510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION

DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k111260

B. Purpose for Submission:

New device

C. Measurand:

Vitamin B_6

D. Type of Test:

Quantitative Enzymatic Assay

E. Applicant:

AntiCancer Inc.

F. Proprietary and Established Names:

A/C Enzymatic Vitamin B_6 Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CDD	II	862.1810	75

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The A/C Enzymatic Vitamin B_6 Assay is intended for the quantitative determination of pyridoxal 5'-phosphate (PLP, vitamin B_6) in EDTA-plasma.

The device will monitor vitamin B_6 (PLP) status in human plasma for aid in the diagnosis of vitamin B_6 deficiency. The A/C Enzymatic Vitamin B_6 Assay is for in vitro diagnostic use only.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

A 96-well plate absorbance reader with filter wavelength at 660-680 nm.

I. Device Description:

The kit consists of Binding Buffer and Assay Buffer, Apo-enzyme Lyophilized powder, Dl-homocystine powder, Chromogen I, and Chromogen II, which are mixed as instructed by the user before testing. The kit also contains two level controls (high and low) and six level calibrators. These are supplied in 0.2 mL ready to use vials. Calibrators and controls contain human sourced and/or potentially infectious components. Donor units of components sourced from human plasma have been tested and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV and anti-HIV-1/HIV-2.

J. Substantial Equivalence Information:

1. Predicate device name(s):

ALPCO IMMUNOASSAYS Vitamin B_6 REA

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

k955561

3. Comparison with predicate:

Reagent Similarities and Differences		
Item	Candidate device: A/C Enzymatic Vitamin B_6 Assay	Predicate Device: ALPCO IMMUNOASSAYS Vitamin B_6 REA
Indications for Use/Intended Use	aid in the diagnosis of vitamin B_6 deficiency	same
Sample type	Human EDTA-plasma	same
Assay Principle	Enzymatic Assay	Radio-enzymatic assay

Calibration	6 point calibration	3 point calibration
Measurement Range	15.6-200 nmol/L	0-192 nmol/L
Throughput	96 tests/2hrs	40 tests/4hrs

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

CLSI EP5-T2 Evaluation of precision performance of clinical chemistry devices

CLSI EP EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The Enzymatic Vitamin B6 Assay is based on PLP-dependent recombinant homocysteine-α,γ-lyase (rHCYase), which is prepared in the apo-enzyme form by stripping off the cofactor PLP (vitamin B6). The restoration of enzymatic activity by reconstitution of the holoenzyme depends on the amount of PLP in the plasma bound to apo-enzyme (Reaction 1) and produces hydrogen sulfide (H2S). H2S combines with N,N – dibutyl phenylene diamine (DBPDA), which then forms a chromophore (Reaction 2). The absorbance of this compound is read at 660 – 680 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was determined as described in Clinical and Laboratory Standards Institute (CLSI) Protocol EP5-T2 Evaluation of precision performance of clinical chemistry devices. Three PLP levels plasma samples were assayed in duplicate for two runs per day for 20 days. The results are summarized below.

PLP Levels	Mean Conc. (nmol/L)	Within Run		Total Run	
		SD	CV%	SD	CV%
Low	26.6	2.05	7.5	3.22	12.1
Medium	53.1	2.98	5.6	5.77	10.9
High	111.2	7.22	6.5	10.83	9.7

b. *Linearity/assay reportable range:*

Linearity was performed according to the CLSI protocol EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. Pooled human plasma sample was spiked with PLP to a concentration above the measuring range of the device and diluted with calibrator 0 to make six intermediate levels. Each level was measured with the device and the mean observed values were compared to the expected values. Samples tested ranged from 251.3 to 7.85 nmol/L and the observed results were within ±10% of the expected values for each of the concentrations tested in the claimed measuring range. The results were plotted as a least-squares linear regression of the expected concentration versus the observed concentration with a resulting regression equation of: $Y = 0.999X + 0.000056$, $r = 0.999$.

The device claims a measuring range from 15.6 to 200 nmol/L.

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

There is currently no internationally recognized reference method or reference material for pyridoxal 5'-phosphate (PLP). Calibrator values are assigned by HPLC measurement for each calibrator. Multiple measurements are performed and the mean value is calculated for value assignment. These calibrators are used to assign the target values and ranges for the quality control samples. These products contain human sourced and/or potentially infectious components. Donor units of components sourced from human plasma have been tested and found to be nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV and anti-HIV-1/HIV-2.

Real-time stability testing was conducted by the firm and the protocols and acceptance criteria have been reviewed and found to be acceptable. The sponsor stated that their acceptance criteria for all stability studies was <10% difference from the day 0 value. The reagents, calibrators, and controls are stable until the date shown on the label when stored as instructed. Open vial stability testing was performed and showed that the kit is stable for one month when stored at 2-8°C after opening.

Stability of stored specimens (frozen storage and refrigerated) and the effect of freezing/thawing on the measurement of PLP by the A/C Enzymatic Vitamin B6 Assay were evaluated. EDTA-plasma specimens were shown to be stable for at least one year frozen at -70°C and for two weeks when refrigerated (2-8°C). The sponsor's acceptance criteria for this evaluation are recovery within ±10%. The sponsor notes care should be taken to limit the number of freeze-thaw cycles when stored at -70°C

d. Detection limit:

The limit of blank (LoB) and limit of detection (LoD) were evaluated as recommended in CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation. The calculations resulted in an LoB of 9.28 nmol/L and an LoD of 13.1 nmol/L. The limit of quantitation (LoQ) was also evaluated as recommended in CLSI EP17-A and resulted in an LoQ of 15.6 nmol/L, which met the sponsor's requirement of a % CV of < 20%.

e. Analytical specificity:

Compound	Final Concentration (nmol/L)	% Cross-reactivity
pyridoxal,	1,000.0	< 0.5
pyridosamine	1,000.0	< 0.5
pyridoxine	1,000.0	< 0.5
pyridoxamine-phosphate	1,000.0	< 3.2

The cross reactants shown below were each spiked into a plasma sample with 50 nmol/L PLP. These samples were then evaluated with the device and the cross reactivity was calculated. Results are summarized below.

Triglyceride, bilirubin, and hemoglobin interference was evaluated by adding each interferent to EDTA plasma samples with PLP concentrations of 32.5 nmol/L and 115.0 nmol/L. The concentration of each interferent tested and the results are summarized below.

Interfering Substance		Interference %	
		Sample 1 32.5 nmol/L PLP	Sample 2 115.3 nmol/L PLP
Intralipid mg/dL	1000	8.2	7.5
	500	5.2	3.5
Hemoglobin mg/dL	500	12.2	10.9
	250	6.3	6.0
Bilirubin mg/dL	20	3.4	3.2
	10	1.3	1.1

This study demonstrated that Hemoglobin samples above 250 mg/dL reduced the recovery of PLP in plasma samples by more than 10%. The package

insert states that hemoglobin concentrations above 250 mg/dL cause interference >10% and that hemolyzed samples should not be used.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed by testing 48 patient plasma samples and 2 patient samples diluted to the low end of the measuring range. Samples tested ranged from 16.3 nmol/L to 189.3 nmol/L and spanned the measuring range of the device. A linear regression was performed with the following results: $y = 0.969x + 7.6$, $r^2 = 0.909$.

b. Matrix comparison:

Not applicable. EDTA plasma is the only intended sample type.

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 package insert states that the normal reference range is 20-120 nmol/L according to literature*.

* Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. A report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on folate, Other B Vitamins, and Choline and Subcommittee on Upper Reference Levels of Nutrients. Washington, DC: National Academy Press, 1998.

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.