

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

A. 510(k) Number:

k041627

B. Purpose for Submission:

New Device

C. Analyte:

Cystatin C

D. Type of Test:

Quantitative

E. Applicant:

DakoCytomation Denmark A/S

F. Proprietary and Established Names:

Cystatin C Immunoparticles

Cystatin C Control Set

Cystatin C Calibrator

G. Regulatory Information:

1. Regulation section:

Cystatin C Immunoparticles 21 CFR 862.1225

Cystatin C Control Set 21 CFR 862.1660

Cystatin C Calibrator Kit 21CFR 862.1150

2. Classification:

Cystatin C Immunoparticles – Class II

Cystatin C Control Set- Class I

Cystatin C Calibrator Kit- Class II

3. Product Code:

Cystatin C Immunoparticles- NDY

Cystatin C Control Set- JJX

Cystatin C Calibrator Kit- JIT

4. Panel:

75

H. Intended Use:

1. Intended use(s):

Cystatin C Immunoparticles are intended for the quantitative determination of cystatin C in human serum, heparinized plasma and EDTA plasma by turbidimetry and nephelometry. Cystatin C measurements are used as an aid

in the diagnosis and treatment of renal diseases. For In Vitro Diagnostic Use. For Professional Use Only.

Cystatin C Control Set is an assayed bi-level control intended to monitor and evaluate the precision and accuracy of the quantitative immunological determination of human cystatin C by turbidimetry or nephelometry. For In Vitro Diagnostic Use. For Professional Use Only.

Cystatin C Calibrator is intended for establishing calibration curves for the quantitative immunological determination of human cystatin C by turbidimetry or nephelometry. For In Vitro Diagnostic Use. For Professional Use Only.

2. Indication(s) for use:
See Intended Use Statements above.
3. Special condition for use statement(s):
For prescription use only.
4. Special instrument Requirements:
Hitachi 911, Hitachi 917, Cobas MIRA Plus, IMMAGE Immunochemistry System, Modular P or other commercially available nephelometer or turbidimeter.

I. **Device Description:**

Cystatin C Immunoparticles are purified immunoglobulin fraction of rabbit antiserum directed against cystatin C covalently coupled to uniform polystyrene particles distributed in 10 ml vials. In the DakoCytomation Cystatin C Assay, human serum or plasma is mixed with the Cystatin C Immunoparticles to form an immune complex that is measured by turbidimetry or nephelometry.

Cystatin C Control set contains three 1 mL of control 1 and three 1 mL of control 2. It is a bi-level control to monitor and evaluate human cystatin C by turbidimetry or nephelometry. The controls are liquid pools of delipidated human serum enriched with recombinant human cystatin C produced in *E. coli* and preserved with 15 mmol/L sodium azide. Each donor was tested and found negative for Human immunodeficiency virus (HIV) 1 and 2, Hepatitis B virus (HBV) and Hepatitis C virus (HCV) in European blood banks. The test used were cleared for *in vitro* diagnostic use in the EU (in compliance with the European Directive 98/79/EC on *in vitro* Diagnostic Medical Devices as Annex II, List A products) and are also approved by the Paul-Ehrlich-Institute in Germany.

Cystatin C Calibrator kit is a liquid pool of delipidated human serum enriched with recombinant human cystatin C produced in *E. Coli* and preserved with 15 mmol/L sodium azide. Each donor was tested and found negative for HIV 1 and 2, HBV and HCV in European blood banks. The test used were cleared for *in vitro* diagnostic use in the EU (in compliance with the European Directive 98/79/EC on *in vitro* Diagnostic Medical Devices as Annex II, List A products) and are also approved by the Paul-Ehrlich-Institute in Germany.

J. Substantial Equivalence Information:

1. Predicate device name(s):
 N Latex Cystatin C Test Kit, Dade Behring Inc. k003503
 N Cystatin C Control, Dade Behring Inc. k003503
 N Protein Standard UY, Dade Behring Inc. k003501
2. Predicate K number(s):
 k003503
 k003503
 k003501
3. Comparison with predicate:

Cystatin C Immunoparticles

Similarities		
Item	Device	Predicate
Intended Use	For In Vitro Diagnostic Use. Cystatin C Immunoparticles are intended for the quantitative determination of cystatin C in human serum, heparinized plasma and EDTA plasma by turbidimetry and nephelometry. Cystatin C measurements are used as an aid in the diagnosis and treatment of renal diseases.	N latex Cystatin C is an in vitro diagnostic test kit for the quantitative determination of cystatin C in human serum and heparinized plasma by means of particle enhanced immunonephelometry. Cystatin C measurements are used in the diagnosis and treatment of renal diseases.
Sample	Serum or heparin/ EDTA plasma	Serum or heparin plasma
Analyte	Cystatin C	Cystatin C
Differences		
Item	Device	Predicate
Methodology	Human serum or plasma is mixed with the Cystatin C immunoparticles to form immuno complexes that are measured by turbidimetry or nephelometry. The concentration of cystatin C in the sample is calculated using a standard curve.	Polystyrene particles are coated with antibodies to cystatin C are agglutinated when mixed with samples containing Cystatin C. The intensity is measured using immunonephelometry. The concentration of cystatin C is determined by comparison with dilutions of a standard of known concentration.
GFR Evaluation Method	Iohexol Clearance	Iothalamate Clearance
Assay Range	~0.4-7.5 mg/L	~0.23 -8 mg/L

Instrument	Hitachi 911, Hitachi 917. Cobas Mira Plus, IMMAGE Immunochemistry System, Modular P or other commercially available nephelometer or turbidimeter	Behring Nephelometer Systems
Test	Nephelometry or Turbidimetry	Nephelometry

Cystatin C Control Set

Similarities		
Item	Device	Predicate
Intended Use	Cystatin C Control Set is intended as a bi-level control for the quantitative immunological determination of human Cystatin C by turbidimetry or nephelometry	N Cystatin C Control is intended to monitor and evaluate the precision and accuracy of the N Latex Cystatin C Test Kit
Analyte	Cystatin C	Cystatin C
Differences		
Item	Device	Predicate
Matrix	Pool of delipidated human serum	Polygeline with urine proteins of human origin and preservative.
Confidence Interval	+/- 15%	+/- 20%

Cystatin C Calibrator

Similarities		
Item	Device	Predicate
Intended Use	Cystatin C Calibrator is intended for establishing calibration curves for the quantitative immunological determination of human cystatin C by turbidimetry or nephelometry.	N Protein Standard UY is intended for preparing reference curves for the immunophelometric determination of alfa-microglobulin, Cystatin C and Beta-trace protein using the Behring Nephelometer System as well as for the assay of alfa-microglobulin

		by radial immunodiffusion on (RID).
Analyte	Cystatin C	Cystatin C, beta-trace protein, alpha1-microglobulin.
Differences		
Item	Device	Predicate
Matrix	Pool of delipidated human serum	Polygeline with urine proteins of human origin and preservative.

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

NCCLS standard, "EP9-A2: Method Comparison and Bias Estimation Using Patient Samples".

NCCLS, Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline EP5-A:1999;19(2):1-43.

NCCLS, Evaluation of the Linearity of Quantitative Analytical Measurement Procedure: A Statistical Approach; Approved Guideline EP6-A:2003;23(16):509-575.

NCCLS, User demonstration of Performance for Precision and Accuracy; Approved Guideline NCCLA EP9-A Second Edition. Vol. 15(17).

NCCLS, Identification and Quantification fo Matrix Effects; Proposed Guidelines, EP14-P 1993.

NCCLS, Assessment of Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; Approved Guideline GP10-A:1995; Vol. 15(19).

ISO17511; Metrological Traceability of Values Assigned to Calibrators and Control Materials.

EN 13640 "Stability Testing of In Vitro Diagnostic Reagents"

L. Test Principle:

Human Serum or Plasma is mixed with the Cystatin C Immunoparticles. The resulting immuno complexes are measured by turbidimetry or nephelometry. The signal generated is correlated with the concentration of cystatin C in the sample. By interpolation on a standard curve, the concentration of cystatin C in the sample is calculated. A conversion equation is given in the package insert but is recommended that each individual laboratory determine its own conversion formula.

M. Performance Characteristics (if/when applicable):1. Analytical performance:a. *Precision/Reproducibility:*

Precision was tested on the Hitachi 917 following the NCCLS EP5-A and the results are listed in the chart below on two control materials and three frozen serum samples (low, medium and high). These samples were analyzed in duplicates twice daily for twenty days. Precision was tested on the Cobas MIRA Plus and Hitachi 911 using a modified NCCLS document EP5-A on two controls and 3

frozen serum samples that were analyzed in duplicates once a day for 10 days. Imprecision was conducted on Modular P and IMMAGE Immunochemistry System using a 6 x 6 precision analysis on two controls and two frozen samples. The % CV for each instrument is listed in the chart below.

