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

A. 510(k) Number:

k080294

B. Purpose for Submission:

New Device

C. Measurand:

Biotinidase

D. Type of Test:

Semi-quantitative colorimetric assay

E. Applicant:

Astoria-Pacific, Inc.

F. Proprietary and Established Names:

SPOTCHECK Biotinidase Microplate Reagent 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 (77)

H. Intended Use:
1. Intended use(s):
   See Indications for use statement below.

2. Indication(s) for use:
   This method is for the semi-quantitative determination of biotinidase, EC 3.5.1.12, activity in dried whole blood spots using a spectrophotometer. 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.

This device is for use by trained, qualified laboratory personnel.

3. Special conditions for use statement(s):
   For Prescription Use Only

   This kit is not to be used for monitoring purposes. This test cannot be used to screen for biotinidase deficiency in patients who have received a transfusion or in patient being treated with certain sulfonamide antibiotics. All samples below the partial activity cutoff require confirmation and follow-up testing according to local, state and federal requirements. The Astoria-Pacific Biotinidase Microplate Reagent Kit test procedure cannot classify any particular genotype.

4. Special instrument requirements:

   A microplate absorbance reader capable of reading at 550 and 690 nm is needed but not provided.

   The performance data provided in this submission was generated using a TECAN sunrise absorbance reader.

I. Device Description:

The kit includes sufficient reagent for the analysis of 20 plates, or 1920 individual measurements of patient samples, calibration standards and quality control material.

The kit includes:
Color Reagent 1 (containing sodium nitrate)
Color Reagent 2 (containing ammonium sulfamate)
Color Reagent 3 (containing N-1-naphthylethylenediamine dihydrochloride)
Biotin-PAB Substrate (containing biotinyl-p-aminobenzoate and tetrasodium ethylenediaminetetraacetate)
Color Reagent 1 Diluent (containing surfactant and preservative)
Color Reagent 2 Diluent (containing N hydrochloric acid)
Substrate Diluent (containing Pro-clin 300 in water)
Substrate Buffer (containing potassium sulfate, monobasic and potassium sulfate, dibasic)
PABA Stock Standard (containing p-aminobenzoic acid)
30% TCA (trichloroacetic acid) in water

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Astoria-Pacific SPOTCHECK Biotinidase Kit, 50 hour

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

k010844

3. Comparison with predicate:

Differences		
Characteristic	**Predicate device (k010844)**	**Proposed device**
Incubation	In microplate, on combination incubator/shaker	On flow analyzer system
Matrix interference mitigation	Chemical precipitation and manual vacuum filtration	Manual vacuum filtration and dialysis on the flow analyzer system
Incubation Temp	37°C	40°C
Absorbance measurements	Spectrophotometric plate reader, 550 nm (reference at 690 nm)	Flow through split-beam spectro-photometer, 550 nm
Unit of measurement	Enzyme response unit (ERU) 1 ERU defined as the azo dye formed from 1 μmol of p-aminobenzoic acid produced from Biotin-PAB per dL per ~120 min. of incubation at 40°C	Microplate response unit (MRU) 1 MRU defined as the azo dye formed from 1 μmol of p-aminobenzoic acid produced from Biotin-PAB per dL per 240 min. of incubation at 37°C
Incubation Time	240 minutes	~120 minutes

Similarities		
Characteristic	Predicate device (k010844)	Proposed device
Sample collection and handling	Use standardized filter paper, S&S®903™ Follow CLSI document LA4-A2: *Blood Collection on Filter Paper for Neonatal Screening*	Same
Deficient cutoff	10% of normal activity	Same
Near Deficient cutoff – Clinical decision level	37% of normal activity	Same
Sample	2 x 1/8" punched blood spots	Same
Color Reagents	Sodium nitrite, acidic ammonium sulfate, NED	Same
Incubation substrate	Buffered Biotinyl-p-Aminobenzoate	Same

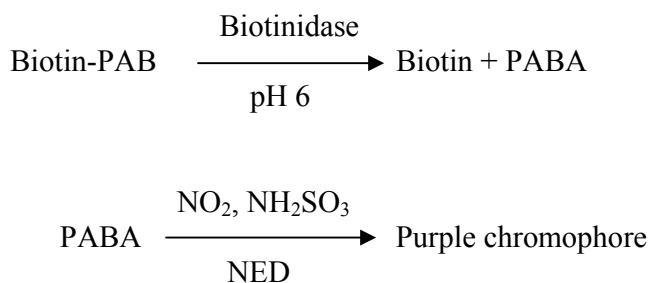**K. Standard/Guidance Documents Referenced (if applicable):**
CLSI Documents:
• *User Evaluation of Precision Performance of Clinical Chemistry Devices: Approved Guideline* (EP5-A2)
• *Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach: Approved Guideline* (EP6-A2)
• *Interference Testing in Clinical Chemistry: Approved Guideline* (EP7-A2)
• *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* (EP-17A)

