

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

**A. 510(k) Number:**

k080811

**B. Purpose for Submission:**

New device

**C. Measurand:**

Cystatin C

**D. Type of Test:**

Quantitative immunoturbidimetric assay

**E. Applicant:**

Roche Diagnostics Corp.

**F. Proprietary and Established Names:**

Tina-quant Cystatin C

Calibrator f.a.s. Cystatin C

Cystatin C Control Set

**G. Regulatory Information:**

<b>Product Code</b>	<b>Classification</b>	<b>Regulation Section</b>	<b>Panel</b>
NDY	Class II	21 CFR 862.1225 Test, Cystatin C	Clinical Chemistry
JIT	Class II	21 CFR 862.1150 Calibrator	Clinical Chemistry
JJX	Class I	21 CFR 862. 1660 Quality Control Material	Clinical Chemistry

**H. Intended Use:**

1. Intended use(s):

See indications for use statement below.

2. Indication(s) for use:

Immunoturbidimetric assay for the quantitative in vitro determination of cystatin C in human serum and lithium-heparin plasma on Roche automated clinical chemistry analyzers. Cystatin C measurements are used as an aid in the diagnosis and treatment of renal diseases.

Calibrator:

Cfas (Calibrator for automated systems) Cystatin C is for use in the calibration of quantitative Roche methods on Roche clinical chemistry analyzers as specified in the value sheets.

Control:

Cystatin C Control Set is for use in quality control by monitoring accuracy and precision for the quantitative methods as specified in the value sheets.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Roche Hitachi 917

**I. Device Description:**

The Roche Tina-quant Cystatin C kit is a ready to use dual reagent assay. Reagent 1 is a polymer solution in MOPS- buffered saline, preservatives and stabilizers.

Reagent 2 is a glycine buffer containing latex particles coated with anti-cystatin C antibody (rabbit), preservatives and stabilizers. Test principle is a particle enhanced immunoturbidimetric assay.

The C.f.a.s. Cystatin C calibrator is a liquid ready to use calibrator based on pooled delipidated human serum enriched with recombinant human cystatin C produced in E. coli. Each donor was tested and found negative for HIV 1 and 2, HCV and HBsAg by FDA-approved methods or by methods cleared in compliance with the European Directives 98/79/EC Annex II, List A.

The Cystatin C Control Set is a ready to use bi-level control that consists of four 1 mL vials of a low and high control. The controls are liquid pools of delipidated human serum enriched with recombinant human cystatin C produced in E. coli and

preserved with preservatives. Each donor was tested and found negative for HIV 1 and 2, HCV and HBsAg by FDA-approved methods or by methods cleared in compliance with the European Directives 98/79/EC Annex II, List A.

**J. Substantial Equivalence Information:**

	k080811- proposed device Roche Tina-quant Cystatin C, C.f.a.s. Cystatin C calibrators, Cystatin C Control Set	k041627 – predicate device Dako Cytomation Cystatin C Immunoparticles, Cystatin C Control Set, Cystatin C Calibrator
<b>Similarities</b>		
Indications for use	Immunoturbidimetric assay for the quantitative in vitro determination of cystatin C in human serum and plasma on Roche automated clinical chemistry analyzers. Cystatin C measurements are used as an aid in the diagnosis and treatment of renal diseases.	Same
Analyte	Cystatin C	Same
Principle	Particle enhanced immunoturbidimetric assay.	Same
<b>Differences</b>		
Measuring Range	0.4 to 8.0 mg/L	0.4 to 7.5 mg/L
Analyzers	Hitachi 917	Hitachi 911, Hitachi 917, Modular P, Cobas Mira Plus and IMMAGE
Specimen	Serum and Lithium-heparinized plasma	Serum, heparinized plasma, EDTA plasma

