510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K242585

B Applicant

SENTINEL CH. S.p.A.

C Proprietary and Established Names

Cystatin C

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NDY	Class II	21 CFR 862.1225 – Creatinine Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Cystatin C

C Type of Test:

Quantitative turbidimetric method

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Cystatin C assay is an in vitro diagnostic test used in the quantitative immunoturbidimetric determination of cystatin C in human serum and plasma on the Alinity c system.

Measurement of cystatin C aids in the diagnosis and treatment of renal diseases.

For laboratory professional use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Alinity c system

IV Device/System Characteristics:

A Device Description:

The Cystatin C assay reagent kit is available in two configurations:

	List Number	
	06T3220	**06T3230**
Tests per cartridge	100	250
Number of cartridges per kit	2	2
Tests per kit	200	500
Reagent (R1)	20.8 mL	46.5 mL
Reagent (R2)	7.5 mL	13.7 mL

Each Cystatin C cartridge contains 2 ready to use liquid reagents (R1 and R2). R1 contains buffer, stabilizers, and preservative (sodium azide (< 0.1%)). R2 contains latex particles coated with rabbit IgG against human cystatin C (0.09%), buffer, stabilizers, and preservative (sodium azide (< 0.1%)).

Materials required but not supplied with the reagent kit include:

- Cystatin C assay file
- Cystatin C calibrators
- Cystatin C controls or other control material containing cystatin C
- Saline (0.85% to 0.90% NaCl) for specimen dilution

B Principle of Operation:

The Cystatin C assay is a particle-enhanced turbidimetric immunoassay (PETIA) to measure cystatin C levels in serum and plasma. Latex particles coated with anti-human cystatin C antibody agglutinate when mixed with sample containing human cystatin C. The change in absorbance due to agglutination of the reaction mixture is proportional to the quantity of human cystatin C in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Tina-quant Cystatin C Gen.2

B Predicate 510(k) Number(s):
K161817

C Comparison with Predicate(s):

Device & Predicate Device(s):	K242585	K161817
Device Trade Name	Cystatin C	Roche Tina-quant Cystatin C Gen.2
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative determination of cystatin C in human serum and plasma as an aid in the diagnosis and treatment of renal diseases.	Same
Methodology	Immunoturbidimetric	Same
Standardization	ERM-DA471/IFCC	Same
General Device Characteristic Differences		
Measuring Range	Analytical Measuring Interval (AMI): 0.30-10.00 mg/L Extended Measuring Interval (EMI): 10.00 – 40.00 mg/L	Measuring Range: 0.40 – 6.80 mg/L Extended Measuring Range: 6.80 – 10.20 mg/L

Hook Effect	No prozone effect up to 40.00 mg/L	No prozone effect up to 12 mg/L

VI Standards/Guidance Documents Referenced:

Clinical and Laboratory Standards Institute (CLSI) EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures.

CLSI EP06 2nd Edition. Evaluation of the Linearity of Quantitative Measurement Procedures.

CLSI EP07 3rd Edition. Interference Testing in Clinical Chemistry.

CLSI EP09c 3rd Edition. Measurement Procedure Comparison and Bias Estimation Using Patient Samples.

CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

CLSI EP25 2nd Edition. Evaluation of Stability of In Vitro Medical Laboratory Test Reagents.

CLSI EP28-A3c. Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory.

CLSI EP34. Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking.

CLSI EP35 1st Edition. Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures.

CLSI EP37 1st Edition. Supplemental Tables for Interference Testing in Clinical Chemistry.

ISO 17511 2nd Edition. In vitro diagnostic medical devices – Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Internal 20-day Precision Study

An internal precision study for the Cystatin C assay was conducted following the recommendations in the CLSI EP05-A3 guideline.

Two controls and 4 human serum panels with cystatin C concentrations spanning the analytical measuring range were tested. Each sample was tested in 2 replicates per run, 2 runs per day for 20 days using 1 lot of Cystatin C reagents, 1 lot of Cystatin C Calibrators, 1 lots of Cystatin C Controls and 1 instrument. The result of the study is shown in the table below. Within-laboratory imprecision includes within-run (repeatability), between-run, and between-day variability components.

Sample	N	Mean (mg/L)	Repeatability		Between-Run		Between-Day		Within-Laboratory	
			SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV
Control Level 1	80	0.81	0.01	1.0	0.01	0.9	0.01	1.0	0.01	1.7
Control Level 2	80	4.11	0.03	0.6	0.02	0.4	0.03	0.6	0.04	1.0
Panel A (native)	80	0.49	0.01	1.7	0.00	0.6	0.00	0.0	0.01	1.8
Panel B (native)	80	0.92	0.01	0.8	0.00	0.0	0.00	0.4	0.01	0.9
Panel C (native)	80	5.89	0.03	0.5	0.01	0.1	0.02	0.3	0.03	0.6
Panel D (supplemented)	80	8.95	0.07	0.8	0.03	0.4	0.05	0.5	0.09	1.0

Reproducibility Study

A multi-day precision study for the Cystatin C assay was conducted following the recommendations in the CLSI-EP05-A3 guideline.