L. Test Principle:
Biotinidase activity is determined by measuring the color that develops from p-Aminobenzoic Acid (PABA) after PABA is released from Biotinyl-p-Aminobenzoate (Biotin-PAB). Samples with biotinidase activity develop a purple color. Samples without biotinidase activity remain straw-colored.

Patient samples of whole blood collected on standardized filter paper are eluted in a standard 96 well microplate. The plate is incubated with Biotin-PAB in a pH 6 buffer at 37°C for 240 minutes on a combination incubator/shaker. Following incubation, trichloroacetic acid is added to the sample mixture and the resulting precipitate is removed through filtration. The PABA in the filtrate is subsequently diazotized and coupled to a napthol derivative to form an azo dye by the successive addition of sodium nitrite, acidic ammonium sulfamate and finally, N-1-naphthylethylenediamine dihydrochloride (NED). The azo dye is measured colorimetrically at 550 nm on a commercial microplate absorbance reader with a reference measurement at 690 nm.

$$\text{Biotin-PAB} \xrightarrow[\text{pH 6}]{\text{Biotinidase}} \text{Biotin} + \text{PABA}$$

$$\text{PABA} \xrightarrow[\text{NED}]{\text{NO}_2, \text{NH}_2\text{SO}_3} \text{Purple chromophore}$$

The color developed is proportional to the biotinidase activity in the sample. A standard curve prepared from a stock PABA solution is used to evaluate the results.

M. Performance Characteristics (if/when applicable):
1. Analytical performance:

   a. *Precision/Reproducibility:*
   Precision studies were performed using CLSI Document EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition* as a guideline. Samples at 3 concentration levels of biotinidase activity were analyzed in duplicate on 2 runs per day for 20 non-consecutive days. The concentration level proximate to the biotinidase deficient cutoff of 10 MRU was analyzed over 5 non-consecutive days. Within-run and

total precision was calculated according to CLSI EP5-A2. A total of 80 data points at each level were collected.

Sample blood spot controls used for the study with no activity (deficient) and partial activity are manufactured from stripped human serum that is mixed with whole human red blood cells and subsequently heat deactivated before being spotted on standard filter paper. Normal controls consist of normal human serum mixed with whole red blood cells that are not heated before spotting. Controls with partial activity are manufactured by combining deficient and normal controls before they are spotted. Additionally, dithioerythritol (DTE) is added prior to spotting to preserve the enzymatic activity.

Units for the Microplate Reagent Kit are microplate response units or MRU. A MRU equals 1 µmol of p-aminobenzoic acid produced from Biotin-PAB per dL per 240 minutes of incubation at 37°C.

Biotinidase (MRU)	Deficient (5 days)	Partial Activity (20 days)	Normal (20 days)	High Activity (20 days)
Mean (MRU)	10.3	18.3	50.7	124.9
SD (within-run)	0.6	1.2	2.2	4.7
C.V. (within-run)	5.8%	6.3%	4.4%	3.8%
B (daily mean)	0.8	1.1	3.1	7.2
SD (total)	1.1	1.7	3.7	8.9
C.V. (total)	9.7%	9.4%	7.3%	7.1%

b. *Linearity/assay reportable range:*
The linearity study was performed using CLSI Document EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline* as a guideline. The claimed measuring range for this device is 3 - 213 MRU. Two pools of manufactured blood spots, one with high biotinidase activity and one with low biotinidase activity were extracted and then proportionately mixed to provide 18 concentrations at equally spaced intervals. Four replicates at each level were analyzed in a single run to provide data to determine linearity according to CLSI EP6-A.

The absolute values of the differences between each replicate were calculated for each level, followed by the mean difference. Next, the sum of the squared differences and its mean were calculated as well as the sum of the squared percent differences and its mean. Finally, first, second and third order regressions of the data were performed to evaluate whether any non-linear terms were significant. Results were determined according to the method outlined in CLSI EP6-A.

