510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k090123

B. Purpose for Submission:
New device

C. Measurand:
Biotinidase (BTD)

D. Type of Test:
Semi-quantitative fluorometric assay

E. Applicant:
Wallac Oy, Division of PerkinElmer, Inc.

F. Proprietary and Established Names:
PerkinElmer Neonatal Biotinidase Kit

G. Regulatory Information:

Device Name	Product Code	Classification	Regulation Section	Panel
Neonatal Biotinidase Kit	NAK	Class II	21 CFR § 862.1118	Chemistry (75)
Biotinidase Calibrators	JIT	Class II	21 CFR § 862.1150	Chemistry (75)
Biotinidase Controls	JJX	Class I, Reserved	21 CFR § 862.1660	Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See Indication(s) for use below.

2. Indication(s) for use:
   The Neonatal Biotinidase kit is intended for the semi-quantitative determination of biotinidase activity in blood specimens dried on filter paper as an aid in screening newborns for biotinidase deficiency.

3. Special conditions for use statement(s):
   For *in vitro* diagnostic use only, for prescription use only, and the package insert contains the following black box warning: Warning: Neonate albumin levels above normal (2.8 to 4.4 g/dL) can interfere with this test by increasing biotinidase activity. This could result in the misclassification of a patient with a

biotinidase result near the cut-off value as 'normal' when in fact, the patient should be classified as 'deficient'. A patient with known or clinically suspected elevated blood albumin concentration should be screened with an alternative method and confirmed according to local requirements for follow-up testing.

4. Special instrument requirements:
   A fluorometer, capable of measuring the fluorescence from the top of the well and using the excitation central wavelength of 355 nm and the emission central wavelength of 460 nm

   The performance data provided in this submission was generated using the 1420 VICTOR^2 D fluorometer.

I. Device Description:

The Neonatal Biotinidase kit has two package configurations: Model number 3018-0010 (960 assays) and model number 3018-001B (4800 assays).

The kit contains the following components:

Six calibrators (prepared from porcine whole blood with a preservative spotted onto filter paper) that contain BTD at the following concentrations (approximate values, 1U = nmol/min/dL): A (10 U), B (30 U), C (130 U), D (180 U), E (250 U) and F (350 U).

Two controls (prepared from human blood (hematocrit adjusted to 55%) with a preservative spotted onto filter paper) that contain BTD at the following concentrations (approximate values, 1U = nmol/min/dL): Normal (275 U) and Abnormal (50 U).

Biotinidase Reconstitution Buffer
Biotinidase Substrate Reagent
Black microplates (uncoated, 96 wells)
Barcode labels for the plate
Adhesive microplate covers
Lot specific quality control certificate

All human source materials used in the preparation of kit components was tested and found to be non-reactive for the presence of HBsAg, anti-HIV 1 and 2, and HCV by FDA-approved methods.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Astoria-Pacific SPOTCHECK® Biotinidase 50-Hour Reagent Kit

2. Predicate K number(s):
k010844

3. Comparison with predicate:

Item	Device	Predicate Device
Similarities		
Intended Use	The Neonatal Biotinidase kit is intended for the semi-quantitative determination of biotinidase activity in blood specimens dried on filter paper as an aid in screening newborns for biotinidase deficiency.	The Astoria-Pacific® SPOTCHECK Biotinidase 50 Hour Reagent Kit (k010844) is intended for the semi-quantitative determination of biotinidase, EC 3.5.1.12, activity in dried whole blood spots using the Astoria-Pacific SPOTCHECK® Analyzer. Measurement of biotinidase activity is primarily for the diagnosis and treatment of biotinidase deficiency in newborns. This method is intended for in vitro diagnostic use to aid in screening for decreased levels of biotinidase activity and not for monitoring purposes.
Specimen type	Whole blood specimen spotted on filter paper	Same
Assay technology	Enzymatic	Same
Interpretation of results	Calibration curve	Same
Differences		
Test principle	1-step enzymatic assay were the BTD in the sample cleaves the substrate biotin 6-aminoquinoline (6-AQ) generating a fluorescent 6-AQ product.	2-step assay were BTD releases p-Aminobenzoic acid (PABA) from biotinyl-p-aminobenzoate. The PABA is diazotized and coupled to a napthol derivative to form a purple chromophore.
Detection technique	Fluorometric	Colorimetric
Instrumentation requirement	Fluorometer with excitation central wavelength of 355 nm and the emission central wavelength of 460 nm	Astoria-Pacific SPOTCHECK® Analyzer
Screening outcome	Normal and Deficient	Normal, Partial Deficient and Profound Deficient
Measuring unit	U	ERU
Calibrator matrix	Dried blood spots (DBS) prepared from porcine blood.	Liquid standards. PABA stock standard. 1.0 mM p-Aminobenzoic acid diluted with Tris Buffer.
Calibrator concentrations	Six levels, ready to use 10 U 30 U 130 U 180 U	Six levels to be prepared from PABA stock standard 0 ERU 5 ERU 25 ERU

