

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

A. 510(k) Number:

k072270

B. Purpose for Submission:

New device

C. Measurand:

Cystatin C

D. Type of Test:

Quantitative, particle-enhanced turbidimetric immunoassay

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme Cystatin C Assay

Diazyme Cystatin C Calibrator

Diazyme Cystatin C Controls

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
Test, Cystatin C (NDY)	Class II	21 CFR 862.1225 Creatinine test system	Clinical Chemistry (75)
Product Code	Classification	Regulation Section	Panel
Calibrator (JIT)	Class II	21 CFR 862.1150 Calibrator	Clinical Chemistry (75)
Product Code	Classification	Regulation Section	Panel
Controls (JJX)	Class I	21 CFR 862.1660 Quality control material (assayed and unassayed)	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

Refer to Indications for use below.

2. Indication(s) for use:

Diazyme Cystatin C Assay

The Diazyme Cystatin C Assay is an in-vitro diagnostic test for the quantitative determination of Cystatin C in serum or plasma on Hitachi 717 analyzers by latex enhanced immunoturbidimetric method. The measurement of Cystatin C is used as an aid in the diagnosis and treatment of renal disease.

Calibrator

Diazyme Cystatin C Calibrator is intended for the in-vitro diagnostic use on Hitachi 717 analyzer. Cystatin C Calibrator is used for the calibration of Diazyme Cystatin C Assay in serum or plasma.

Controls

Diazyme Cystatin C Control is intended for the in-vitro diagnostic use on Hitachi 717 analyzer. Cystatin C Control is used as a quality control to monitor precision of the Diazyme Cystatin C Assay.

3. Special conditions for use statement(s):

For Prescription use only

4. Special instrument requirements:

Hitachi 717 analyzer

I. Device Description:

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

Ready-to-use five levels of liquid calibrator materials (0.5, 1.0, 2.0, 4.0, 8.0 mg/L) are provided with the device. The sponsor recommends using these calibrator materials and saline as the zero calibrator.

The sponsor separately from the reagent kit provides two levels of control materials (expected range: 0.76 – 1.04 mg/L for control 1; 2.17 – 2.94 mg/L for control 2) for validating the performance of the Cystatin C reagents. The source for the calibrators and controls is from Recombinant Human Cystatin C, which is immersed in HEPES-KOH buffer with sodium azide.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Dako Cytomation Cystatin C Immunoparticle

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

k041267

3. Comparison with predicate:

Characteristics	New Device (k072036)	DakoCytomation Cystatin C Immunoparticle (k041627)
Product Type	Turbidimetric Immunoassay	Turbidimetric Immunoassay

Characteristics	New Device (k072036)	DakoCytomation Cystatin C Immunoparticle (k041627)
Intended Use (Reagent)	An in-vitro diagnostic test for the quantitative determination of Cystatin C in serum or plasma on Hitachi 717 analyzers by latex enhanced turbidimetric immuno assay. The measurement of Cystatin C is used in the diagnosis and treatment of renal disease.	Cystatin C Immunoparticles are intended for the quantitative determination of cystatin C in human serum, heparinized plasma and EDTA plasma by turbidity and nephelometry. Cystatin C measurements are used as an aid in the diagnosis and treatment of renal diseases.
Intended Use (Calibrators)	Cystatin C Calibrator is used for the calibration of Diazyme Cystatin C Assay in serum or plasma.	Cystatin C Calibrator is intended for establishing calibration curves for the quantitative immunological determination of human cystatin C by turbidimetry of nephelometry.
Measuring Range	0.15 – 7.8 mg/L	0.4 – 7.5 mg/L
Specimen Type	Serum or heparin/ EDTA plasma	Serum or heparin/ EDTA plasma
Calibrator Levels	5 levels in liquid form	liquid form

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

- CLSI EP5-A: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline Vol 19, No 2.
- CLSI EP6-A: Evaluation of the Linearity of Quantitative Analytical Measurement Procedure: A Statistical Approach; Approved Guideline, Vol 23 No 16.
- CLSI EP17-A: Protocol for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. Vol 24, No 34.

L. Test Principle:

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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Following CLSI EP5-A, the sponsor evaluated the precision using three levels of serum specimens containing about 1.0, 4.7, and 6.0 mg/L Cystatin C with 2 runs per day with duplicates for 20 days. The sponsor’s acceptance was $\leq 5\%$ total CV for all three levels. The results are tabulated below.

Sample mg/L	N	Mean (µg/mL)	Within Run		Between Run	
			SD	%CV	SD	%CV
Level 1 (1.0)	80	1.0	0.04	3.9	0.05	4.8
Level 2 (4.7)	80	4.63	0.10	2.1	0.17	3.7
Level 3 (6.0)	80	5.95	0.16	3.9	0.22	3.7

b. *Linearity/assay reportable range:*

Following instructions in CLSI Document EP6-A, the sponsor conducted studies on Hitachi 717 instrument to evaluate the dilution linearity of the Cystatin C assay. The sponsor used a serum sample containing 7.8 mg/L Cystatin C and prepared 11 test concentration levels (7.8 mg/L – 0.78 mg/L) with saline. Testing was done using one reagent lot and running triplicates of each diluent level and saline for 0 mg/L. The results demonstrated that the sample values fell within the sponsor’s acceptance criteria of Slope =0.90-1.10; $R^2 \geq 0.95$. The linear regression analysis generated the equation, $y=1.0475x-0.1746$ with regression coefficient (R^2) of 0.9964. Based on the linearity data and the Limit of Detection (LOD) and Limit of Quantitation (LOQ) described below, the sponsor established the assay reportable range for Cystatin C assay as 0.27 mg/L – 7.8 mg/L.