Order	Coefficient	Value	Standard Error of the Regression	Degrees of Freedom	t-Test
First	b_o	-7.241			
First	b_1 (x term)	12.251	1.026	70	
Second	b_o	-7.148			
Second	b_1 (x term)	12.224			
Second	b_2 (x^2 term)	0.001	1.033	69	0.228*
Third	b_o	-7.526			
Third	b_1 (x term)	12.434			
Third	b_2 (x^2 term)	-0.026			
Third	b_3 (x^3 term)	0.001	1.035	68	0.837*

*does not exceed the limits in the table for the "Students t" distribution at the 95% level

Sample level (n=4)	Mean MRU	Std. Deviation	Difference from Predicted MRU	% Difference
1	4.96	0.417	0.066	1.33
2	17.67	0.680	0.043	0.25
3	28.92	0.481	0.022	0.08
4	41.63	0.798	0.005	0.01
5	54.33	0.680	-0.010	-0.02
6	66.63	0.798	-0.021	-0.03
7	78.50	0.680	-0.030	-0.04
8	90.38	0.798	-0.036	-0.04
9	103.50	0.680	-0.039	-0.04
10	115.58	1.076	-0.039	-0.03
11	127.04	0.798	-0.036	-0.03
12	139.54	1.049	-0.030	-0.02
13	152.25	1.076	-0.021	-0.01
14	164.13	0.798	-0.010	-0.01
15	176.21	1.848	0.005	0.00
16	188.50	2.041	0.022	0.01
17	201.21	1.423	0.043	0.02
18	213.71	1.848	0.066	0.03

The results of the t-test for b_2 (x^2 term) on the second order regression analysis and for b_3 (x^3 term) on the third order regression analysis show that there are no significant nonlinear terms. There are no concentrations at which the percentage difference between the first and second order regressions exceeds 5%. In the range of 5 to 213 MRU, the system is judged to be linear.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   Controls: Positive or negative control materials are not included with this assay. The manufacturer states that laboratories include normal and abnormal

dried blood spot quality control material with each run to validate results. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations.

Calibrator:
a. Traceability: Users dilute the stock PABA solution provided in the kit to generate a series of calibrators added to wells of each microplate. The PABA stock solution is made with commercially available p-aminobenzoic acid free acid. This solid is weighed on certified balances and dissolved in a solution containing hydrochloric acid and preservative. There is no current standard for PABA available.
b. Value assignment: multiple replicates of each new lot of PABA stock solution as well as replicates of the currently marketed lot and an archived primary lot are tested with an FDA cleared device for biotinidase screening. New lots of PABA stock solution must have values ± 2% of the primary lot.

Stability: Astoria-Pacific makes two claims of shelf life on the labeling:

Shelf life for unopened kits is 2 years. Recommended storage for unopened kits is 2-8°C. Prepared reagents are stable for 14 days. The study protocol, acceptance criteria and summary of results was reviewed and found to be acceptable.

d. *Detection limit:*
The Limit of the Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were determined using CLSI Document EP-17A: *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* as a guideline.

Over the course of 3 non-consecutive days, 5 samples deficient in biotinidase activity were analyzed in 5 batches of 12 replicates each for a total of 60 observations. Analyzed concurrently were 60 replicates of a blank control sample. Results were used to determine the detection limit of the analytical system per CLSI document EP-17A.

LoB	
Number of Observations (N)	60
Mean (μ)	0.2639
Standard Deviation (σ)	1.1388
Limit of Blank (LoB) {LoB = μ + 1.645σ}	2

LoD and LoQ	
Number of Observations (N)	60
Number of Samples (K)	5
Standard Deviation (SD)	0.5438

Degrees of Freedom (f) {f = N – K}	55
c_β {c_β = 1.645 / (1 - 1 / (4 x f))}	1.653
Limit of Detection (LoD) {LoD = LoB + c_β SD}	3
Total Error (TE), 2 SD (95.45% confidence limit)	1.0875
Total Error, 3 SD (99.73% confidence limit)	1.6313
Total Error, 4 SD (99.99% confidence limit)	2.1750
LoQ (Limit of Quantitation) {TE < LoD, so LoQ = LoD)	3

The LoD and LoQ for biotinidase activity was calculated as 3 MRU, determined using the guidelines in CLSI EP17-A. Proportions of false positives (α) are less than 0.1% and false negatives (β) are less than 0.1%, based on 120 determinations, with 60 blank and 60 low-level samples. The LoB was calculated as 2 MRU.

e. *Analytical specificity:*
   Study protocols to evaluate the potential interference from various compounds used CLSI Document EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition* as a guideline. To determine the potential interference from various compounds, the following procedure was used: six lots of dried blood spots were created for two blood samples with different levels of partial biotinidase activity (at the lower side of the partial deficiency range at ~18 MRU and at the higher side of the range at ~48 MRU) were prepared with either no added compound or two different concentrations of added compound (see table below for each compound and added concentration/quantity). Eight replicates at each level were analyzed in a single run to provide data to determine the effects of the compound on biotinidase analysis.