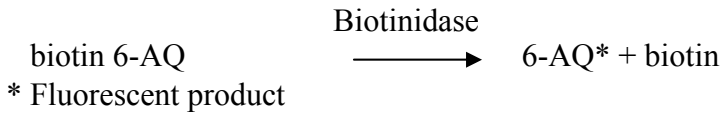
	250 U 350 U	50 ERU 100 ERU 200 ERU
Quality control materials	Included in the kit. DBS samples prepared from human blood. Normal 275 U Abnormal 50 U	Provided separately

K. Standard/Guidance Document Referenced (if applicable):

- CEN 13640, Stability Testing of In Vitro Diagnostic Reagents. 2002.
- Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition (CLSI EP5-A2).
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A).
- Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition (CLSI EP7-A2).
- Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (CLSI EP9-A2).
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A).

L. Test Principle:

The Neonatal Biotinidase kit is based on a semi-quantitative fluorometric assay. A schematic presentation of the BTD enzymatic reaction is shown below.



BTD is found in the blood sample itself. Filter paper disks from newborn DBS samples, calibrators and controls are punched into the wells of a microplate. When biotin substrate reagent containing biotin 6-aminoquinoline (6-AQ) is added to a well containing a punched DBS, the reagent extracts and reconstitutes the proteins and enzymes in the spot. The BTD enzyme in the sample cleaves the substrate to biotin and fluorescent 6-AQ. The addition of the ethanol stops the reaction and precipitates the proteins to cover the bottom of the well and the extracted spot. The fluorescent product (6-AQ) formed during the reaction is measured with a fluorometer. The BTD activity of the sample is determined by comparing the fluorescence intensity of the sample to a calibration curve. The measurement is expressed as U. 1 U = 1 nmol of the end-product that is formed during one minute and in one deciliter of blood (1 nmol/min/dL).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the proposed device was determined according to CLSI EP5-A2. The variation of the assay was determined using DBS samples with 6 different activity levels, 3 kit lots, 3 instruments, and 3 operators. The study was performed on 20 separate measurement days. Each run included 4 replicates per sample level. A nested, balanced random effects analysis of variance (ANOVA) was used to calculate the estimates of precision. The results are summarized below:

mean (U)	**n**	**Measured (U)**		**Within Run Variation**		**Within Lot* Variation**		**Total Variation**	
		Min	**Max**	**SD**	**CV%**	**SD**	CV%	**SD**	**CV%**
23	108	17	29	1.4	6.3	2	8.8	2.3	9.9
54	108	44	72	4.4	8.2	5.2	9.7	5.5	10
75	108	50	97	5.9	7.9	7.8	10	8.4	11
95	108	71	121	7.9	8.3	10	11	11	11
144	108	112	175	10	7.2	14	9.8	16	11
287	108	217	358	21	7.4	30	11	35	12

* Within lot variation consists of within run, between run, between day, between instrument, and between operators.

As part of the precision study, the kit quality control (QC) materials were assayed for run acceptance and the sponsor calculated the variance in precision. The results are summarized below:

Kit QC material	n	Mean (U)	Within Run Variation		Within Lot Variation	
			SD	CV %	SD	CV %
Normal	108	267	21	7.8	26	9.8
Abnormal	108	55	5.1	9.2	5.3	9.7

b. Linearity/assay reportable range:

The linearity was determined in accordance with CLSI EP6-A. A regression analysis was performed with DBS samples ranging from 13.8 to 390 U. The fitted regression model is: $y = 0.998x - 0.66$ ($r^2 = 0.999$) where:
y = observed BTD activity
x = expected observed BTD activity
The sponsor concluded that the assay is linear from 16 U to 390 U. The claimed measuring range is 16 U up to the highest calibrator (nominal value approximately 350 U).