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

STANDARDS			
Title and Reference Number			
None were referenced.			
GUIDANCE			
Document Title	Office	Division	Web Page
Guidance for Industry and FDA Staff; Replacement Reagent and Instrument Family Policy	OIVD		<a href="http://www.fda.gov/cdrh/oivd/guidance/950.html">http://www.fda.gov/cdrh/oivd/guidance/950.html</a>
Guidance for Industry and FDA Staff - Assayed and Unassayed Quality Control Material	OIVD		<a href="http://www.fda.gov/cdrh/oivd/guidance/2231.html">http://www.fda.gov/cdrh/oivd/guidance/2231.html</a>
Guidance for Industry - Abbreviated 510(k) Submissions for In Vitro Diagnostic Calibrators; Final	OIVD		<a href="http://www.fda.gov/cdrh/ode/calibrator.html">http://www.fda.gov/cdrh/ode/calibrator.html</a>
CLSI EP17-A			

**L. Test Principle:**

The Roche Tina-quant Cystatin C test principle is a particle enhanced immunoturbidimetric assay. Human cystatin C agglutinates with latex particles coated with anti-cystatin C antibodies. The precipitate is determined turbidimetrically. 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.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

All performance data was determined with the Hitachi 917 analyzer. Additional information can be obtained from k041627.

*a. Precision/Reproducibility:*

Precision for the Tina-quant Cystatin C was conducted on the Hitachi 917 analyzer with a single lot and single instrument. Within run imprecision of the Tina-quant Cystatin C test system was determined with two control materials and two serum samples run in duplicates of 21. Between-day imprecision was determined with two controls and two serum samples run in triplicate (median value used). Total imprecision was determined with two controls and two serum samples run once a day in triplicates for 21 days. Results for the studies are shown in the table below.

Within Run Precision				
Material used	control 1	control 2	sample 1	sample 2
N	21	21	21	21
MEAN (mg/L)	4.48	0.95	0.75	5.14
SD (mg/L)	0.04	0.01	0.01	0.03
CV (%)	0.91	0.97	1.71	0.67
Between- Day Precision				
Material used	control 1	control 2	sample 1	sample 2
N	21	21	21	21
MEAN (mg/L)	4.37	0.4	0.73	4.98
SD (mg/L)	0.09	0.03	0.02	0.10
CV (%)	2.01	2.79	2.83	2.05
Total Precision				
Material used	control 1	control 2	sample 1	sample 2
N	63	63	63	63
Total mean (mg/L)	4.36	0.94	0.73	4.98
W-R SD(mg/L)	0.1	0.0	0.0	0.0
W-R CV(%)	1.4	1.9	2.0	0.9
Total SD(mg/L)	0.11	0.03	0.03	0.12
Total CV(%)	2.5	3.13	3.76	2.36

b. *Linearity/assay reportable range:*

Linearity was assessed via percent recovery of three serum samples (low, medium and high levels) of different ranges of cystatin C concentrations. Low and mid levels dilution series were prepared with human serum containing low concentrations of cystatin C and saline as a diluent. The high level dilution series were prepared using human serum spiked with Cystatin C and saline as a diluent. Samples were diluted in 9 steps with saline and were analyzed in with the Hitachi 917 analyzer. The sponsors acceptance criteria for samples ranging from 0.40 to 1.00 mg/L is a recovery  $\leq 0.20$  mg/L and for samples between 1.00 to 8.00 is a recovery  $\leq 10\%$ . The results are shown in the tables below and support the sponsors claimed linear range of 0.40 to 8.0 mg/L.

The sponsor conducted a high dose (hook-effect) study to determine if high does of cystatin C interfered with the assay. Human serum samples were

spiked to Cystatin C concentrations of 26.5 mg/L and 31.2 mg/L and diluted with saline. There was no interference observed for concentrations up to 20 mg/L for the Hitachi 917.

