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

A. 510(k) Number:

k180209

B. Purpose for Submission:

New Device

C. Measurand:

1,5-Anhydroglucitol (1,5-AG)

D. Type of Test:

Quantitative, colorometric, pyranose oxidase (PROD)

E. Applicant:

Diazyme Laboratories Inc.

F. Proprietary and Established Names:

Diazyme 1,5-AG Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.7470; Glycosylated hemoglobin assay

2. Classification:

   Class II

3. Product code:

   NOZ; Assay, 1,5-Anhydroglucitol

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   Diazyme 1,5-anhydroglucitol (1,5-AG) Assay is an enzymatic method intended for the quantitative determination of 1,5-anhydroglucitol (1,5-AG) in serum or plasma. The 1,5-AG Assay is for the intermediate term (preceding 1-2 weeks) monitoring of glycemic control in people with diabetes. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   - For in vitro diagnostic use only
   - Prescription use only

4. Special instrument requirements:

   Beckman AU680

I. Device Description:

Diazyme's 1,5-AG Assay is an enzymatic method consisting of a two-reagent test kit (Reagent 1 and Reagent 2) and is to be used with a fully automated chemistry analyzer. The test system also includes a calibration standard and a two-level control set, both of which are purchased separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   GlycoMark

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

   k031604

3. Comparison with predicate:

Similarities/Differences		
Item	Diazyme 1,5-AG Assay	GlycoMark™ (K031604)
Indications for use	The intermediate term monitoring of glycemic	Same

Similarities/Differences		
Item	Diazyme 1,5-AG Assay	GlycoMark™ (K031604)
	control in people with diabetes	
Sample	Serum or plasma	Same
Test principle	Pyranose oxidase (PROD)	Same
Linearity	0.5-110 ug/mL	Same
Methodology	Colorimetric	Same
Instruments	Beckman AU680	Roche Hitachi 917

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

Clinical and Laboratory Standards Institute EP5-A2 – Evaluation of Precision Performance of Clinical Chemistry Devices-Approved Guideline-Second Edition

Clinical and Laboratory Standards Institute EP6-A – Evaluation of the Linearity of Quantitative Analytical Methods; Approved (2003)

Clinical and Laboratory Standards Institute EP7-A2 – Interference Testing in Clinical Chemistry; Approved Guideline (2002)

Clinical and Laboratory Standards Institute EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

Clinical and Laboratory Standards Institute C28-A3 – Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition

L. Test Principle:

Diazyme's 1,5-AG assay uses the enzyme pyranose oxidase (PROD) to oxidize the 2nd position hydroxyl group of 1,5-AG and to detect the generated hydrogen peroxide by colorimetry using peroxidase (POD). To eliminate reactive glucose in sample, it is pretreated by enzymatic reactions using hexokinase and pyruvate kinase (PK). Hexokinase uses adenosine triphosphate (ATP) to convert glucose into non-reactive glucose-6-phosphate (G-6-P), generating adenosine diphosphate (ADP). The reaction is driven to completion with PK, as ADP is phosphorylated to ATP during the conversion of phosphoenolpyruvate (PEP) into pyruvate.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>
2.
   a. *Precision/Reproducibility:*

Precision studies were performed using six serum samples containing the following concentrations of 1,5-AG: 3.1, 5.7, 11.6, 23.2, 61.8 and 97.3 ug/mL. Two levels of quality control (QC) material (3.6 and 12.3 ug/mL) and one level of calibrator solution (21.1 ug/mL) were also tested. Samples were tested in duplicate using three reagent lots over 20 days with two runs per day (20x2x2 design). Samples were analyzed using the Diazyme 1,5-AG Assay run on a Beckman AU680 analyzer. The results of data analysis of the combined three lots (n=240) are summarized below:

Sample	Mean	Within-run		Between- Run		Between-Day		Between-Lot		Total	
		SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
QC1	3.6	0.07	2.0	0.05	1.4	0.03	0.7	0.09	2.5	0.09	2.5
QC2	12.3	0.09	0.8	0.10	0.8	0.03	0.3	0.14	1.1	0.14	1.1
Calibrator	21.2	0.12	0.6	0.11	0.5	0.09	0.4	0.18	0.9	0.19	0.9
Serum 1	3.1	0.07	2.3	0.07	2.3	0.11	3.5	0.14	4.7	0.15	4.8
Serum 2	5.7	0.07	1.3	0.08	1.4	0.07	1.2	0.13	2.2	0.13	2.2
Serum 3	11.6	0.09	0.8	0.11	1.0	0.05	0.5	0.15	0.3	0.15	1.3
Serum 4	23.2	0.14	0.6	0.15	0.6	0.07	0.3	0.22	0.9	0.22	0.9
Serum 5	61.8	0.32	0.5	0.36	0.6	0.42	0.7	0.63	1.0	0.64	1.0
Serum 6	97.3	0.48	0.5	0.51	0.5	0.62	0.6	0.93	1.0	0.94	1.0

b. Linearity/assay reportable range:

Linearity was evaluated using high and low 1,5-AG serum pools that were mixed to create the following eleven samples containing 1,5-AG concentrations spanning the measuring range: 0.2, 10.9, 22.1, 34.4, 45.3, 58.5, 68.6, 81.1, 92.7, 103.2, and 116.7 ug/mL 1,5-AG. Samples were measured in triplicate using one lot of reagent on a Beckman AU860 analyzer. The results of the linear regression analysis are summarized below:

$y = 1.0014x – 0.8351$, $R^2 = 0.9998$

The results of the linearity study support the claimed measuring range of 0.5 to 110 ug/mL.

