an aid in screening because it might assist practitioners in making earlier decisions that diagnostic testing is needed for patients with severe combined immunodeficiency disorder and lead to earlier appropriate treatment.
Summary of Risk(s)	Associated device risks include erroneous false negative results due to device or user error or false positive results due to device or user error. A patient with a false positive result could have unnecessary additional testing. A patient with a false negative result could fail to be detected as a possible SCID case and be delayed from timely therapy. Proposed special controls and labeling are appropriate for risk mitigation as a class II device.
Summary of Other Factors	Analytical performance studies were considered as part of overall device review.
Conclusions Do the probable benefits outweigh the risks?	Yes. Based on the pivotal clinical study, proposed special controls, labeling, and analytical validation, the probable benefits outweigh the probable risks.

T. Conclusion:

The information provided in this de novo submission is sufficient to classify this device into class II under regulation 21 CFR 866.5930. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. This device is classified under the following:

Product Code:	PJI—Severe Combined Immunodeficiency Disorder (SCID) Newborn Screening Test System
Device Type:	Newborn screening test for SCID
Class:	II (special controls)
Regulation:	21 CFR 866.5930

(a) *Identification*. A newborn screening test for severe combined immunodeficiency syndrome (SCID) is a prescription device intended to measure T-cell receptor excision circle (TREC) DNA obtained from dried blood spot specimens on filter paper using a polymerase chain reaction (PCR) based test as an aid in screening newborns for SCID. Presumptive positive results must be followed-up by diagnostic confirmatory testing. 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.

(b) *Classification*. Class II (special controls). A newborn screening test for severe combined immunodeficiency must comply with the following special controls:

1) Premarket notification submissions must include the following information:

i. The intended use must indicate:

A) The test is not intended for diagnostic use, or for screening of SCID-like syndromes, such as DiGeorge syndrome or Omenn syndrome, and

B) The test is not intended to screen for less acute SCID syndromes, such as leaky SCID or variant SCID.

ii. A detailed description of all components in the test that includes:

A) A detailed description of the test components, all required reagents, instrumentation and equipment, including illustrations or photographs of non-standard equipment or methods.

B) Detailed documentation of the device software including, but not limited to, standalone software applications and hardware-based devices that incorporate software.

C) Specifications for the filter paper, which must be appropriately labeled for in vitro diagnostic use, to be used in specimen collection and how it will be used in specimen collection validation. These specifications must include: descriptive characteristics of the filter paper, instructions on how a lab should choose the appropriate filter paper, chemical properties of the filter paper, interference concerns associated with the

chemicals in the filter paper, and absorption properties of the filter paper, punch size, absorption capacity, testing for homogeneity of punches, diameter of the circle for the dried blood spot aliquot, absorption time, physical composition, and number and size of punches to be tested.

D) Methodology and protocols for detection of T-cell receptor excision circles and methods for determination of results. The cut-off must be selected before conducting clinical and analytical studies.

E) A description of the result outputs along with sample reports. Sample reports must include the scale used in reporting of results (e.g., TREC copies/µL) and the range of values that will be reported out.

F) A description of appropriate internal and external controls that are recommended or provided. The description must identify those control elements that are incorporated into the testing procedure.

iii. Information that demonstrates the performance characteristics of the test, including:

A) Data that demonstrates the clinical validity of the device, using well characterized prospectively or retrospectively obtained clinical specimens representative of the intended use population. A minimum of 10-15 confirmed positive specimens must be obtained from more than one site, including relevant annotation, and, at one year or beyond, a SCID diagnosis by flow cytometry or clinically meaningful information regarding the status of the subject must be obtained. Additional specimens should have been obtained that are characterized by other disorders that can be found by screening specimens that have low or absent TREC (e.g., other T-cell lymphopenic disorders) to supplement the range of results. The clinical validation study must have a pre-specified clinical decision point (i.e., cut-off to distinguish positive and negative results). Results must be summarized in tabular format comparing interpretation of results to the reference method. Point estimates together with two-sided 95 percent confidence intervals must be provided for the positive percent agreement (PPA), negative percent agreement (NPA) and overall percent agreement (OPA). Data must include the retest rate, the false positive rate before retest, the final false positive rate, and the false negative rate.