Total %CV	Cystatin C Control 1	Cystatin C Control 2	Human Serum Pool Low	Human Serum Pool Medium	Human Serum Pool High
Hitachi 917	2.1	2.6	5.9	2.0	2.3
Hitachi 911	2.0	2.8	5.7	2.6	1.7
Cobas MIRA Plus	2.0	1.8	2.4	2.2	1.5
Modular P	2.1	2.3	4.3	Not conducted	3.3
IMMAGE Immunochemistry System	3.4	5.9	3.3	Not conducted	3.5

Two lots of DakoCytomation- Controls 1 and 2 were run in duplicates in all five instruments and compared to the assigned values in the chart below.

% Mean Difference	Cystatin C Control 1	Cystatin C Control 2	Cystatin C Control 1	Cystatin C Control 2
Hitachi 917	2.15	-5.29	0.56	-2.54
Hitachi 911	3.42	0	5.89	8.90
Cobas MIRA Plus	8.48	-4.33	5.22	0.42
Modular P	1.01	0.48	Not conducted	Not conducted
IMMAGE Immunochemistry System	2.7	2.0	Not conducted	Not conducted

b. Linearity/assay reportable range:

Linearity was tested on serum samples low, medium and high for examination of different ranges of cystatin C concentrations. Samples were diluted in 11 steps with either low serum sample or saline and were analyzed in triplicates.

The Hitachi 917 (representing turbidimetry) and IMMAGE (representing nephelometry) were diluted with both saline and a low concentration serum to obtain 4 ranges of linearity. The Hitachi 911 and the Cobas MIRA Plus were studied using dilutions with saline samples.

Linearity equations for the Hitachi 917 for serum dilutions and saline dilutions were $y=0.060X + 0.823$ and $y=0.06411X + 0.063$ respectively. Linearity equations for the IMMAGE Immunochemistry System for serum and saline dilutions was $Y=0.07032X + 0.701$ and $Y=0.07848X - .087$ respectively. Linearity

equations for the Hitachi 911 and the Cobas MIRA Plus with saline were $Y=0.06519X + 0.183$ and $Y= 0.06662X + 0.140$ respectively. A hook effect study was conducted on all five instruments and it was not found to interfere with concentrations up to 28.3 mg/L for the Hitachi 917 and Hitachi 911.

c. *Traceability (controls, calibrators, or method):*

The Cystatin C Control Set is traceable to the DakoCytomation Cystatin C Calibrator, which is traceable to a pure recombinant human cystatin C reference preparation made in-house.

Stability

Real Time stability was the only stability conducted. The Cystatin C Immunoparticles, Cystatin C Calibrator and the Cystatin C Control Set real time stability study supports the 12 month shelf life when stored at 2-8° C. One lot of Cystatin C immunoparticles and three lots of the Cystatin C Calibrator and Cystatin C Control set were studied for shelf life. Two new lots of Cystatin C Immunoparticles are ongoing stability studies. On-board stability study for Cystatin C immunoparticles was determined to be at room temperature to be 90 days for the Hitachi 917 and 911. The on-board stability was 21 days on the IMMAGE Immunochemistry system due to the lack of a cooling system on the instrument. There is no cooling system on the Cobas MIRA Plus. The study was conducted with an initial calibration and tested with 4 individual samples- Cystatin C Control 1 and control 2 and two serum samples (high and low). They were measured in duplicates.

d. *Detection limit:*

The detection limit for each instrument was performed by analyzing dilutions from the lowest standard and down to 0 mg/L with 7 determinations for each of the 11 concentrations. The Hitachi 917, 911 and the Cobas MIRA Plus used 11 sample dilutions each. The IMMAGE Immunochemistry system used 32 hospital samples the estimation of the detection limit.

Using a linear regression analysis, the detection limits is 0.034 mg/L on the Hitachi 917, 0.080 mg/L on Hitachi 911 and 0.079 mg/L on Cobas MIRA Plus. The detection limit for the IMMAGE Immunochemistry system was approximately 0.073 mg/L.

e. *Analytical specificity:*

Interference studies were conducted on 4 instruments. The studies used one to 3 serum pools with low, medium and high cystatin C concentrations. Potentially interfering substances such as hemoglobin, triglycerides, conjugated and unconjugated bilirubin and rheumatoid factor were added in varying concentrations were analyzed in triplicates. No interference was found on the Hitachi 911, Hitachi 917 and Cobas MIRA Plus for hemoglobin up to 10 g/L, triglycerides up to 15 g/L, conjugated and non-conjugated

bilirubin up to 600 mg/L or rheumatoid factor up to 1200 IU/mL. No interference was detected on the IMMAGE Immunochemistry system for hemoglobin up to 10 g/L, triglycerides up to 10 g/L, conjugated and non-conjugated bilirubin up to 600 mg/L or rheumatoid factor up to 1200 IU/mL.