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

The Diazyme 1,5-AG Assay is traceable to an internal standard manufactured using highly purified 1,5-AG, referred to as the Master Calibrator. The Master Calibrator was prepared by spiking the appropriate amount of pure 1,5-AG into a phosphate buffer solution. The Master Calibrator was subsequently value assigned.

d. Detection limit:

The Limit of Blank (LoB) study was performed with five serum samples, which were dialyzed to removed endogenous 1,5-AG, and two reagent lots. The samples were

tested in quadruplicate over three days (n=60 samples per reagent lot). The LoB was determined to be 0.3 ug/mL.

The Limit of Detection (LoD) study was performed with five serum samples that were prepared by diluting native serum samples with serum that was dialyzed to removed endogenous 1,5-AG, with two reagent lots. Samples were tested in quadruplicate over three days (n=60 samples per reagent lot). The LoD was calculated as the LoB + (1.653 * SD of LoD samples). The LoD was determined to be 0.45 ug/mL.

The Limit of Quantitation (LoQ) study was performed using five serum samples ranging from approximately 1 to 16 times the claimed LoB. Samples were either native serum or serum diluted with dialyzed serum to obtain the relevant 1,5-AG concentrations. The diluted serum samples were then tested in 8 replicates per day over 5 days using two lots of reagents. The sponsor defines LoQ as the lowest concentration which meets an imprecision (%CV) of <20%. The LoQ was determined to be 0.6 ug/mL.

e. *Analytical specificity:*

Interference studies were conducted with serum samples adjusted to three levels of 1,5-AG (approximately 5.0, 23.0, and 50 ug/mL). Each sample was divided into a test pool and a control pool, and potentially interfering substances were added to the test pool. Test pool samples with various concentrations of substances were compared to non-spiked controls. The sponsor defines significant interference as greater than ±10% bias between the test and control samples measured using the Diazyme 1,5-AG Assay on a Beckman AU680 analyzer. The following substances were tested up to the levels indicated and demonstrated no significant interference:

Substance	Highest concentration of substance tested that did not demonstrate significant interference (mg/dL)
Bilirubin	5
Conjugated Bilirubin	5
Hemoglobin	125
Triglycerides	1000
Ascorbic Acid	37.5
Glucose	1000
Maltose	500
Uric Acid	20
Creatinine	10
Urea	20

Based on the testing completed, the sponsor has included the following limitation in the labeling:

WARNING: Significant negative bias (>10%) was observed at bilirubin levels

> 5 mg/dL. This should be taken into consideration for patients with conditions that cause hyperbilirubinemia (Gilbert's disease, Jaundice, etc.).

f. Assay cut-off:

Not applicable.

3. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was conducted by comparing the results from the 1,5-AG Assay to the to the predicate method (GlycoMark 1,5-AG, K031604) on a Beckman AU680 analyzer. Serum samples ranging in 1,5-AG concentration from 1.7 to 36.2 ug/mL were collected from 91 diabetic and non-diabetic subjects. In order to obtain sufficient samples in the high 1,5-AG concentration range, 11 additional samples were spiked to obtain concentrations between 39.0 and 103.7 ug/mL 1,5-AG. Samples were tested in singlet. The results of linear regression analysis are as follows:

$y = 1.0164x – 0.2042$; $R^2 = 0.9995$

b. Matrix comparison:

To evaluate anticoagulant effects, paired serum/K_2EDTA plasma and serum/Lithium Heparin plasma samples were tested on the Beckman AU680 analyzer with the Diazyme 1,5-AG Assay. Fifty-two serum vs. K_2EDTA plasma and 52 serum vs. Lithium Heparin plasma paired sample sets were evaluated. The results of the linear regression analysis are presented below:

Tube Type	Regression	R^2	Test range ug/mL
Lithium-heparin plasma vs serum	y = 1.0059x + 0.0465	0.997	0.8 - 109
K_2EDTA plasma vs serum	y = 1.0071x – 0.6457	0.994	1.7 - 105.6

The study data supports the sponsor's claim that the Diazyme 1,5-AG Assay is suitable for use with human specimens consisting of serum, K_2EDTA plasma, and Lithium Heparin plasma.

4. 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.

5. Clinical cut-off:

Not applicable.

6. Expected values/Reference range:

To establish the reference interval of 1,5-AG for a normal population, serum samples from 280 apparently healthy adults, 140 males and 140 females, were tested using the Diazyme 1,5-AG Assay on the Beckman AU680 analyzer. Using non-parametric 5th-95th percentiles, the reference interval was established to be from 8.19 to 32.19 ug/mL for males and 6.00 to 29.10 ug/mL for females.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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