B) Device reproducibility data generated, using a minimum of three sites of which at least two must be external sites, with two operators at each site. Each site must conduct a minimum of 5 runs per operator over 5 non-consecutive days evaluating a minimum of six different relevant TREC concentrations that span and are well distributed over the measuring range and include the clinical cut-off. Specimens must include cord blood and cord blood diluted with ABO matched adult blood specimens. Identical specimens from the same sample panel must be tested at each site. Each specimen must be run in triplicate and include controls run in triplicate. Results must be reported as the standard deviation and percentage coefficient of variation for each level tested. Results must also be displayed as a dichotomous variable around the cut-

off. Total variation must be partitioned into the sum of within-lab and between-lab variations with pre-specified acceptance criteria and 95 percent confidence intervals for all data. Pre-specified acceptance criteria must be provided and followed.

C) Device precision data using clinical samples to evaluate the within-lot, between-lot, within-run, between run and total variation. A range of TREC levels of the specimen must include samples within the measuring range, samples above and below the measuring range, as well as with samples very near above and below the cut-off value. At least three replicates of each specimen must be tested with controls and calibrator(s) according to the device instructions for use. The precision study must use well characterized samples using different lots, instruments and operators. Results must be summarized in tabular format. Pre-specified acceptance criteria must be provided and followed.

D) Linearity of the test must be demonstrated using a dilution panel from clinical samples. The range of dilution samples must include samples within the measuring range, samples above and below the measuring range, as well as with samples very near above and below the cut-off value. Results of the regression analysis must be summarized in tabular format and fitted into a linear regression model with the individual measurement results against the dilution factors. Pre-specified acceptance criteria must be provided and followed.

E) Device analytic sensitivity data, including limit of blank, limit of detection, and limit of quantification.

F) Device specificity data, including interference, carryover, cross-contamination, and in silico analysis of potential off-target genomic sequences.

G) Device stability data, including real-time stability of samples under various storage times, temperatures, and freeze-thaw conditions. A separate shipping stability study must be performed.

H) Lot-to-lot reproducibility study of each filter paper that will be validated with the test. The lot-to-lot study must include a minimum of three lots of each blood spot card that will be validated with the test and be conducted over 5 non-consecutive days. The sample panel must consist of specimens with a range of TREC levels and include samples within the measuring range, samples above and below the measuring range, and samples very near above and below the cut-off value. Multiple punches must be obtained from each card for demonstration of homogeneity of the analyte across the dried blood spot. Comparability of the test performance for each filter paper must be demonstrated. Stability and storage of TREC DNA on each blood spot card must be demonstrated. Results of the lot-to-lot study must be summarized providing the mean, standard deviation, and percentage coefficient of variation in a tabular format. Data must be calculated for the within-run, between-run, within-lot, and between-lot. Data demonstrating the concordance between results across different filter papers must be provided. Study acceptance criteria must be provided and followed.

I) If applicable, a thermocycler reproducibility study must be performed using thermocyclers from three independent thermocyler manufacturers. The sample panel must consist of specimens with a range of TREC levels and must include samples within the measuring range, samples above and below the measuring range, and samples very near above and below the cut-off value. The study must be done using 3 filter paper lots and conducted over 5 non-consecutive days. Results of the thermocycler reproducibility study must be summarized providing the mean, standard deviation, and percentage coefficient of variance in a tabular format. Data must be calculated for the within-run, between-run, within-lot, between-lot and between thermocycler manufacturer study results. Study acceptance criteria must be provided and followed.

iv. Identification of risk mitigation elements used by your device, including a description of all additional procedures, methods and practices incorporated into the directions for use that mitigate risks associated with testing.

2) Your 809.10 compliant labeling must include:

i. A warning statement that reads, "This test is not intended for diagnostic use, pre-implantation or prenatal testing, 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."

ii. A warning statement that reads, "Test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, and clinical evaluation as appropriate."

iii. A description of the performance studies listed in section 1(iii) and a summary of the results.

iv. A description of the filter paper specifications required for the test.