

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

A. 510(k) Number:

k071388

B. Purpose for Submission:

New Device

C. Measurand:

Cystatin C

D. Type of Test:

Quantitative, particle enhanced turbidimetric immunoassay

E. Applicant:

Gentian AS

F. Proprietary and Established Names:

Gentian Cystatin C Immunoassay

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1225

2. Classification:

Class II

3. Product code:

NDY

4. Panel:

Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indication for use below.

2. Indication(s) for use:

The Gentian cystatin c immunoassay is an in-vitro diagnostic test for quantitative determination of cystatin c in human serum and plasma. The measurement of cystatin c is used in the diagnosis and treatment of renal disease.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Performance characteristics was provided for the Roche Modular P Automated Analyzer and the Abbott Architect c8000 Automated Analyzer

I. Device Description:

The Gentian Cystatin C Immunoassay kit consists of the following:

1. Cystatin C immunoparticles: a purified immunoglobulin fraction that is directed against cystatin c attached to the polystyrene particles. It is provided as a ready to use suspension, preserved with 15 mmol/L sodium azide and antibiotics.
2. Cystatin C assay buffer: a MOPS[3-{N-Morpholino}-propanesulfonic acid] buffered saline, preserved with sodium azides and is ready to use.
3. Cystatin C calibrator: a delipidated human serum pool spiked with human cystatin c. It is preserved with antibiotics and is ready to use.
4. Cystatin C control set: consists of cystatin c control low and high. It is made from a delipidated human serum pool spiked with human cystatin c. It is preserved with antibiotics and is ready to use.

All human source materials were tested and found to be negative for HIV 1/2, HBsAg, and HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Dade Behring N Latex Cystatin C

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

k003503

3. Comparison with predicate:

Similarities and Differences between the candidate device and the predicate device		
Item	Gentian Cystatin C Immunoassay (Candidate device)	Dade Behring N Latex Cystatin C (Predicate)
Intended use	An <i>in vitro</i> diagnostic test for the quantitative determination of cystatin c in human serum and plasma. Cystatin c measurements are used in the diagnosis and treatment of renal diseases.	An <i>in vitro</i> diagnostic assay for the quantitative determination of cystatin c in human serum and heparinized plasma. Cystatin c measurements are used in the diagnosis and treatment of renal diseases.
Method	Particle enhanced turbidimetric immunoassay.	Particle enhanced immunonephelometry.
Instruments	Roche Modular P and Abbott Architect c8000	Dade Behring BN systems
Calibrators	Gentian cystatin c calibrator	N protein standard UV
Controls	Gentian cystatin c control set (2 levels)	N latex cystatin c control set (2 levels)
Measuring range	Roche Modular P analyzer: 0.37-8.4 mg/L Abbott Architect c8000 analyzer: 0.33-8.8 mg/L	Approx. 0.23-8 mg/L
Reference interval	0.52-0.98 mg/L	0.53-0.95 mg/L
Antibodies	Avian	Rabbit

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 Measurement Procedures: A Statistical Approach; Approved Guideline, Vol 23, No 16

CLSI EP9-A2, Method Comparison and Bias Estimation Using Patient samples; Approved Guideline-Second Edition, Vol 22, No 19

CLSI EP10-A2, Preliminary Evaluation of Quantitative Clinical Laboratory Methods; Approved Guideline-Second edition, Vol 22, No 29

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, Vol 24, No 34

CLSI C28-A, How to define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline-Second Edition, Vol 15, No 4

European Standard EN 13640, Stability testing of in vitro diagnostic reagents, 2002

L. Test Principle:

When cystatin c from the sample is mixed with the Gentian cystatin c immunoparticles, agglutination occurs. The created complex particles absorb light, and by turbidimetry the absorption is related to the cystatin c concentration via interpolation on an established standard calibration curve. The instrument platform used for Gentian cystatin c measurement will automatically calculate the results.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were designed according to the CLSI EP5-A guideline. Three serum pools and two control levels were measured twice a day in duplicate on the Roche Modular P analyzer for 20 days and on the Abbott Architect c8000 analyzer for 5 days (4 serum pools and 2 control levels were used). Results are summarized below:

1. Precision table for Roche Modular P analyzer:

Sample ID	Mean cys C (mg/L) (N=80)	Within- run (CV %)	Between -day (CV %)	Between -run (CV %)	Total precision (CV %)
Low serum	0.88	2.23	1.92	2.25	3.70
High serum	5.28	1.17	1.54	0.84	2.11
Medium serum	2.05	1.70	2.24	2.51	3.77
Low Control	1.17	1.81	1.25	3.54	4.17
High Control	3.23	1.68	1.45	1.33	2.58