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

The sponsor provides calibrators and control materials for quality control procedure. The calibrator materials are provided in five levels (0.5, 1.0, 2.0, 4.0, 8.0 mg/L) and the sponsor recommends using this calibrator with saline (zero calibrator) to maintain assay calibration procedure. The sponsor provided the protocols for preparation and value assignment for calibrators and controls. The sponsor used a primary measurement standard quantified using molar absorptivity method to prepare manufacturers working calibrator (master lot). Controls and calibrator lots are made with reference to the master lot.

Using three lots of reagent, the sponsor conducted all stability studies including real time stability up to 14-month period and accelerated stability studies. The results demonstrated that reagents including calibrators were stable for at least 10 days at 37°C, one month at 25°C, and 14 month at 4°C. On-board stability studies at 2-8°C chamber inside Hitachi 717 instrument demonstrated at least 30-day stability. Using

one lot of reagent, the sponsor conducted studies to determine the reagent calibration frequency using controls (0.9 and 2.5 mg/L). The results demonstrated that calibration curve is stable and accurate for 14 days. Using three lots, calibrator stability studies were conducted real time at 4°C, 25°C, and 37°C and the results demonstrated stability of 12 months, one month, and 10 days, respectively. A similar study conducted for controls demonstrated same stability claims as for the calibrators.

d. Limit of Detection:

To demonstrate the lower limit of the assay range, the sponsor performed the limit of detection (LOD), limit of quantitation (LOQ), and limit of blank (LOB) tests following CLSI document EP17-A. A serum sample was selected (0.429 mg/L) and diluted into 5 other concentrations (0.031 – 0.429 mg/L) and the 6 samples were run over 5 days with 4 runs per day for a total of 120 total replicates. The data were analyzed using EP Evaluator Software's Limit of Quantitation Module, which generated an estimated LOQ based on the entered data points from the 6 concentrations. Using the calculated percent CV from the 6 concentrations it fits a curve that calculates at which concentration of Cystatin C, where the upper 95% confidence level for the curve has a CV of 15%. The estimated LOQ is 0.27 mg/L. The sponsor conducted LOD studies using five serum samples each diluted 100 times to obtain low samples ranging from 0.01 mg/L to 0.06 mg/L. Tests were run on Hitachi 717 with each dilution replicated 12 times to obtain 60 data points. LOD was determined using the algorithm, $LOD = LOB + [1.645 \times s]$. Based on 12 replicates of a zero calibrator, LOB was determined to be 0.13 mg/L and using this value, LOD was determined to be 0.158 mg/L.

e. Analytical specificity:

The sponsor evaluated the effect of known endogenous interferences on Hitachi 717 System using three levels of serum samples. The interferences and the test range included triglycerides (2400 mg/dL), hemoglobin (1000 mg/dL), rheumatoid factor (450 IU/mL), free bilirubin (18.2 mg/dL), bound bilirubin (19.6 mg/dL), and ascorbic acid (50 mg/dL). The low, medium, and high Cystatin C serum samples were spiked with varied concentrations of interfering substances. Each sample spiked with different substances was run in triplicates. The sponsor's acceptance criteria was <10% deviation from the unspiked samples. Based on the acceptance criteria of $\pm 10\%$ of non-interfered value, the results demonstrated that there was no interference for the above interferences and up to the concentrations tested.

f. Assay cut-off:

Not Applicable.

2. Comparison studies:

a. Method comparison with predicate device:

To demonstrate the substantial equivalence to the predicate device, Dako Cytomation Cystatin C assay, the sponsor conducted a method comparison study using Hitachi 717 System. A total of 47 serum samples (range: 0.5-7.7 mg/L) was used for this study. The analysis of data using Passing & Bablok method generated regression equation $y = 0.9375x + 0.15$ with 95% CI of 0.0784 to 0.2500 for intercept and 95% CI of

0.900 to 0.973 for the slope. Linear regression analysis produced the equation $y = 0.9304x + 0.1709$ with correlation coefficient of 0.9838.

b. Matrix comparison:

The sponsor conducted matrix comparison studies using 33 matched serum and plasma samples preserved separately with EDTA and heparin. Serum tubes without anticoagulants were used as the control. Cystatin C values for serum samples ranged from 0.74 to 4.22 mg/L. The regression analysis of data generated for plasma (EDTA) (y) vs. serum (x) and plasma (heparin) vs. serum produced equations $y = 0.962x - 0.0505$ ($r = 0.997$) and $y = 0.9707x + 0.0143$ ($r = 0.998$), respectively.

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):

4. Clinical cut-off:

Not Applicable.

5. Expected values/Reference range*:

The expected values of Cystatin C were based on literature*. The expected value is 0.5 - 1.0 mg/L. However, the sponsor recommends in the labeling that each laboratory should establish a range of normal values for the population in their region.

* Tietz NW. Clinical Guide to Laboratory Tests. 4th ed. St. Louis, MO: WB Saunders Company; 2005.

N. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

O. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

P. Conclusion:

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