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

A. 510(k) Number:
k111664

B. Purpose for Submission:
New Device

C. Measurand:
Cystatin C

D. Type of Test:
Quantitative, Immunoturbidometric

E. Applicant:
Diazyme Laboratories

F. Proprietary and Established Names:
Cystatin C POC Test, Cystatin C POC Test Control Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NDY Test, Cystatin C	Class II	21 CFR § 862.1225	Clinical Chemistry (75)
JJX Single (Specified) Analyte Controls (Assayed and Unassayed)	Class I, reserved	21 CFR § 862.1660	Clinical Chemistry (75)

H. Intended Use:

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

2. Indication(s) for use:
   The Diazyme Cystatin C Point-of Care (POC) Test is an *in-vitro* diagnostic test for the quantitative determination of Cystatin C in venous whole blood by latex enhanced immunoturbidimetric method. The measurement of Cystatin C is used as an aid in the diagnosis and treatment of renal disease. For *in vitro* Diagnostic Use Only.

The Diazyme Cystatin C POC Test Control Kit is intended for use as quality controls for the Cystatin C POC Test. For *in vitro* Diagnostic Use Only.

3. Special conditions for use statement(s):
   For prescription use only.

4. Special instrument requirements:
   For use on SMART Analyzer (k092911) only.

I. Device Description:

The Diazyme Cystatin C POC Test Kit consists of the following three components: (1) twenty DRS cuvettes prefilled with Reagent 1 (100 mM TrisCl buffer, 0.125% Triton), (2) twenty DRS caps prefilled with Reagent 2 (Suspension of anti-human Cystatin C chicken polyclonal antibody coated latex particles) and (3) one preprogrammed Radio Frequency ID (RFID) card which contains a lot specific calibration curve.

The Diazyme Cystatin C POC Test Control Kit is sold separately and is comprised of two levels of controls (1.0 mL each of 1.0 mg/L for level 1 and 2.5 mg/L for level 2) which are lyophilized and need to be reconstituted prior to use. The controls are prepared from human whole blood. Each donor unit of blood in the preparation of this material was tested by FDA-approved methods and found negative for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies.

SMART Analyzer (k092911) is a compact cuvette based spectrophotometer (10 inches x 5.5 inches x 5.5 inches) machine for point-of-care testing designed to analyze readings from single use reagent cuvette. The instrument only uses the Diazyme Reagent System (DRS) cuvette and caps and performs assay with a preprogrammed Radio Frequency ID (RFID) card. The lot specific RFID card contains reagent addition time, mixing time, reading time and calibration curve for estimating cystatin C concentration.

J. Substantial Equivalence Information:

Predicate devices name	Predicate 510(k) number
Diazyme Cystatin C Assay	K093680

Comparison with predicate:

Similarities and Differences		
Item	Proposed Device	Predicate Device (k093680)
Intended Use/ Indications for use	Same	For the *in vitro* quantitative determination of Cystatin C. The measurement of Cystatin C is used as an aid in the diagnosis and treatment of renal disease.
Sample Type	EDTA Whole Blood	Serum or Plasma
Assay Principle	Same	Latex enhanced immunoturbidimetric method.
Measuring Range	0.30 to 7.65 mg/L	0.27 to 7.8 mg/L
Limit of Blank Limit of Detection Limit of Quantitation	0.045 mg/L 0.109 mg/L 0.30 mg/L	0.04 mg/L 0.068 mg/L 0.19 mg/L
Analyzer	SMART Analyzer	Hitachi 917 analyzer
Antisera	Same	Latex particles coated with anti-human Cystatin C chicken polyclonal antibodies
Calibrator	Each kit has individual lot specific RFID preprogrammed calibration card.	Five levels of recombinant Cystatin C antigen in buffered aqueous matrix.
Control	Two levels prepared from whole blood.	Two levels of recombinant Cystatin C antigen in buffered aqueous matrix.