2. Precision table for Architect c8000 analyzer:

Sample ID	Mean cys C (mg/L) (N=80)	Within- run (CV %)	Between -day (CV %)	Between -run (CV %)	Total precision (CV %)
High serum	5.71	1.12	2.23	3.35	4.18
Medium serum	3.38	1.93	1.62	2.77	3.75
Low serum	1.35	0.97	1.30	2.12	2.67
Low serum	0.69	1.51	1.07	2.11	2.81
Low Control	0.88	1.42	1.51	3.09	3.72
High Control	3.58	0.71	0.10	1.21	1.41

b. *Linearity/assay reportable range:*

A linearity study was performed according to the CLSI EP6-A guideline. Three different cystatin c serum samples (8.4, 6.3, 7.5 mg/L) were serially diluted and tested in duplicate on the Roche Modular P analyzer. One high serum sample of 8.77 mg/L was serially diluted and tested in duplicate on the Abbott Architect c8000 analyzer. Acceptance criterion was a difference between the expected values and the observed values of $\pm 12\%$. The linear regression analysis between the expected values and the observed values are shown as follows:

For Roche Modular P analyzer:

$$Y = 1.0366X - 0.0486, \text{ sample range tested} = 0.57 \text{ to } 8.4 \text{ mg/L}$$

For Abbott Architect c8000 analyzer:

$$Y = 1.0198X + 0.00034, \text{ sample range tested} = 0.63 \text{ to } 8.8 \text{ mg/L}$$

In addition, a low end linearity study was performed to determine the low end linearity range of the Gentian cystatin c assay on the Modular P and Architect c8000 analyzers. One serum sample of 1.53 mg/L cystatin c was serially diluted and tested in duplicate on the Roche Modular P analyzer. Another serum sample of 1.35 mg/L cystatin c was serially diluted and tested in

duplicate on the Abbott Architect c8000 analyzer. The linear regression analysis between the expected values and the observed values are shown as follows:

For Roche Modular P analyzer:

$$Y = 0.9546X - 0.0005, \text{ sample range tested} = 0.34 \text{ to } 1.53 \text{ mg/L}$$

For Abbott Architect c8000 analyzer:

$$Y = 1.018X - 0.02639, \text{ sample range tested} = 0.27 \text{ to } 1.33 \text{ mg/L}$$

The sponsor concluded that the linearity range of the Gentian cystatin c assay on the Modular P analyzer is 0.34 – 8.4 mg/L and on the Architect c8000 analyzer is 0.27-8.8 mg/L.

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

The Gentian cystatin c calibrator and control sets are calibrated by the use of pure human cystatin c isolated from human urine and have a value assignment traceable to an internal transfer protocol as recommended in ISO 17511 document. No international cystatin c reference material is available currently. Gentian cystatin c calibrator and control set are made from delipitated human serum pool spiked with human cystatin c.

The shelf life of the Gentian cystatin c immunoassay using the accelerated stability study of the reagents showed that the reagents are stable for at least 18 months when stored at 2-4°C. Real-time stability study is still on going.

The open vial stability and the calibration curve stability was evaluated and the open vial stability for the Gentian cystatin c immunoassay, calibrator, and controls is 9 weeks at 2-8°C. The calibration frequency is every 4 weeks.

Sample stability was also performed and the sponsor claimed that the sample is stable for at least 14 days at room temperature and 21 days at 2-8°C.

d. Detection limit:

A detection limit study was performed to assess the limit of detection for the Gentian cystatin c assay on the Roche Modular P analyzer and the Abbott Architect c8000 analyzer according to the CLSI EP17-A guidelines. LoD is defined as the smallest amount of an analyte that the method can reliably detect to determine presence or absence of the analyte. LoD was determined by measuring 60 replicates of a zero cystatin c solution (saline) and 21 replicates of 8 dilutions of a normal serum within 4x LoB. LoQ was defined as the lowest actual amount of an analyte that can be reliably detected and at which the total error meets the requirements for accuracy. The LoQ criterion was set to be the lowest cystatin c concentration corresponding to a CV below 6%. LoQ was conducted by running 3 additional dilutions of the same serum in replicates of 15. The calculated LoD for the Modular P analyzer is 0.039

mg/L and LoQ is 0.37 mg/L. The calculated LoD for the Architect c8000 analyzer is 0.031 mg/L and LoQ is 0.40 mg/L.

