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

A. 510(k) Number:

k131284

B. Purpose for Submission:

New device

C. Measurand:

Biotinidase activity

D. Type of Test:

Quantitative, time-resolved fluoroimmunoassay

E. Applicant:

Wallac Oy, a subsidiary of PerkinElmer, Inc.

F. Proprietary and Established Names:

GSP Neonatal Biotinidase kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1118 Biotinidase Test System

2. Classification:

   Class II

3. Product code:

   NAK

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use statement below.

2. Indication(s) for use:

   The GSP Neonatal Biotinidase kit is intended for the quantitative in vitro determination of human biotinidase activity in blood specimens dried on filter paper as an aid in screening newborns for biotinidase deficiency using the GSP instrument.

3. Special conditions for use statement(s):

   For prescription use only.

The data obtained using the GSP Neonatal Biotinidase kit should be used as an aid to other medically established procedures and results interpreted in conjunction with other clinical data available to the clinician.

The GSP Neonatal Biotinidase kit should be used to screen newborns for biotinidase deficiency. It should not be used to distinguish partial from profound biotinidase deficiency.

Glutathione levels above normal (>30 mg/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 glutathione concentration should be screened with an alternative method and confirmed according to local requirements for follow-up testing.

4. Special instrument requirements:

   For use on the GSP Instrument.

I. Device Description:

The GSP Neonatal Biotinidase kit consists of the following reagents (sufficient to perform 1152 assays):

- **Biotinidase Calibrators** have been prepared from human whole blood using ProClin 300 as preservative. The biotinidase calibrators' approximate activities are: 10, 25, 50, 100, 175 and 325 U/dL.
- **Biotinidase Controls** have been prepared from human whole blood using ProClin 300 as preservative. The low control is approximately 40 U/dL and the high control approximately 160 U/dL.
- **Biotinidase Substrate Reagent** – 3 ready-to-use vials, 2.8 ml each of Eu-labeled biotin in Tris-HCl buffered salt solution containing BSA and sodium azide.
- **Biotinidase SA Reagent** – 3 ready-to-use vials, 2.8 ml each of streptavidin in Tris-HCl buffered salt solution containing BSA, bovine gammaglobulin, Tween 40, inert red dye and sodium azide.
- **Assay Buffer** – 3 bottles, 120 ml each of Tris-HCl buffered salt solution containing BSA, bovine gammaglobulin, Tween 40, inert red dye and sodium azide.
- **Anti-SA Microtitration Strips** – 12 plates with wells coated with anti-streptavidin IgG (mouse monoclonal).

All human source materials used in the preparation of kit components were tested and found to be negative for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 derivatives by FDA approved methods.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   PerkinElmer, Inc. Neonatal Biotinidase kit

2. Predicate 510(k) number(s):

   k090123

3. Comparison with predicate:

Similarities		
Item	Proposed Device	Predicate Device (k090123)
Indications for use	For the determination of human biotinidase activity in blood specimens dried on filter paper as an aid in screening newborns for biotinidase deficiency	Same
Specimen Type	Dried blood spot	Same
Interpretation of Results	Calibration curve	Same
Calibrator levels	Six	Same

Differences		
Item	Proposed Device	Predicate Device (k090123)
Test principle	Combines an enzyme reaction with a solid phase time-resolved immunofluorescence assay. Biotinidase in the sample cleaves the amide bond in the Eu-labeled biotin substrate. The end product is captured using Streptavidin and Anti-streptavidin coated plate. DELFIA® Inducer dissociates the molecules into the solution where the europium fluorescence is measured	1-step enzymatic assay were the biotinidase in the sample cleaves the substrate biotin 6-aminoquinoline generating a fluorescent 6-aminoquinoline product
Calibrator matrix	Dried blood spots prepared from human whole blood	Dried blood spots prepared from porcine blood
Measuring range	14.8 U/dL – 325 U/dL	16 U – 350 U
Measuring unit	U/dL	U
Instrument	GSP Instrument (k090846)	Manual fluorometer
Detection method	Time-resolved fluorescence	Fluorometer with excitation central wavelength of 355 nm and the emission central wavelength of 460 nm

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

- CLSI document EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*
- CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*
- CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry*
- CLSI Protocol EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*