The package insert includes the following recommendations:

- Samples that result in values below 16 U are recommended to be re-assayed as duplicates. If the values are still below the limit of detection, they are recommended to be reported as "< 16 U". The result should be considered positive [for biotinidase deficiency].
- Sample values above the measuring range are reported as the activity of the highest calibrator of the kit. These samples should be considered negative [for biotinidase deficiency]. To accurately quantitate values above the calibration curve, another method should be used.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
There is no international reference preparation or reference method for BTD. The Neonatal Biotinidase Kits are traceable to commercially available 6-AQ. The BTD activity is assigned to the units using synthetically prepared reagent, 6-AQ (reaction end-product), as the gravimetric standard. The purity of the 6-AQ reagent is analyzed using quantitative ^{1}H-NMR.

Calibrators are prepared from porcine whole blood with a preservative spotted onto filter paper. Kit quality control materials are prepared from human blood (hematocrit adjusted to 55%) with a preservative spotted onto filter paper.

Value Assignment: Each level of control and calibrator material is assayed multiple times on multiple fluorometers. The mean of the data obtained is calculated and presented in the quality control certificates for the calibrators and controls. The secondary calibrators (which are value assigned against the primary calibrators) are used to value assign the kit controls and calibrators. The SD presented for the controls is taken from the precision study performed to characterize the assay's precision.

Real-time stability including shelf-life, transport, and in-use stability studies for the entire kit were performed, including the controls and calibrators. Additionally, accelerated stability studies were performed separately for the kit calibrators and controls.

The shelf life of the kit (including calibrators and controls) is 18 months when stored at -16 to -30 ºC. Once opened, the calibrators and controls are stable for one month when stored at 2 to 8 ºC.

d. Detection limit:
The limits of blank (LoB) and limit of detection (LoD) were determined according to CLSI EP17-A.

For the LoB, 180 measurements were carried out using 5 analyte-free DBS samples on filter paper. The limit of blank (LoB) was determined as the 95th percentile of the ranked results. The LoB of the assay is 12 U.

For the LoD, 48 measurements were carried out on each of 8 low level samples (DBS samples on filter paper). The LoD was defined as the lowest sample data where less than 5% of the values were below the LoB. The LoD of the assay is 16 U. The CV % at this level was < 10%.

e. Analytical specificity:

The proposed device was evaluated for interference in accordance with CLSI document EP7-A2. Whole blood pools with approximate target BTD activities of 20, 55, 80 and 160 U were used in the studies. The potential interfering compounds were added to the one set of blood pools to create the test pools. Solvent only was added to the other set of blood pools to create the control pools. The test and control pools were used to prepare DBS samples on filter paper. The following potential interfering compounds were evaluated:

Compound Used	Concentrations tested
Adrenocorticotropic hormone	15 ng/dL
Ampicillin	0.1 to 5.6 mg/dL
Ascorbic Acid	3 mg/dL
Bilirubin conjugated	30 mg/dL
Bilirubin unconjugated	20 mg/dL
Biotin	500 ng/dL
EDTA	1 g/dL
Gammaglobulin	0.43 to 6.0 g/dL
Gentamicin sulphate	0.5 mg/dL
Glutathione	22.5 to 150 mg/dL
Hemoglobin	200 mg/dL
Heparin	37.5 mg/dL
HSA	1.5 to 6.0 g/dL
Kanamycin sulphate	3 mg/dL
Penicillin G potassium	6.25 to 25 mg/dL
Phenobarbital sodium	5.5 mg/dL
Phenytoin	0.625 to 2.5 mg/dL
Sulfamethoxazole	5 to 40 mg/dL
Sulfisoxazole	3.8 to 30 mg/dL
Triglycerides	0.15 to 3.5 g/dL
Trimethoprim	2 mg/dL
Valproic acid	25 mg/dL
Vitamin K_1	0.2 mg/dL

To address the observed interference, the sponsor included the following black box warning, limitations and statement in the package insert:

Black box warning: This warning was included under the intended use statement, under "limitations of the assay" and in the "interference" section of the product insert.