e. Analytical specificity:

Interference studies were designed according to the CLSI EP7-A guideline. Two pools of human serum with different concentrations of cystatin c were tested with the known interference substances and analyzed in triplicate on the Modular P and Architect c8000 analyzers. No significant interference was defined as the observed value <7.5 % of the control value for low cystatin c sample (<1 mg/L) and < 5% for high cystatin c sample(>1 mg/L).

i.)The sponsor claimed that there was no significant interference by the following interferents:

For the Roche Modular P analyzer:

- Hemoglobin up to 7 g/L
- Bilirubin up to 800 mg/L
- Triglycerides up to 12.5 mmol/L
- Intralipid up to 16 g/L

For the Architect c8000 analyzer:

- Hemoglobin up to 8 g/L
- Bilirubin up to 420 mg/L
- Triglycerides up to 15 mmol/L
- Intralipid up to 11 g/L

ii.) The sponsor claimed that there was no significant interference by the following drugs and anticoagulants:

Summary of drug interference table:

Drug	Test concentration (mg/L)	Low cystatin C sample			High cystatin C sample		
		Acceptable difference (mg/L)	Observed difference (mg/L)	CI observed difference (mg/L)	Acceptable difference (mg/L)	Observed difference (mg/L)	CI observed difference (mg/L)
Acetaminophen	200	0.072	0.00	-0.064-0.064	0.28	-0.043	-0.17-0.083
Acetylcysteine	150	0.071	-0.003	-0.0192-0.024	0.26	-0.14	-0.25-(-0.034)
Acetylsalicylic acid	1000	0.072	0.013	-0.049-0.076	0.28	-0.02	-0.147-0.107

		Low cystatin C sample			High cystatin C sample		
Drug	Test concentration (mg/L)	Acceptable difference (mg/L)	Observed difference (mg/L)	CI observed difference (mg/L)	Acceptable difference (mg/L)	Observed difference (mg/L)	CI observed difference (mg/L)
Ampicillin	1000	0.071	0.003	-0.018-0.023	0.26	0.023	-0.026-0.072
Ascorbic Acid	300	0.071	0.02	0.0-0.040	0.26	-0.048	-0.148-0.053
Ca Dobesilate	200	0.071	-0.01	-0.027-0.007	0.26	0.005	-0.10-0.11
Cefoxitin	2500	0.071	0.01	-0.045-0.065	0.26	0.023	-0.109-0.154
Cyclosporine	5	0.072	0.01	-0.041-0.061	0.28	-0.05	-0.148-0.042
Doxycycline	50	0.071	0.003	-0.015-0.020	0.26	0.045	-0.12-0.21
Heparin	5000U	0.071	0.01	-0.005-0.025	0.26	-0.01	-0.077-0.057
Ibuprofen	500	0.071	0.01	-0.012-0.032	0.27	-0.045	-0.27-0.18
Levodopa	20	0.071	0.01	-0.005-0.025	0.26	-0.003	-0.11-0.11
Methyldopa	200	0.071	-0.005	-0.039-0.029	0.26	-0.125	-0.27-0.020
Metronidazole	200	0.060	-0.007	-0.052-0.039	0.24	-0.07	-0.25-0.11
Phenylbutazone	400	0.072	0.013	-0.040-0.066	0.28	0.01	-0.071-0.091
Rifampicin	60	0.071	0.003	-0.032-0.037	0.27	0.053	-0.089-0.19
Theophylline	100	0.073	-0.015	-0.075-0.046	0.27	-0.018	-0.21-0.18

Summary of anticoagulant interference table:

		Low cystatin C sample			High cystatin C sample		
Anticoagulant	Test concentration	Acceptable difference	Observed difference	CI observed difference	Acceptable difference	Observed difference	CI observed difference
Heparin	1000 U/ml	0.063	-0.037	-0.091-0.017	0.21	-0.103	-0.37-0.17
Disodium EDTA	2 g/L	0.065	0.00	-0.013-0.013	0.21	0.06	-0.033-0.153
Sodium Citrate	38 g/L	0.065	-0.033	0.090-0.024	0.21	-0.15	-0.35-0.059
Sodium Fluoride	10 g/L	0.063	0.003	-0.057-0.063	0.21	0.15	-0.13-0.42

