

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

A. 510(k) Number:

k093680

B. Purpose for Submission:

Modifications to the Diazyme Cystatin C assay (previously cleared in k072270)

C. Measurand:

Cystatin C

D. Type of Test:

Quantitative, Immunoturbidimetric

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme Cystatin C Assay

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1225, Creatinine Test System

2. Classification:

Class II

3. Product code:

NDY; Test, Cystatin C

4. Panel:

Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indications for use below

2. Indication(s) for use:

The Diazyme Cystatin C Assay is an in-vitro diagnostic test for the quantitative determination of Cystatin C in serum or plasma by latex enhanced immunoturbidimetric method. The measurement of Cystatin C is used as an aid in the diagnosis and treatment of renal disease.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Hitachi 917 analyzer

I. Device Description:

The device is a ready-to-use, two-reagent kit. Reagent 1 contains a Tris-buffer solution while Reagent 2 contains a suspension of anti-human Cystatin C polyclonal antibody coated latex particles.

Five levels of ready-to-use calibrator material (0.5, 1.0, 2.0, 4.0, 8.0 mg/dL) are provided with the kit. The calibrators are prepared with recombinant Cystatin C antigen in a buffered aqueous matrix.

Two levels of ready-to-use control material (1.0 and 2.5 mg/L) are provided separately. The controls are prepared with recombinant Cystatin C antigen in a buffered aqueous matrix.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Diazyme Cystatin C Assay

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

k072270

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate device	Predicate device (k072270)
Intended Use/Indications for use	The Diazyme Cystatin C Assay is an in-vitro diagnostic test for the quantitative determination of Cystatin C in serum or plasma by latex enhanced immunoturbidimetric method. The measurement of Cystatin C is used as an aid in the diagnosis and treatment of renal disease.	Same
Sample Types	Serum or Plasma	Same
Limit of Quantitation	0.19 mg/dL	0.22 mg/dL
Measuring Range	0.2 to 8.0 mg/L	0.27 to 7.8 mg/L
Sample Volume	3 µL	3 µL
Methodology	Latex enhanced immunoturbidimetric method	Same
Instrument Platforms	Hitachi 917 analyzers	Hitachi 717 analyzers
Antibodies	Latex particles coated with anti-human Cystatin C chicken polyclonal antibodies	Suspension of anti-human Cystatin C rabbit polyclonal antibody coated latex particles
Calibrators	5 levels of calibrators	Same
Controls	2 levels of controls	Same

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

Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition (CLSI EP5-A2)

Evaluation of the Linearity of Quantitative Measuring Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)

Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition (CLSI E7-A2)

Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (CLSI EP9-A2)

Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP-17-A)

L. Test Principle:

The Diazyme Cystatin C assay is based on a latex enhanced immunoturbidimetric assay. 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 instrument calculates the Cystatin C concentration of a patient specimen by interpolation of the obtained signal on a 6-point calibration curve generated from use of saline and a 5-point calibrator set.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The sponsor performed precision studies in accordance with the CLSI EP5-A2 guideline. Three control samples containing 0.90, 2.50, and 5.40 mg/L Cystatin C were analyzed in duplicate, twice a day, over 20 days (N=80) on the Hitachi 917. Results are summarized below:

Samples	Mean (mg/L)	Within Run		Between Run	
		SD (mg/L)	% CV	SD (mg/L)	% CV
Level 1	0.91	0.03	3.5	0.04	4.6
Level 2	2.51	0.06	2.5	0.08	3.0
Level 3	5.40	0.11	2.0	0.25	4.6

b. *Linearity/assay reportable range:*

The sponsor performed linearity studies in accordance with the CLSI EP6-A guideline. Eleven levels of serum based samples were prepared by diluting a serum control sample containing 8.0 mg/L Cystatin C with Cystatin C negative serum. The linearity samples were tested in triplicate on the Hitachi 917 over the range of 0.00 to 8.0 mg/L. The recovered Cystatin C values were plotted against the expected values and an appropriate line fitted by standard linear regression resulting in: $y = 1.0069x - 0.0071$; $R^2 = 0.9973$.

The linearity data and the LoQ data support the sponsor's claim that the measuring range of this device is 0.2 to 8.0 mg/L.