L. Test Principle:

The GSP Neonatal Biotinidase test system measures biotinidase activity, combining an enzyme reaction with a solid phase time-resolved immunofluorescence assay. The GSP Neonatal Biotinidase assay is based on the ability of the biotinidase enzyme to cleave the amide bond in Eu-labeled biotin. The enzyme reaction is stopped by addition of streptavidin which has high affinity for biotin (either Eu-labeled or free biotin). The streptavidin-biotin complexes are captured by the solid phase monoclonal antibody directed against streptavidin. DELFIA Inducer dissociates the molecules into the solution where the europium fluorescence is measured. The measured fluorescence is inversely proportional to the biotinidase activity of the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The study was performed using three lots of the GSP Neonatal Biotinidase kit, three operators, and three GSP instruments in 27 runs over 20 non-consecutive days. Altogether 8 dried blood spot samples were included in the precision runs. The six precision samples (PS1-6) were selected to simulate the characteristics of the appropriate clinical samples. The precision samples were prepared by mixing of adult human whole blood and biotinidase deficient adult whole blood. The hematocrit value of the adult whole blood used in the sample preparation was adjusted to 40-55% to correspond to the hematocrit of neonates. The samples were spotted on to Whatman 903 filter paper and dried overnight in room temperature in vertical laminar flow cabinet and stored thereafter at -80 to -60°C in sealed bags containing desiccant. Targets for the study samples were determined with four GSP instruments and 3 kit lots (total of 51 replicates per sample) before the study. The Kit Controls Low and High were included as samples in the study and they were also used as run controls.

Samples PS1 and PS2 are below the approximate screening cut-off point (i.e.,

considered screen positive samples or potentially affected newborn), sample PS3 is near the approximate screening cut-off, and samples PS4 through PS6 are higher than the cut-off (i.e. considered a screen negative sample or unaffected newborn) with PS6 near the end of the measuring range.

Separate estimations for the precision of GSP Neonatal Biotinidase assay were calculated with two options: 1) a full calibration curve in duplicate for each plate and 2) a full calibration curve in duplicate for every batch of two plates. Precision results are summarized in the tables below:

1. Precision results using a full calibration curve in duplicate for each plate:

Sample	n	Mean biotinidase activity (U/dL)	Within run		Within lot		Between lot		Total variation	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
PS1	216	15.4	1.3	8.3	1.6	10.4	0.2	1.2	1.6	10.4
PS2	216	28.0	2.4	8.6	2.8	9.9	0.6	2.0	2.8	10.1
PS3	216	47.9	2.7	5.5	4.2	8.8	2.0	4.1	4.7	9.7
PS4	216	136.4	9.6	7.0	16.2	11.9	6.0	4.4	17.3	12.7
PS5	216	216.9	14.2	6.6	24.6	11.3	10.6	4.9	26.8	12.3
PS6	216	303.6	11.9	3.9	18.8	6.2	12.7	4.2	22.7	7.5

2. Precision results using a full calibration curve in duplicate for every batch of two plates run 24 hours apart:

Sample	n	Mean biotinidase activity (U/dL)	Within run		Within lot		Between lot		Total variation	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
PS1	216	15.3	1.4	9.1	1.7	10.9	0.1	0.9	1.7	10.9
PS2	216	28.0	2.5	9.0	2.7	9.8	0.5	1.6	2.8	9.9
PS3	216	48.2	3.2	6.5	4.2	8.6	1.9	4.0	4.6	9.5
PS4	216	138.3	12.2	8.8	16.5	11.9	3.9	2.8	16.9	12.2
PS5	216	218.7	18.7	8.5	24.8	11.3	6.9	3.2	25.7	11.8
PS6	216	301.2	12.3	4.1	18.6	6.2	16.0	5.3	24.6	8.2

b. Linearity/assay reportable range:

The claimed measuring range for the GSP Neonatal Biotinidase kit is 14.8 U/dL – 325 U/dL based on the Limit of Quantitation (see d. below) and Linearity studies.

The linearity was assessed in this study following the principles described in CLSI EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. The study used one kit lot and one GSP instrument. A full calibration curve in duplicate was included in the plate. Each sample and control were measured in four replicates, the samples were positioned in a random order on the plate.

The samples used were dried blood spot specimens prepared from heparinized whole blood. Blood drawn from one apparently healthy adult represented a "high activity" sample. The hematocrit value of the blood was adjusted to 40% - 55% to correspond to the hematocrit of neonates. Blood drawn from biotinidase deficient (profound) adult represented a "low activity" sample. 16 dried blood spot samples with intermediate concentrations were prepared by mixing the "low activity" sample with the "high activity" sample and then spotting on collection cards and dried.