Low-level Dilution					
No	Dilution low	Dilution high	Measured Value	Theoretical Value	Recovery [%]
0	8.50	1.50	0.330	0.360	91.667
1	8.00	2.00	0.430	0.443	97.065
2	7.00	3.00	0.640	0.610	104.918
3	6.00	4.00	0.770	0.777	99.099
4	5.00	5.00	0.980	0.943	103.924
5	4.00	6.00	1.140	1.110	102.703
6	3.00	7.00	1.310	1.277	102.584
7	2.00	8.00	1.450	1.443	100.485
8	1.00	9.00	1.600	1.610	99.379
9	0.00	10.00	1.730	1.777	97.355
Mid-level Dilution					
No	Dilution low	Dilution high	Measured Value	Theoretical Value	Recovery [%]
0	10.00	0.00	0.760	0.935	81.283
1	9.00	1.00	1.560	1.670	93.413
2	8.00	2.00	2.350	2.405	97.713
3	7.00	3.00	3.250	3.140	103.503
4	6.00	4.00	4.090	3.875	105.548
5	5.00	5.00	4.780	4.610	103.688
6	4.00	6.00	5.400	5.345	101.029
7	3.00	7.00	6.190	6.080	101.809
8	2.00	8.00	6.690	6.815	98.166
9	1.00	9.00	7.210	7.550	95.497
High-level Dilution					
No	Dilution low	Dilution high	Measured Value	Theoretical Value	Recovery [%]
0	10.00	0.00	1.300	1.315	98.859
1	9.00	1.00	2.450	2.450	100.000
2	8.00	2.00	3.690	3.585	102.929
3	7.00	3.00	4.940	4.720	104.661
4	6.00	4.00	6.000	5.855	102.477
5	5.00	5.00	6.990	6.990	100.000
6	4.00	6.00	7.880	8.125	96.985
7	3.00	7.00	8.920	9.260	96.328
8	2.00	8.00	9.990	10.395	96.104
9	1.00	9.00	10.810	11.530	93.755

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

#### Traceability

There is no internationally recognized reference standard for Cystatin C. The ready to use secondary calibrator materials are traceable to an in-house gravimetric (dry-mass) reference preparation of delipidated human serum enriched with recombinant human Cystatin C. The Cystatin C Control Set is traceable to the Cfas Cystatin C Calibrator (secondary calibrator materials).

#### Value assignment

The Cystatin C Calibrator and Control Set value assignments were determined by turbidimetry on the Hitachi 917. The reference preparation of pure recombinant human Cystatin C was prepared and is used to value assign a master calibrator or primary calibrators. The Primary calibrator is used to assign the value of the secondary calibrator (Cfas Cystatin C). A daily calibration curve is prepared from 6 dilutions of the master calibrator and of the secondary calibrator. All dilutions are measure in duplicate. This is repeated over 3 days and the final target value of the secondary calibrator is the grand mean of all determinations for the value with the highest concentration.

The Cystatin C Control Set is value assigned from the Cystatin C Calibrator based on six calibrations. The grand mean value is used to value assign the Cystatin C Control tested and the uncertainty for the grand mean is noted. The control from another lot is measured in duplicates. The recommended confidence interval for control accuracy is the assigned value +/- 15%.

#### Stability

Real-time stability for the C.f.a.s. Cystatin C and the Cystatin C Control set were conducted for 25 months and the results support the sponsor's shelf-life claims of 24 months.

Closed reagent stability was assessed with three lots of the Cystatin C latex reagent at day 0, month 2, 6,12,13,18, 24 and 25. The real-time stability testing is still on-going and the results support the sponsor's shelf-life claims of 24 months.

On-board reagent stability was assessed and the results support the sponsor's open vial claims of 8 weeks when stored on-board or at 2-8°C.

#### *d. Detection limit:*

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined in accordance with the CLSI EP17-A requirements.

The LoB study was conducted to determine the highest observed measurement values for samples free of analyte. The LOB was determined as the 95<sup>th</sup> percentile of measurements of bank samples. A blank sample was assayed on two Hitachi 917 analyzers in six runs over 3 days per instrument for a total of 30 replicates. The LoB was determined to be  $\leq 0.3$  mg/L.