Compound	Concentrations of compound added to samples	
Albumin	30 g/L (3g/dL)	60 g/L (6 g/dL)
Hemoglobin	2 g/L (0.2 g/dL)	1 g/L (0.1 g/dL)
Lipids (Intralipid)	18.5 mmol/L (1635 mg/dL)	37 mmol/L (3250 mg/dL)
Direct Bilirubin	171 µmol/L (14.4 mg/dL)	342 µmol/L (28.8 mg/dL)
Indirect Bilirubin	171 µmol/L (10 mg/dL)	342 µmol/L (20 mg/dL)
Gamma globulin	30 g/L (3g/dL)	60 g/L (6 g/dL)
Sulfonamides	0.79 mmol/L (200 µg/mL)	1.58 mmol/L (400 µg/mL)
Trimethoprim	69 µmol/L (20 µg/mL)	138 µmol/L (40 µg/mL)

Analysis of the data shows that direct and indirect bilirubin, gamma globulin and hemoglobin at the levels tested levels had no statistically significant effect on biotinidase activity determination.

Albumin: The endogenous levels of albumin in the samples tested were 1.6 g/dL (4.5 MRU sample), 1.7 g/dL (for the 54.2 MRU sample) and 1.8 g/dL (for the 17.4 MRU sample).
(The total albumin for each spiked test lot is adjusted in the table below to represent the total albumin concentration during analysis.)

Deficient Biotinidase Activity	Endogenous only	+ 3 g/dL albumin	+ 6 g/dL albumin
	Control Lot 1 1.6 g/dL albumin	Test lot 4.6 g/dL albumin	Test lot 7.6 g/dL albumin
Mean MRU (n=8)	4.50	9.92	13.8
Standard Deviation	0.89	0.63	0.295
Coefficient of Variation	0.198	0.064	0.021
Mean Difference from Control	---	5.4	9.2

Partial Biotinidase Activity	Endogenous only	+ 3 g/dL albumin	+ 6 g/dL albumin
	Control Lot 1 1.8 g/dL albumin	Test lot 4.8 g/dL albumin	Test lot 7.8 g/dL albumin
Mean MRU (n=8)	17.4	22.4	25.9
Standard Deviation	0.39	0.39	0.63
Coefficient of Variation	0.022	0.017	0.024
Mean Difference from Control (MRU)	---	5.0	8.5

Moderate Biotinidase Activity	Endogenous only	+ 3 g/dL albumin	+ 6 g/dL albumin
	Control Lot 1 1.7 g/dL albumin	Test lot 4.7 g/dL albumin	Test lot 7.7 g/dL albumin
Mean MRU (n=8)	54.2	59.2	63.4
Standard Deviation	1.72	2.92	1.09
Coefficient of Variation	0.032	0.049	0.017
Mean Difference from Control (MRU)	---	5.0	9.2

The results of the study show a mean increase of 1.6 MRU per 1 g/dL added albumin for all three sample levels tested. The sponsor has included that information in the labeling, along with the following statement: "[a] patient with high blood albumin concentrations should be screened with an alternative method if its result is proximate to the established partial activity cutoff" and other

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prominent warnings.

Lipid: analysis of the data shows that a lipid concentration of 37 mmol/L caused an apparent decrease in biotinidase activity of approximately 65%. A lipid concentration of 18.5 mmol/L caused an apparent decrease in biotinidase activity of approximately 44%. A sample with partial biotinidase activity with very high lipid levels could potentially be classified as deficient, which would result in confirmatory testing. Confirmatory testing mitigates the risk of a patient being misclassified due to interference from high lipid levels.

Sulfamethoxazole: added sulfamethoxazole caused a significant increase in patient response at both the 1.58 mmol/L and 0.79 mmol/L concentration level. Sulfonamides react with the color developing reagents to give an intense purple color. Sulfamethoxazole combined with trimethoprim, sulfisoxazole, and any sulfonamide with a free or hydrolyzable primary aromatic amino group may interfere in this fashion. As a consequence, for patients being treated with sulfonamides, an alternate biotinidase screening method should be employed. This is a well-known interference and it is prominently included this constraint in the labeling.