- Warning: Neonate albumin levels above normal (2.8 to 4.4 g/dL) can interfere with this test by increasing biotinidase activity. This could result

in the misclassification of a patient with a biotinidase result near the cut-off value as 'normal' when in fact, the patient should be classified as 'deficient'. A patient with known or clinically suspected elevated blood albumin concentration should be screened with an alternative method and confirmed according to local requirements for follow-up testing.

Limitations: These statements were included under "limitations of the assay" and in the "interference" section of the product insert.

- Kanamycin sulphate, glutathione, sulfamethoxazole, sulfisoxazole, and trimethoprim can interfere with this test by increasing biotinidase activity. This could result in the misclassification of a patient with a biotinidase result near the cut-off value as 'normal' when in fact, the patient should be classified as 'deficient'. Patients or mothers known to have received kanamycin sulphate, glutathione, sulfamethoxazole, sulfisoxazole or trimethoprim should be screened with an alternative method and confirmed according to local requirements for follow-up testing.
- Gammaglobulin (1.8 g/dL at biotinidase activity level of 55 U and 1.5 g/dL at biotinidase activity levels of 80 U and 160 U) caused a decrease in biotinidase activity. This could result in a false positive result (biotinidase deficient) in normal patients with biotinidase values near the cut-off value. However no interference was observed with gammaglobulin (6 g/dL) at a biotinidase activity level of 20 U.
- Triglycerides (Intralipid at 150 mg/dL for biotinidase activity levels of 55 U and 80 U, 300 mg/dL at biotinidase activity of 20 U and 400 mg/dL at biotinidase activity of 160 U) caused a decrease in biotinidase activity. This could result in a false positive result (biotinidase deficient) in normal patients with biotinidase values near the cut-off value.
- Biotin (500 ng/dL) at biotinidase activity level of 55 U caused a decrease in biotinidase activity. This could result in a false positive result (biotinidase deficient) in normal patients with biotinidase values near the cut-off value.
- Ampicillin (0.56 mg/dL), penicillin G potassium (18.75 mg/dL), sodium phenobarbital (5.5 mg/dL) and phenytoin (1.88 mg/dL) at biotinidase activity level of 160 U caused an increase in response, whereas no interference was observed at biotinidase levels of 20 U, 55 U and 80 U.
- Valproic acid (25 mg/dL) at biotinidase activity levels of 80 U and 160 U caused an increase in response, whereas no interference was observed at biotinidase activity levels of 20 U and 55 U.

Statement: This statement was included in the "interference" section of the product insert.

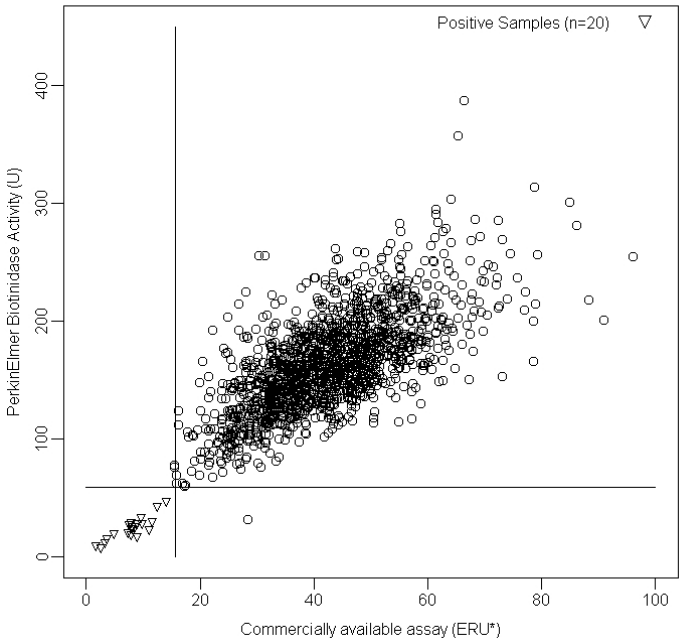
- The following substances were found not to interfere at the concentrations indicated; adreno-corticotropic hormone (15 ng/dL), ampicillin (2.8 mg/dL for biotinidase activities of 20 U, 55 U and 80 U), ascorbic acid (3 mg/dL), biotin (500 ng/dL for biotinidase activities of 20 U, 80 U and 160 U), conjugated bilirubin (30 mg/dL), unconjugated bilirubin (20 mg/dL),