A polynomial evaluation of linearity was used for the data analysis. The mean results for each sample assayed constituted the data for the statistical analysis. The assumption of constant variance across all levels is not fulfilled in the GSP Neonatal Biotinidase kit. Rather, the variance is proportional across different measurement levels therefore, weighted regression models were used. First, it was examined which analysis fits the data better – nonlinear polynomial or linear. A linear regression line and second and third order polynomials were fitted to the data. The results of regression analyses were compared. The fitted models are:

Linear: $y = 8.1 + 525.9\ x$
Second order: $y = 7.3 + 567.0\ x - 86.4\ x^2$
Third order: $y = 7.3 + 565.9\ x - 78.8\ x^2 - 9.4\ x^3$

where y = biotinidase activity (U/dL) and x = dilution point.

The significance of the second and third order polynomials were evaluated by performing a t-test. The regression analysis results showed that the second order polynomial had statistically significant non-linear terms (β2). Thus, the second order model was compared with the linear model. The comparison results between the second order model and fitted linear model shows that for all dilution levels the ±20% acceptance criteria for linearity is fulfilled.

The measured biotinidase activities are compared to the expected activities for a regression analysis (measured vs. expected biotinidase activity); the fitted regression model is:

$y = 1.04x + 1.9$ ($R^2 = 0.998$)

where y = Measured biotinidase activity (U/dL) and x = Expected biotinidase activity (U/dL).

This study and the results of the Limit of Detection evaluation (see 1d below) support the linearity over the claimed measuring range of 14.8 – 325 U/dL.

Samples that result in values below 14.8 U/dL are reported as "<14.8 U/dL". These results are not recommended to be considered accurate, but the specimen can be considered screen positive for biotinidase deficiency.

Samples that result in values above 325 U/dL are reported as ">325 U/dL". These results are not recommended to be considered accurate, but the specimen can be considered screen negative for biotinidase deficiency.

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

Traceability of calibration: There is no available internationally accepted reference material or a reference method for the determination of biotinidase activity. The calibration of the GSP Neonatal Biotinidase kit has been traceable to the in-house reference calibrator series (primary calibrators). The reference calibrator series include six levels, A-F, as dried blood spots on filter paper. The reference calibrators have been manufactured using adult human blood (endogenous biotinidase activity in serum) and washed red blood cells as blood matrices.

Value assignment: The in-house reference materials of the GSP Neonatal Biotinidase kit include primary calibrators, secondary calibrators, level calibrators 1-4, QA controls Low and High and deficient control R. All materials are dried blood spots.

The values of the first manufactured lot of the GSP Neonatal Biotinidase secondary calibrators, level calibrators, QA controls and deficient control were assigned against the primary calibrators using the GSP Neonatal Biotinidase assay.

The activities of following secondary calibrator lots are determined against the previous secondary calibrator lot using the GSP Neonatal Biotinidase assay. The activities of new level calibrator lot and QA control lot are determined against the secondary calibrator lot, and adjusted to the previous level calibrator and QA control lots, respectively. The activities of following deficient control lots are determined using approved kit combinations.

A two-stage calibration procedure is used for kit calibrators and controls. The initial kit calibrator and kit control activities are assigned against the secondary calibrators using the GSP Neonatal Biotinidase assay. QA controls are used to validate the run.

When a reagent kit lot is built by combining kit calibrators along with kit controls and

kit lot specific reagents, a second calibration step, combination calibration, is performed. In combination calibration, the level calibrators are measured against the reagent lot calibration curve using the GSP Neonatal Biotinidase assay. QA controls are used to validate the run. The initial kit calibrator activities are adjusted in a manner that the level calibrator activities read from the new calibrator curve are closest to their set target values.

The final kit lot specific values for the kit calibrators (6 levels) and kit controls (2 levels) are assigned against the final kit calibration curve using multiple plates and instruments.

Biotinidase calibrators (Level A to F) have the following target values:
10, 25, 50, 100, 175 and 325 U/dL

Biotinidase controls (2 levels) have the following target values:
40 and 160 U/dL

Stability: The results of the accelerated and real time stability studies support a shelf life of at least 24 months for all components of GSP Neonatal Biotinidase kit when stored at -30 to -16 °C (calibrators and controls) and at 2 to 8°C (other kit components). After opening, the Kit Calibrators and Controls should be stable at 2 to 8 °C for 7 days and Anti-SA Microtitration Strips for 14 days. The Biotinidase Substrate Reagent, Biotinidase SA Reagent and Assay buffer are stored on-board the GSP in the reagent storage carousel for up to 14 days after opening. Microplates with punched Kit Calibrators and sample disks in wells have been demonstrated to be stable in the GSP instrument plate storage for up to 15 hours.