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

CLSI EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods*
CLSI EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures*
CLSI EP7-A2: *Interference Testing in Clinical Chemistry*
CLSI EP9-A2: *Method Comparison and Bias Estimation Using Patient Samples*
CLSI EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*

L. Test Principle:

Diazyme Cystatin C POC Test is based on a latex enhanced immunoturbidimetric assay. The EDTA whole blood sample is lysed upon mixing with reagent R1. Cystatin C in the sample binds to the specific anti-Cystatin C antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of Cystatin C in the sample. The SMART Analyzer calculates the Cystatin C concentration of a patient specimen by use of a lot specific calibration curve that is stored in a Radio Frequency Identification Card (RFID) card provided with each Cystatin C POC Test kit.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the Diazyme Cystatin C POC Test was evaluated according to CLSI EP5-A guideline in two different precision studies. The first precision study was performed in-house. Six whole blood specimens (0.70, 1.00, 1.25, 2.70, 4.70 and 6.20 mg/L Cystatin C) were tested with 2 runs per day with duplicates over 10 days. Testing was performed on three different SMART Analyzers. The results are summarized in the following table.

Summary of Precision Study #1 Results

Cystatin C (mg/L)	N	Mean (mg/L)	Within Run Precision		Total Precision	
			SD	%CV	SD	%CV
0.70	40	0.696	0.0444	6.37%	0.0478	6.87%
1.00	40	0.988	0.0553	5.60%	0.0577	5.85%
1.25	40	1.217	0.0563	4.63%	0.0590	4.85%
2.70	40	2.720	0.0694	2.55%	0.0780	2.87%
4.70	40	4.717	0.1148	2.43%	0.1467	3.11%
6.20	40	6.131	0.1353	2.21%	0.2145	3.50%

The second precision study was performed at three physician office laboratories by intended users. Six whole blood samples containing Cystatin C levels ranging from low to high were used for the precision study. At each site, four whole blood samples were tested. Each sample was run four times in five days using three SMART Analyzers. The results are summarized in the following tables.

Summary of Precision Study #2 - Combined POC sites Results

Whole Blood Sample	N	Mean (mg/L)	Within Run Precision		Total Precision	
			SD	%CV	SD	%CV
1	40	0.568	0.585	10.29%	0.500	8.80%
2	40	0.95	0.07	7.3%	0.07	7.7%
3	40	1.363	0.929	6.82%	0.0914	6.71%
4	40	3.23	0.18	5.5%	0.22	6.9%
5	40	4.979	0.2821	5.67%	0.2740	5.50%
6	40	6.11	0.41	6.8%	0.45	7.4%

Summary of Precision Study #2 - Three POC site Results

Site 1

Whole Blood Sample	N	Mean (mg/L)	SD	%CV
5	20	3.365	0.1940	5.80%
6	20	6.365	0.3531	5.60%
7	20	0.572	0.0523	9.10%
9	20	5.092	0.3420	6.7%

Site 2

Whole Blood Sample	N	Mean (mg/L)	SD	%CV
4	20	0.927	0.0546	5.90%
5	20	0.95	0.2500	8.0%
8	20	1.400	0.0776	5.50%
9	20	4.866	0.1261	2.60%

Site 3

Whole Blood Sample	N	Mean (mg/L)	SD	%CV
4	20	0.979	0.0520	5.30%
6	20	5.870	0.1652	2.8%%
7	20	0.565	0.0488	8.70%
8	20	1.327	0.0956	7.20%

b. *Linearity/assay reportable range:*
The linearity of the Diazyme Cystatin C POC Test was analyzed over the range of 0.04 to 7.65 mg/L Cystatin C. A set of eleven levels of linearity materials were prepared by diluting a whole blood sample containing 8 mg/L

of Cystatin C with saline according to CLSI EP6-A and were tested with the Diazyme Cystatin C POC Test in triplicate on the SMART Analyzer. The mean of the obtained Cystatin C results (obtained on SMART analyzer) were plotted against the expected values and an appropriate line fitted by standard linear regression resulted in the following equation.
$y = 0.9643x + 0.0456$; $R^2 = 0.9977$.