		Low cystatin C sample			High cystatin C sample		
Anticoagulant	Test concentration	Acceptable difference	Observed difference	CI observed difference	Acceptable difference	Observed difference	CI observed difference
Potassium Oxalate	2 g/L	0.063	-0.07	-0.062-0.009	0.21	0.05	-0.22-0.32

iii.) Rheumatoid factor- The sponsor claimed that there is no RF interference in the Gentian cystatin c immunoassay because the antibodies that they used were originated from chicken egg (avian IgG should not cross react with mammalian IgG). The above information is listed in the Interference section of the package insert with the following reference: Larsson A et al: Poultry Science 1993; 72:1807-1812.

iv.) Hook effect- The hook effect of the Gentian cystatin C immunoassay was determined by using a spiked serum sample (80 mg/L) and making a serial dilution and tested on the Modular P analyzer in duplicate. The sponsor claimed that there is no hook effect observed in concentration above 16 mg/L.

v.) Carry over- Sample carry over of the Gentian cystatin C immunoassay was evaluated on the Modular P analyzer and the Architect c8000 analyzer. The acceptance criteria were set to $\leq 2.5\%$, precision $\leq 5\%$, and a non-significant Student t-test. The experiment was designed based on CLSI EP10-A2 protocol. The total sample carry over was calculated to be 0.10% on Modular P and 0.33% on Architect c8000 analyzer.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison study was performed based on the CLSI EP9-A2 guideline. Dade-Behring cystatin c method on the BN ProSpec analyzer was used to compare the results performed using the Gentian cystatin c immunoassay on the Roche Modular P and Abbott Architect analyzers. 74 serum samples were used for the comparison between the Modular P and BN ProSpec analyzers. 87 serum samples were used for the comparison between the Architect and BN ProSpec analyzers. Samples concentration range tested for Modular P and Architect were 0.59 -7.47 mg/L and 0.52-7.91 mg/L respectively. The method used to fit the linear regression line was Passing Bablok. A summary of the regression statistics is provided below:

Passing-Bablok regression	Term	Coefficient	95% CI of Coefficient
Modular P vs. BN ProSpec N=74 0.59 -7.47 mg/L Architect vs. BN ProSpec N=87 0.52-7.91 mg/L	Slope	1.010	0.984 to 1.043
	Intercept	-0.030	-0.058 to 0.003
	Slope	0.983	0.959 to 1.005
	Intercept	-0.062	-0.099 to 0.023

b. Matrix comparison:

A matrix comparison study was conducted using 63 paired serum and EDTA plasma samples from the same individuals. Testing was performed using the Gentian cystatin c assay on the Modular P analyzer and Dade-Behring cystatin c method on BN ProSpec analyzer. Results were analyzed using the Passing Bablock regression and linear regression. Sample range tested was 0.46 – 0.91 mg/L. The acceptance criteria are: the plasma sample regression analysis falls within the 95% confidence interval of the slope and intercept of the serum analysis. Results are summarized as follows:

	N	R	Slope	Slope 95% CI	Intercept	Intercept 95% CI
Passing Bablock	63	0.96 (Pearson)	1.00	0.955 to 1.074	0.00	-0.050 to -0.031
Linear regression	63	0.96	0.973	0.898 to 1.047	0.018	-0.033 to 0.069

The sponsor states in the labeling that serum and EDTA plasma samples can be used for the Gentian cystatin c determination.

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):

An instrument variation study was performed to detect the instrument variations between Roche Modular P analyzer and Abbott Architect c8000 analyzer.

74 serum samples were run on the Roche Modular P analyzer and Abbott

Architect c8000 analyzer. The Passing Bablock regression analysis yielded the following correlation equation: $Y = 0.999X - 0.019$, $r=0.9991$, sample range tested = 0.61-7.50 mg/dL.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

In the labeling, the reference range is stated as 0.53 – 0.95 mg/L.*

*The reference range is based on the literature, on a reference interval study performed by Dade Behring (predicate device) and referred to in the Dade Behring N Latex Cystatin C Assay Package Insert. This reference interval was determined from a population of ostensibly healthy subjects with no history of renal disease. A total of 413 samples obtained from 194 males and 219 females ranging in age from 1 to 78 years were tested at three evaluation sites. The reference interval was calculated non-parametrically and represents the central 95% of the population tested. The sponsor claims that they followed the CSLI C28-A guideline to determine the transferability of the reference range.

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.