No interference was detected when prescription and over-the-counter drugs were spiked into samples containing lower concentrations of cystatin C.

f. *Assay cut-off:*

N/A

2. Comparison studies:

a. *Method comparison with predicate device:*

190 split heparinized plasma samples measured with the DakoCytomation Cystatin C Assay on Modular P and Dade Behring N Latex Cystatin C Test Kit on the Dade Behring Prospec Nephelometer. An equation of $Y=0.6954X + 0.214$ was obtained with a R of .9865.

Using 127 patients that were undergoing an iohexol clearance procedure for Glomerular Filtration Rate (GFR) evaluation, ROC plots of the DakoCytomation Cystatin C assay and the Dade Behring N Latex Cystatin C Test Kit was conducted in order to distinguish between normal and reduced GFR. The area under the ROC curve was estimated to be 0.96 (95% CI 0.927, 0.988) for the DakoCytomation Cystatin C Assay and for the Dade Behring N Latex Cystatin C Test Kit to be 0.97 (95% CI 0.946, 0.994).

DakoCytomation Cystatin C Assay was also compared to Roche Diagnostics Corp Creatinine Plus Assay (K953239) in two studies. Study A consisted of 294 subjects and study B contained 531 subjects. Using Deming regression analysis, the two methods were compared and study A and B results are in the table below.

	Slope (95% CI)	Intercept	R	Cystatin C Range (mg/L)	N
Study A	0.0056 +/- 0.0002 (0.0052, 0.0059)	0.59 +/-0.03 (0.52,0.65)	0.87	0.50-4.66	294
Study B	0.0051 +/- 0.0001 (0.0048, 0.0053)	0.78 +/- 0.02 (0.74, 0.82)	0.86	0.70-5.25	531

ROC plots for the diagnostic accuracy of cystatin C in the DakoCytomation Cystatin C Assay and creatinine using the Roche Diagnostic Corp. Creatinine Plus Assay was conducted. The area under the ROC curve was estimated to be 0.94 (95% CI 0.914, 0.955) for the DakoCytomation Cystatin C Assay and 0.93 (95% CI 0.911, 0.954) for the Roche Diagnostic Corp. Creatinine Plus Assay.

b. *Matrix comparison:*

10 paired serum, EDTA plasma and heparinized plasma samples were analyzed in duplicates on the Hitachi 917, Hitachi 911, Cobas MIRA Plus and on the Modular P. The mean values are summarized in the chart below.

	Serum	EDTA Plasma	% Deviation
Hitachi 917	3.48	3.35	-3.8
Hitachi 911	3.61	3.47	-4.1
Cobas Mira Plus	3.78	3.72	-1.7
Modular P	0.65	0.68	5.4

3. Clinical studies:

a. *Clinical sensitivity:*

N/A

b. *Clinical specificity:*

N/A

c. *Other clinical supportive data (when a and b are not applicable):*

Previously GFR was estimated from the plasma creatinine concentration. Several formulas (e.g. Cockcroft and Gault) have been derived to allow GFR calculation in mL/min from the cystatin C concentration in mg/L.

274 heparinized plasma samples from patients that were undergoing iohexol clearance procedure for GFR evaluation were assayed using the DakoCytomation Cystatin C Assay on Modular P. The correlation between the glomerular filtration rate measured by iohexol clearance and the cystatin C concentration measured with the DakoCytomation Cystatin C Assay allowed for a conversion equation of $GFR (mL/min) = 69.9 \times (\text{cystatin C (mg/L)})^{-1.5274}$ with an R^2 of 0.88. The 95% confidence intervals for each constant are as follows: 69.9 (67.9 to 72.2) and -1.5274 (-1.5951 to -1.4597).

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

69 normal GFR subjects less than 50 years of age and 94 normal GFR subjects greater than 50 years of age were used in a study to determine the reference range intervals. Healthy individuals 50 years old and under generally have a glomerular filtration rate above 80 mL/min/1.73 m².

Individuals 1-50 years of age: 0.55-1.15 mg/L

Individuals >50 years of age: 0.63-1.44 mg/L.

127 patients that were undergoing iohexol clearance procedure for evaluation of GFR were also used to determine how GFR decreased with age in healthy individuals.

Subjects ≤50 years of age have GFR ≥ 80 mL/min

Subjects 51-61 years of age have GFR ≥60 mL/min

Subjects ≥65 years of age have GFR ≥50 mL/min.

N. Conclusion:

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