The recovery ranged from -0.020 to 0.453 mg/L (or the percent recovery ranged from -1.29 to 7.04%).

Based upon the linearity data and the LoQ study, the sponsor claims a measuring range of 0.30 to 7.65 mg/L.

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

Traceability and Value Assignment:
Reagents: Cystatin C POC test calibration system is traceable to the higher order IFCC International Reference Preparation for Cystatin C in the reference material ERM® DA471/IFCC. Cystatin C POC Test utilizes a Calibration Radio Frequency Identification Card (RFID) card that is preprogrammed with a reagent lot specific calibration curve and is supplied in each kit. RFID cards are programmed at the manufacturer site and are subject to the same quality control checks as the reagents and controls.

Lot specific calibrator values generated for RFID card calibration curve programming are traceable to reference Diazyme Cystatin C calibrator values (k093680) and Cystatin C POC calibration curve values are assigned. The whole blood Cystatin C values are normalized to plasma Cystatin C values.

Controls: The lyophilized (freeze-dried) Cystatin C POC Test Controls are made from pooled human whole blood and value assigned in the following manner. Pooled human whole bloods samples are tested with the Diazyme Cystatin C POC Test kit to determine initial Cystatin C values. The samples are adjusted with Cystatin C antigen to the manufacturing target values for control level 1 and control level 2. With a reference lot of the Diazyme Cystatin C POC Test kit and three SMART Analyzers, the reconstituted bi-level control materials are then subjected to extensive replicate analysis to obtain mean values and ranges.

Stability:
Reagents: Two lots of the Diazyme Cystatin C POC Test reagents were evaluated to determine the shelf life. Reagents from each lot were filled in SMART cartridges and kept in incubators at 37°C and 2-8°C. Two levels of Whole Blood Cystatin C controls were tested in duplicate with the stressed reagents. At the indicated time, the Diazyme Cystatin C POC Test kits were removed from storage and tested with the two levels of the Cystatin C controls

above. Based upon the accelerated study results, the sponsor claims a shelf life of 10 days at 37°C or 12 months when stored at 2-8°C. Real time stability studies at 2-8°C are ongoing.

Controls: To determine the shelf life of the Cystatin C POC Test Controls, stress model tests were performed. Representative number of vials from one lot of the Diazyme Cystatin C controls (level 1 and level 2) was subject to stress in an incubator at 37°C. At predetermined times, vials were removed from storage, reconstituted and tested in duplicate with Diazyme Cystatin C POC Test reagents stored at 2-8°C (unstressed). Based on the accelerated study results, the sponsor claims a shelf life of 7 days at 37°C or 12 months when stored at 2-8°C for the lyophilized Diazyme Cystatin C controls. Real time stability studies at 2-8°C are ongoing

d. Detection limit:

The Limit of Blank, Limit of Detection and Limit of Quantitation of Diazyme Cystatin C POC Test were determined according to *CLSI EP17-A* in the following manner.

To calculate the Limit of Blank (LoB) of the Diazyme Cystatin C SMART Whole Blood Assay, the True Blank Sample (7.5% BSA) was tested with 20 replicates daily for three days. LoB was calculated as the mean of the 57th and 58th highest values for the true blanks. Based upon the results, the sponsor claims a LoB = 0.045 mg/L.

To calculate the Limit of Detection (LoD) of the Diazyme Cystatin C POC Test, five Low Samples were tested with 4 replicates daily for three days. LoD = LoB + (1.645* SD of Low samples). Based upon the results, the sponsor claims a LoD = 0.109 mg/L.