Stability testing protocols and acceptance criteria for stability testing have been reviewed and found to be acceptable.

Sample stability: The objective of the study was to determine the short term stability of intact Biotinidase dried blood spot specimens stored in various conditions.

The sample stability study was performed using one GSP Neonatal Biotinidase kit lot and two GSP instruments. At the zero time point each sample was analyzed in 16 replicates (4 replicates per plate) and in the other 7 time points (1, 4, 7, 11, 14, 21, 29, and 35 days) each sample was analyzed in 8 replicates (4 replicates per plate).

Five dried blood spot samples with different biotinidase activities were prepared from heparinized whole blood drawn from an apparently healthy adult or heparinized whole blood diluted with deficient whole blood. The zero time point samples were analyzed immediately after the overnight drying while the samples for the other 8 time points were stored after the overnight drying in various storage conditions. The measured mean biotinidase activity at different time points/storage conditions was compared to the mean biotinidase activity measured at the zero time point.

The following information from this study is provided the package insert:

"Special attention must be paid to the storage and transportation conditions of the dried blood spot (DBS) samples. Storage of specimens in an environment with elevated temperatures and humidity increases the risk of false positive biotinidase screening results. A decrease in biotinidase activity of 20% may be observed after 3 days at room temperature with low humidity (<30%). High humidity increased the sample degradation since approximately 50% of activity may be lost after 3 days at room temperature with high humidity. When stored at high temperature (+37° C) and high humidity (RH above >50%) more than 50% of the activity may be lost during the first four days, and after seven days approximately 80% of the activity may be lost.

"The influence of storage time, temperature, and humidity on biotinidase activity was studied using several dried whole blood spot samples."

The results of one sample demonstrating the stability of a normal biotinidase activity level at the various storage conditions evaluated are presented in a graph in the package insert.

d. Detection limit:

The objective of the study was to determine the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the GSP Neonatal Biotinidase kit. The analysis of the LoB, LoD and LoQ were performed in accordance with CLSI document EP17-A, P*rotocols for Determination of Limits of Detection and Limits of Quantitation*; Approved Guideline.

The study used three lots of GSP Neonatal Biotinidase Kit and three GSP instruments.

Limit of the Blank (LoB): The samples for LoB study were dried blood spots specimens prepared from washed red blood cells in saline solution. The hematocrit value was adjusted to 40-55% and dried blood spots were prepared.

The samples were measured with two GSP Neonatal Biotinidase kit lots using two GSP instruments. Each plate included a full calibration curve in duplicate and the results were analyzed using a plate specific calibration curve. Repeated measurements (n = 60/sample) were carried out for the blank (analyte free) dried blood spot samples on filter paper. The Kit Controls Low and High, in quadruplicates (n = 4), were included in each plate and used for run acceptance. The five LoB samples were assayed with six replicates in ten separate runs performed over five operating days.

As the GSP Neonatal Biotinidase assay truncates results at zero i.e. values less than zero are not reported, the data is non-Gaussian with an asymmetric distribution of blank values. Therefore, a nonparametric principle based on the ordered values was

used to determine the LoB. The measurements were ordered according to their value and the 95th percentile was estimated as the value of the observation with rank value determined using equation:

The LoB was estimated to be 9.5 U/dL.

Limit of Detection (LoD) and Limit of Quantitation (LoQ): The samples for the LoD and LoQ studies were dried blood spot specimens prepared by mixing adult whole blood drawn from apparently healthy adults with adult biotinidase deficient blood. The LoD samples covered approximately the range from LoB to 3 x LoB.

In absence of a recognized reference method, a functional sensitivity study was used to define the LoQ. LoQ is the lowest biotinidase activity that can be measured with acceptable total variation of the assay. The LoD/LoQ samples were measured with three GSP Neonatal Biotinidase kit lots using three GSP instruments. Repeated measurements (n = 216/sample) were carried out using five low level DBS. Each plate included a full calibration curve in duplicate and the results were analyzed using a plate-specific calibration curve and a calibration curve for the batch of two plates. The Kit Controls Low and High in quadruplicate (n = 4) were included in each plate and used for run acceptance. Altogether 27 runs were assayed during 20 operating days using three GSP instruments and three GSP Neonatal Biotinidase kit lots.

The normality of the measurement distributions was evaluated visually and the data was Gaussian.