Trimethoprim: analysis shows that at trimethoprim concentrations of 138 μmol/L there was approximately an 23% decrease in biotinidase response. At trimethoprim concentrations of 69 μmol/L there was approximately an 8% decrease in biotinidase response. A patient with low biotinidase activity and with very high trimethoprim levels could potentially be classified as having partial activity, which would result in confirmatory testing. Confirmatory testing mitigates the risk of a patient being misclassified due to interference from high trimethoprim levels.

The possible interference by phenytoin, ampicillin, gentamicyn sulfate, vitamin K, penicillin G potassium, kanamycin sulfate, adrenocorticotropic hormone, valproic acid and sodium phenobarbital were evaluated and found to not interfere with this test at therapeutic concentrations.

    f.   *Assay cut-off:*
        Not applicable.

2. Comparison studies:
    a. *Method comparison with predicate device:*
        A method comparison study was performed to determine the correlation between the predicate device and the SPOTCHECK Biotinidase Microplate Reagent Kit (proposed device). This study compares the 2 kits if each were used according to their respective labeling. The cutoffs used for patient classification are calculated from the mean result of the 564 patient samples as described in each device's package insert.

        564 patient samples were analyzed using both the Microplate Reagent Kit and the predicate device. The newborn dried blood spots were primarily from two

states' departments of health and were of varying age. All cards containing real patient samples were stored in a freezer when not in use. Included in this study were the assay results for 2 patients clinically-confirmed to be biotinidase deficient and 10 deficient controls provided by the Centers for Disease Control (CDC).

A partial biotinidase activity and profound deficient cutoff were determined on both devices using 37% and 10% of the mean result of 564 dried blood spots. While literature suggests that a partial activity classification can be considered at or below 30% of the mean patient value, the sponsor has opted to recommend 37% as a more conservative cutoff.

Newborn Dried Blood Spots (Presumed Normal)	Microplate Reagent Kit (MRU)
Number of observations	564
Mean result	96
Standard Deviation	33
Range of the Data	27-210

Note: Above table does not include 10 CDC deficient controls or 2 patients known to be deficient in biotinidase activity.

The cut-offs for the new device with the present study material were calculated to be 10 MRU (deficient) and 36 MRU (partial activity).

The results of the comparison study show that 11 of 11 samples classified as deficient by the predicate device were also found to be deficient by the Microplate Kit. Five samples were classified as having partial biotinidase activity by the predicate device; of these samples, four were similarly classified by the Microplate Kit. The single sample that was classified as normal by the Microplate Kit yielded a value of 9 ERU with the predicate device, right at the cutoff of 10 ERU.

The Microplate Kit classified 1 sample as having partial biotinidase activity that the predicate device found to be normal. Similar to the disagreement above, this sample had a result of 35 MRU, right at the partial activity cutoff of 36 MRU.

It should be noted that both devices classified 1 patient sample clinically-determined to be deficient as partial, however this sample result fell on the cutoff of each kit. Most importantly, this patient would have been slated for confirmatory testing regardless of which kit was employed.

		Predicate		
		Deficient	Partial deficient	normal
Proposed	Deficient	11 of 11	0	0
	Partial deficient	0	4 of 5*	1**
	normal	0	1**	559 of 560

As noted in the labeling, it is up to each laboratory to determine their own clinical decision levels based on its regional patient population.

b. *Matrix comparison:*
Not applicable. This device is to be used only with neonatal whole blood.

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:
A partial biotinidase activity and profound deficient cutoff were determined on both devices using 37% and 10% of the mean result of 564 dried blood spots. While literature suggests that a partial activity classification can be considered at or below 30% of the mean patient value, the sponsor has opted to recommend 37% as a more conservative cutoff.
As specified in the package insert, all samples below the partial activity cutoff (37%) require confirmation and follow-up testing according to local, state and federal requirements.

5. Expected values/Reference range:
Each laboratory must determine its range of normal, partial and deficient levels of biotinidase activity, based on its population and analytical variables.
The activity of normal samples varies widely, and the activity of all samples decreases with time under any conditions of storage.

The summary of results from the 564 newborn specimens tested with this kit is shown below:

Newborn Dried Blood Spots (Presumed Normal)	Microplate Reagent Kit (MRU)
Number of observations	564
Mean result	96
Standard Deviation	33
Range of the Data	27-210

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.