The LoD was conducted to determine the lower limit for samples with analyte. The LoD was determined as the lowest amount of analyte in a sample that can be detected with 95% probability. Five human serum samples with low analyte were run on two Hitachi analyzers in six runs over 3 days per instrument for a total of 25 replicates. The LoD was determined to be  $\leq 0.4$  mg/L.

*e. Analytical specificity:*

Pooled human serum samples spiked with varying levels of interferent were tested on the Hitachi 917 analyzer. The effects of interference by hemoglobin, conjugated and unconjugated bilirubin, lipemia (intralipid) and rheumatoid factor were evaluated. The samples were tested in triplicate and the median values were used to calculate recovery. The sponsor states that no significant interference criterion is a recovery of  $\pm 10\%$  of the initial value. No significant interference was found on the Hitachi 917 for hemoglobin up to 700 mg/dL, conjugated and unconjugated bilirubin up to 60 mg/dL, lipemia up to an L index of 700 (corresponds to turbidity, however the sponsor states in the labeling there is a poor correlation between L index and triglycerides) and rheumatoid factor up to 1200 IU/mL.

Eighteen other compounds tested: Acetylsteine, ampicillin Na, ascorbic acid, Ca-Dobesilate, Cefoxitin-Na, Liquemin Na-Heparin, Levodopa, L-a-Methyl dopa, Metronidazole, Phenylbutazone, Doxycycline HCl, Acetylsalicylic acid, Rifampicine, Acetaminophen, Ibuprofen, theophylline and cyclosporine were reported to have no significant interference or cross-reactivity at low levels of cystatin C.

Hook effect was evaluated by testing high dose analyte concentrations up to 31 mg/L on the Hitachi 917 analyzer. There was no high dose hook effect up to 20 mg/L. The sponsor states in the labeling that a high dose hook effect may occur at cystatin C concentrations  $> 20$  mg/L.

*f. Assay cut-off:*

Not applicable.

2. Comparison studies:

*a. Method comparison with predicate device:*

Ninety-four native human plasma (Li heparin) samples from a retrospective collection of banked frozen samples, including multiple donors from both hospitalized and healthy blood donors were measured with the DAKO application (predicate device) on the Hitachi 917 (X) and with the Roche Tina-quant Cystatin C (candidate device) on the Hitachi 917 (Y). The Roche

Tina-quant Cystatin C reagent is the same reagent as the DAKO Cystatin C reagent. The samples ranged from 0.61 to 6.05 mg/L and the results were calculated using the Passing/Bablok regression analysis. The correlation was  $Y=1.009x+0.019$  with a  $r^2= 0.9991$ .

*b. Matrix comparison:*

An anticoagulation study was conducted to validate the use of Li-heparin plasma with the Tina-quant Cystatin C assay. Forty six paired samples were analyzed in singlicate on the Hitachi 917 and the concentrations ranged from 0.53 to 6.66 mg/L. Each plasma sample was compared to the respective serum sample and the percent recovery was calculated. The sponsor's acceptance criterion was a deviation of less than 15%. The recoveries ranged from 93.09 to 113.89% and support the use of Li-heparin samples for the Roche Tina-quant Cystatin C assay.

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 sponsor conducted a reference range study to determine the reference range for the Tina-quant Cystatin C assay. Serum samples were measured in singlicate on the Hitachi 917 analyzer. Samples were drawn from healthy, non-hospitalized donors and the cystatin C results are shown in the table below.

Ref. Panel Cystatin C	sorted by age			sorted by gender	
	All	20-50 years	> 50 years	Male	Female
N	500	310	190	249	251
Min mg/L	0.408	0.466	0.408	0.472	0.408
Perc (2.5) mg/L	0.562	0.557	0.577	0.581	0.537
Median mg/L	0.729	0.703	0.777	0.746	0.716
Perc (97.5) mg/L	0.987	0.895	1.087	1.058	0.953
max mg/L	1.445	1.098	1.445	1.445	1.091

**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.