To calculate the Limit of Quantitation (LoQ) of the Diazyme Cystatin C POC Test, five patient whole blood samples from a commercial source were diluted with 7.5% BSA to targeted concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 mg/L. The diluted samples were tested with the Diazyme Cystatin C SMART reagent on SMART analyzers in 5 runs with 4 replicates per run (20 replicates total per sample). EP Evaluator software 8 was used to estimate the LOQ. Based upon the results, the sponsor claims a LoQ = 0.30 mg/L.

e. Analytical specificity:

The sponsor performed interference studies according to the *CLSI EP7-A2* guideline. Whole blood samples containing approximately 0.85 mg/L and 2.55 mg/L Cystatin C were used. Five levels of each interferant were tested in triplicate on a SMART analyzer. The level of interference was considered not significant by the sponsor if there was no more than 10% difference between the result in the presence of the interferant and the control result. The table below lists the substances tested and the concentrations at which no

significant interference was observed:

Potential Interfering Substance	Concentration at which no significant interference (<10% difference)was observed.
Bilirubin	≤40 mg/dL
Bilirubin Conjugated	≤40 mg/dL
Triglyceride	≤1000 mg/dL
Ascorbic Acid	≤10 mg/dL
Rheumatoid Factor	≤1000 IU/mL
Hemoglobin	≤10.0 g/dL

f. Assay cut-off:
Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
To demonstrate accuracy of the Diazyme Cystatin C POC Test, the candidate device was tested with individual EDTA whole blood samples and the results were compared to plasma results on the predicate device (k093680) using *CLSI EP9-A2* as a guideline.

A method comparison study was performed at Diazyme Laboratories. Fifty-one unaltered and four altered paired human whole blood and plasma samples were tested for comparison. The range of Cystatin C was 0.48 to 6.10 mg/L for whole blood samples and 0.52 to 6.13 mg/L for plasma samples. The whole blood samples were tested with the Diazyme Cystatin C POC Test on SMART analyzer and the corresponding plasma samples were tested with the predicate device (k093680) on Hitachi 917 analyzer.
Linear regression analysis of the results yielded the following:
$y = 0.9535x + 0.0958$, $R^2 = 0.9867$.

Another method comparison study was performed at three physicians' office laboratories by three (3) intended users. One hundred and fifteen unaltered and six altered paired human whole blood-plasma samples were tested for comparison. At each site forty whole blood samples were tested using SMART analyzers. The corresponding plasma specimens were tested with the predicate device (k093680) on Hitachi 917 analyzer at Diazyme Laboratories.

Linear regression analysis of the results yielded the following:

Site	N	Sample Range (mg/L)		Linear Regression Results
		Plasma	Whole Blood	
1	40	0.58-5.64	0.56-5.54	y = 0.9967x + 0.1058, R^2 = 0.9902
2	40	0.51-7.71	0.57-6.81	y = 0.9049x + 0.0731, R^2 = 0.9902
3	40	0.53-7.07	0.52-6.75	y = 0.9617x + 0.0352, R^2 = 0.9937
All Sites	120	0.51-7.71	0.52-6.81	y = 0.955x + 0.0723, R^2 = 0.9872

b. Matrix comparison:
Not applicable – EDTA venous whole blood is the only sample type indicated

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 assay reference interval was determined using human whole blood specimens from 126 apparently healthy adults with age of 19 - 63 years according to CLSI C28-A3 guideline. EP Evaluator 8 Software was used to establish the reference interval. The reference range was established to be 0.46 to 1.06 mg/L, which is similar to the published range of 0.5 to 1.03 mg/L for serum/plasma samples (Ref. *Wu. Alan, H.B., Tietz: Clinical Guide to laboratory Tests: Fourth Edition*, Saunders Elsevier, St. Louis, MO; 2006; 328-329). The sponsor recommends in the labeling that each laboratory should establish its own range of normal values for the population it serves.

N. Proposed Labeling:
The labeling is sufficient and 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.