Estimate of standard deviation for LoD was derived from five low level sample result distributions. There were statistically significant differences among the variances ($p<0.05$ for all three Kit lots), and a pooled estimate of the SDs could not be used. Therefore, the SD was calculated for every sample separately (per kit lot) and the highest observed SD, which gives the most conservative value for the estimation of SD at low level, was used for the calculations of LoD. Type I error (α) and type II error (ß) levels of 5% were used in this study. LoD was calculated from equation:

The LoD was estimated to be 14.8 U/dL.

In absence of a recognized reference method, a functional sensitivity study was used to define LoQ. LoQ is the lowest biotinidase activity that can be measured with acceptable total variation of the assay. The results were analyzed using a plate specific calibrator curve and a calibration curve from another plate run within 24 hours (long run). The total variation CV% less than or equal to 20 % is fulfilled for sample all samples with biotinidase activities equal or higher than 11.0 U/dL using both full calibration curve in duplicate for each plate and using full calibration curve in duplicate for each run, therefore, LoQ = LoD.

The LoQ was estimated to be 14.8 U/dL

The sponsor's claimed measuring range of the device is 14.8 to 325 U/dL.

e. Analytical specificity:

The objective of the study was to evaluate the effect of potential interfering substances in dried blood spot (DBS) samples on the measurement of GSP Neonatal Biotinidase kit. The interference study was performed in accordance with the principles described in CLSI Guideline EP7-A2: *Interference Testing in Clinical Chemistry*; Approved Guideline. The study used one kit lot and one GSP instrument.

Whole blood samples with different biotinidase activity levels (35 and 150 U/dL for initial potential interference screening; 35, 84, and 150 U/dL biotinidase activity for additional dose-response evaluation of potential interferents) were tested for potential interferences using the DBS. Tested samples were spiked with different concentrations of interference substances and results were compared against the control sample without the spiked interference substances. Significant interference is defined as >15% bias between the spiked sample and the control sample.

The following substances were found not to interfere at concentration indicated:

Tested substance	**Added concentration of tested substance**
Adrenocorticotropic Hormone	15 ng/dL
Ascorbic acid	3 mg/dL
Biotin	500 ng/dL
Gammaglobulin	3 g/dL
Gentamicin sulphate	0.5 mg/dL
Hemoglobin	1.6 g/dL
Human serum albumin	6 g/dL
Kanamycin sulphate	3 mg/dL
Penicillin G	25 mg/dL
Phenytoin	2.5 mg/dL
Phenobarbital	5.5 mg/dL
Sulfmethoxazole	20 mg/dL

Tested substance	Added concentration of tested substance
Trimethoprim	2 mg/dL
Valproic acid	19 mg/dL
Vitamin K1	0.1 mg/dL

The package insert states:

> Ampicillin (1.4 mg/dL and above), sulfisoxazole (7.5 mg/dL and above) at low biotinidase activity levels (35 U/dL) and ampicillin (2.8 mg/dL) at high biotinidase activity levels (150 U/dL) were found to interfere with this test by increasing measured biotinidase activity by 19.9%, 32.1% and 15.6%, respectively. Elevated ampicillin (2.8 mg/dL) and sulfisoxazole (15 mg/dL) near the biotinidase cut-off did not exhibit a significant effect (<15%).
>
> Glutathione levels above normal (> 30 mg/dL) can interfere with this test by increasing biotinidase activity by 16.1% or more. 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 glutathione concentration (>30 mg/dL) should be screened with an alternative method and confirmed according to local requirements for follow-up testing.
>
> Unconjugated bilirubin (10 mg/dL) added to whole blood at low biotinidase activity levels (35 U/dL) were found to interfere with this test by increasing measured biotinidase activity by 18.7%.. Elevated unconjugated bilirubin level (20 mg/dL) near the biotinidase cut-off did not exhibit a significant effect (<15%).
>
> Conjugated bilirubin (2.5 mg/dL and above) and triglyceride (250 mg/dL and above) added to whole blood were found to interfere with this test by decreasing measured biotinidase activity by 26.0% and 15.7%, respectively. Elevated conjugated bilirubin (2.5 mg/dL and above) and triglyceride levels (250 mg/dL and above) may cause a false positive screening result for a specimen with measured biotinidase activity near the cut-off.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A study to evaluate the screening performance of the GSP Neonatal Biotinidase

device (GSP BTD) compared to the predicate device was conducted at a U.S. state public health laboratory that routinely performs newborn screening. The sample results for twenty leftover confirmed positive biotinidase deficient samples and 1,988 leftover routine newborn samples are included in the study (511 samples were excluded because of runs that failed QC or inclusion/exclusion criteria). The retrospective positive specimens were dispersed throughout the multiple assay runs and were not run as one batch. All kit controls were included in duplicate in each assay's respective runs and the runs were accepted based on the kit control values. Two GSP instruments and one kit lot of the new device were used.