High-dose hook effect: The potential for a high-dose hook effect was evaluated for this assay. A serum sample containing 50 mg/L of Cystatin C was diluted with

Cystatin C free serum to achieve concentrations of 4.00 to 50.00 mg/L Cystatin C. The samples were analyzed in triplicate on one Hitachi 917 analyzer. Based on the results, the sponsor concluded that Cystatin C levels up to 20 mg/L did not exhibit any high dose effect.

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

The calibrators and controls were prepared with a recombinant human Cystatin C in a buffered matrix and were previously cleared in k072270.

The sponsor states in the labeling that the unopened Cystatin C reagents are stable until the expiration date when stored at 2 to 8°C, and for 4 weeks if stored on-board the analyzer at 2 to 8°C. The product insert instructs not to freeze the reagents and not to mix reagents from different lots.

d. Detection limit:

The sponsor determined the limit of detection (LoD), limit of blank (LoB), and limit of quantitation (LoQ) according to the CLSI EP-17-A guideline.

The limit of the blank (LoB) was determined by analyzing a blank sample (7.5% BSA) with 20 replicates daily for 3 days on the Hitachi 917. The LOB was calculated as the mean of the 57th and 58th highest values for the true blanks and was determined to be 0.04 mg/L.

To determine the LoD five low patient serum samples were diluted, to achieve 0.6 to 1.03 mg/L, and tested in 4 replicates daily for 3 days. The LoD was calculated as $LOD = LOB + (1.645 * SD \text{ of the low samples})$. The results demonstrated that the LoD is 0.068 mg/L.

To determine the LoQ 5 patient serum samples were diluted with 7.5% BSA to targeted concentrations of 0.0075, 0.015, 0.03, 0.06 and 0.15 mg/L. The diluted serum samples were tested with the Cystatin C reagent on the Hitachi 917 in 20 replicates each. The LoQ was calculated as the lowest concentration for which the CV is $\leq 20\%$. The results demonstrated that the LoQ is 0.19 mg/L at 20% CV.

The claimed measuring range of the device is 0.2 to 8.0 mg/L.

e. Analytical specificity:

The sponsor performed interference studies according to the EP7-A2 guideline. Patient serum samples representing low (0.9 mg/L) and high (2.5 mg/L) Cystatin C levels were used. Five levels of each interferent were tested in triplicate on a Hitachi 917. The level of interference was considered not significant if there was no more than 10% difference between the result in the presence of the interferent 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 was observed
Bilirubin (conjugated)	≤ 40 mg/dL
Bilirubin (unconjugated)	≤ 40 mg/dL
Triglyceride	≤ 1000 mg/dL
Ascorbic Acid	≤ 176 m/dL
Hemoglobin	≤ 1000 mg/dL
Rheumatoid Factor	≤ 1000 IU/mL

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Method comparisons between the candidate device and the predicate device were performed according to the CLSI EP9-A guideline. A total of 45 serum samples ranging from 0.60 to 7.34 mg/L were used in the study, including some spiked samples in order to achieve concentrations in the upper portion of the measuring range. The candidate device results on the Hitachi 917 (Y) were compared to the corresponding predicate results on the Hitachi 917(X) and regression analysis resulted in: $y = 0.9999x + 0.0715$; $R^2 = 0.9922$.

This data was also analyzed using Passing and Bablok regression resulting in: $y = 1.0985x - 0.0253$.

b. *Matrix comparison:*

The sponsor performed a matrix comparison study using 37 unaltered paired serum/EDTA plasma/Lithium Heparin plasma samples and spiked samples were used to cover the upper range of the measuring range. Cystatin C samples ranging from 0.76 to 7.05 mg/L were analyzed on the Hitachi 917. The plasma results (Y) were compared to the corresponding serum results (X) and the regression analysis results are summarized in the table below:

Anticoagulant	Regression Analysis
EDTA	$y = 1.0221x - 0.0625$; $R^2 = 0.994$
Lithium Heparin	$y = 0.996x + 0.0065$; $R^2 = 0.9959$

The sponsor claimed that EDTA and Lithium heparin are acceptable anticoagulants.

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 reference range of 0.5 to 1.03 mg/L is included in the labeling with the following literature reference:

Wu. Alan, H.B., Teitz-Clinical Guide to Laboratory Tests; Fourth Edition, Saunders Elsevier, St. Louis, MO; 2006; 328-329.

The sponsor recommends that each laboratory establish its own range of normal values for the population in the region.

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.