EDTA (1 g/dL), gentamicin sulphate (0.5 mg/dL), hemoglobin (200 mg/dL), heparin (37.5 mg/dL), penicillin G potassium (25 mg/dL at biotinidase activities of 20 U, 55 U and 80 U), phenytoin (2.5 mg/dL for biotinidase activities of 20 U, 55 U and 80 U), sodium phenobarbital (5.5 mg/dL for biotinidase activities of 20 U, 55 U and 80 U) and vitamin K_1 (0.2 mg/dL).

f. Assay cut-off:
Not applicable. See clinical cut-off in 4. below.

2. Comparison studies:

a. Method comparison with predicate device:
Studies were performed according to CLSI EP9-A2. The samples used in the study included 1516 newborn DBS specimens representing the US population analyzed as singlicates with the proposed device and with a commercially available assay. The samples consisted of 1496 routine screening specimens and 20 retrospective specimens diagnosed positive for BTD deficiency. The cut-offs were determined according to each method's respective labeling (30 % of the mean ± 2 SD for the proposed device and 37% of the mean for the predicate).



Method comparison data (1516 samples) with the routine and known positive samples. The routine samples are illustrated with (o) and the known positive samples with (▽). The labeling cut-offs are presented with solid lines.

Screening Performance (Screening results and true diagnosis of the proposed device):
The screening summaries according to each method's respective labelling are presented in the table below. The screening positives (+) are samples < cut-off and the screening negatives (-) are samples ≥ cut-off.

Screening result commercially available kit	Screening result proposed device	Total Subjects	Diagnosed BTD deficiency	No diagnosed BTD deficiency
+	+	20	20	0
+	-	2	0	2
-	+	1	0	1
-	-	1493	0	1493
Total		1516	20	1496

The positive percent agreement was 90.9 % (20/22) and the overall percent agreement was 99.8% (20+1493)/1516).

b. Matrix comparison:
Not applicable. The device should only be used with neonatal whole blood spotted on filter paper obtained from heel prick.

3. Clinical studies:
   a. Clinical Sensitivity:
   Not applicable

   b. Clinical specificity:
   Not applicable

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

4. Clinical cut-off:
   BTD deficiency is inherited as an autosomal recessive trait. A newborn with profound BTD deficiency is homozygous and usually has enzyme activity that is less than 10% of the average of the normal population and sometimes there is almost no activity at all. Newborns classified as having partial BTD deficiency are heterozygous and have intermediate levels of BTD activity, typically 10 – 30% of normal.

   The proposed device was not designed to differentiate between partial and profound BTD deficiency. Therefore, the device has one cut-off that differentiates between 'normal' and 'deficient'. The cut-off of the proposed device determined in the submission and recommended in the package insert is 30 % of the mean of the distribution of BTD activity of a normal newborn population ± 2 SD.

The package insert states that samples with BTD activities below the chosen cut-off should be considered as presumptive positive. These samples should be reanalyzed with a different method and confirmed according to local requirements for follow-up testing.

5. Expected values/Reference range:
   The distribution of BTD activity of a normal newborn population (USA) was established using the proposed device by determining the BTD activity of 1496 routine newborn specimens in a neonatal screening laboratory. The following descriptive statistics and percentile cut-offs are included in the package insert:

Activity	n	Range	Mean	Median	Lower percentiles				
					0.1%	0.2%	0.5%	1%	2%
Proposed (U)	1496	31.5 - 388	163.8	160.9	60.4	62.4	70.6	77.8	85.9

The package insert includes precautionary language that each laboratory should establish its own cut-off values and that cut-offs based on data collected with any other BTD assay should not be used.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.