For results generated by the proposed device, specimens were categorized as screen-positive and screen-negative according to three different cut-offs: the lowest 0.5^{th}, 1.0^{th} and 1.5^{th} percentiles. For the predicate device, the specimens were categorized as screen positive or screen negative using a cut-off of 30% of mean + 2SD as recommended in the labeling.

One retrospective confirmed biotinidase deficiency specimen initially tested as screen negative by the proposed device when all three cut-offs were used. Later testing of this sample using two different lots and two different instruments resulted in a screen positive result, as expected. Duplicate punches in the same microtiter well (due to static electricity) may be the potential root cause for the initial false negative result. Laboratories should take this into consideration and follow the instructions under Warnings and Precautions section that recommend using anti-static measures and humidity controls to reduce static electricity.

Screening performance using the 0.5^{th} percentile for the proposed device:

Classification of all test results into screen positive or screen negative when using the 0.5^{th} percentile for GSP BTD and 30% of mean + 2SD for predicate (Total) and classification of known affected samples (Positive) and unaffected samples (Normal):

GSP (cut-off = 74.6 U/dL)	**Predicate** (cut-off = 67.6 U)	**Total**	**Positive**	**Normal**
+	+	23*	19	4
+	-	6	0	6
-	+	2**	1	1
-	-	1977	0	1977
Total		2008	20	1988

* Includes 19 specimens from confirmed positive biotinidase deficient samples
** One specimen from a confirmed positive biotinidase deficient sample had a screen negative result in initial testing, but had a screen positive test result in repeat testing.

Overall percent agreement = (23+ 1977) / 2008* 100% = 99.6%
Positive percent agreement = (23/25) * 100% = 92.0%
Negative percent agreement = (1977/ 1983) * 100% = 99.7%

Screening performance using the 1.0th percentile for the proposed device:

Classification of all test results into screen positive or screen negative when using the 1.0th percentile for GSP BTD and 30% of mean + 2SD for predicate (Total) and classification of known affected samples (Positive) and unaffected samples (Normal):

GSP (cut-off = 84.3 U/dL)	**Predicate** (cut-off = 67.6 U)	**Total**	**Positive**	**Normal**
+	+	23*	19	4
+	-	17	0	17
-	+	2**	1	1
-	-	1966	0	1966
Total		2008	20	1988

* Includes 19 specimens from confirmed positive biotinidase deficient samples
** One specimen from a confirmed positive biotinidase deficient sample had a screen negative result in initial testing, but had a screen positive test result in repeat testing.

Overall percent agreement = (23+ 1966) / 2008* 100% = 99.1%
Positive percent agreement = (23/25) * 100% = 92.0%
Negative percent agreement = (1966/ 1983) * 100% = 99.1%

Screening performance using the 1.5th percentile for the proposed device:

Classification of all test results into screen positive or screen negative when using the 1.5th percentile for GSP BTD and 30% of mean + 2SD for predicate (Total) and classification of known affected samples (Positive) and unaffected samples (Normal):

GSP (cut-off = 92.1 U/dL)	**Predicate** (cut-off = 67.6 U)	**Total**	**Positive**	**Normal**
+	+	23*	19	4
+	-	27	0	27
-	+	2**	1	1
-	-	1956	0	1956
Total		2008	20	1988

* Includes 19 specimens from confirmed positive biotinidase deficient samples
** One specimen from a confirmed positive biotinidase deficient sample had a screen negative result in initial testing, but had a screen positive test result in repeat testing.

Overall percent agreement = (23+ 1956) / 2008* 100% = 98.6%
Positive percent agreement = (23/25) * 100% = 92.0%
Negative percent agreement = (1956/ 1983) * 100% = 98.6%

b. Matrix comparison:

Not applicable.

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:

Not applicable.

5. Expected values/Reference range:

The distribution of biotinidase activity of a normal newborn population was established by testing 1,988 routine newborn specimens in a neonatal screening laboratory in the U.S. using the proposed device (GSP Neonatal Biotinidase kit):

Method	N	Mean	Median	Min	Max	0.5%	1.0%	1.5%
GSP (U/dL)	1942	217.8	222.8	34.4	325.0	73.8	84.2	92.0

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.