A panel of five samples with cystatin C concentrations spanning the analytical measuring range were tested using two (2) reagent lots at three sites. Each sample was assayed in replicates of 4 per run, 2 runs per day for 5 days per reagent lot at each site. The results of the three sites combined are shown in the table below. Within-laboratory imprecision includes within-run (repeatability), between-run, and between-day variability components. Reproducibility includes within-laboratory, between-lot and between-instrument (site) variability components.

Sample	N	Mean (mg/L)	Repeatability		Between-Run		Between-Day		Within-Laboratory	
			SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV
Control level 1	240	0.81	0.01	1.5	0.01	1.1	0.00	0.4	0.02	2.0
Control level 2	240	4.08	0.02	0.6	0.02	0.4	0.02	0.5	0.04	0.9
Panel 1 (native)	240	0.49	0.02	5.0	0.01	1.1	0.01	1.8	0.03	5.4
Panel 2 (native)	240	0.93	0.01	1.3	0.00	0.3	0.01	0.8	0.01	1.6

Sample	N	Mean (mg/L)	Repeatability		Between-Run		Between-Day		Within-Laboratory	
			SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV	SD (mg/L)	%CV
Panel 3 (supplemented)	240	8.80	0.09	1.0	0.04	0.5	0.08	1.0	0.13	1.5

Sample	N	Mean (mg/L)	Between-Lot		Between-Instrument (site)		Overall Reproducibility	
			SD (ng/L)	%CV	SD (ng/L)	%CV	SD (ng/L)	%CV
Control level 1	240	0.81	0.00	0.0	0.00	0.3	0.02	2.0
Control level 2	240	4.08	0.02	0.4	0.02	0.5	0.04	1.1
Panel 1 (native)	240	0.49	0.00	0.0	0.00	0.3	0.03	5.4
Panel 2 (native)	240	0.93	0.00	0.5	0.00	0.0	0.02	1.6
Panel 3 (supplemented)	240	8.80	0.06	0.6	0.00	0.0	0.14	1.6

2. Linearity:

The linearity of the Cystatin C assay was evaluated following the recommendations in the CLSI EP06 2nd Edition guideline.

Linearity was evaluated with three (3) serum sample sets using two (2) reagent lots on two (2) instruments (a total of 12 linearity data set). For each sample set, thirteen levels of serum samples ranging from approximately 0.13 mg/L to 10.76 mg/L were prepared by mixing different proportions of a high cystatin C-containing serum sample pool and a cystatin C-free sample pool.

Each sample in each sample set was measured in replicates of four (4) per reagent lot and instrument combination. The mean of these replicates was compared to the expected values. The data was analyzed using a weighted least square linear regression. Deviations from linearity were never observed to be greater than 9.7% within the claimed analytical measuring range from 0.30 mg/L to 10.00 mg/L for all twelve sets of linearity data.

Prozone Effect

A study was conducted to confirm that no false results will be reported due to prozone effect. A serum sample containing 40.00 mg/L of cystatin C was diluted with cystatin C free serum to create 11 levels of samples. Each sample was analyzed in 4 replicates on one Alinity c instrument using one reagent lot. The results support the labeling claim of no prozone interference for undiluted samples containing up to 40.00 mg/L of cystatin C.

Dilution Recovery

A study was performed to support that samples can be automatically diluted by the instrument or manually diluted using a 1:4 dilution ratio.

3. Analytical Specificity/Interference:

The analytical specificity of the Cystatin C assay was evaluated following the CLSI EP07-3rd Edition guideline.

Interference from endogenous and exogenous substances was assessed using two serum samples with cystatin C at concentrations of 0.79 mg/L and 3.95 mg/L. Two (2) instruments with three (3) reagent, calibrator, and control lot combinations were used in this study. Each serum sample was further divided into two groups: i.e., a test sample (with added interferent) and a control sample (without interferent). The difference and % difference between the mean concentration of the test and control sample was calculated.

At the following concentrations, the difference between the test samples and the control samples was within ±10%.

Potential Interfering Endogenous Substances

Substances	Highest level tested that demonstrated no significant interference
Bilirubin conjugated	60 mg/dL
Bilirubin unconjugated	60 mg/dL
Rheumatoid Factor	550 IU/mL
Hemoglobin	1000 mg/dL
Total protein	10.2 g/dL
Triglycerides	1500 mg/dL

Potential Interference Exogenous Substances

Substances	Highest level tested that demonstrated no significant interference
Acetaminophen	250 mg/L
Acetylcysteine	150 mg/L
Acetylsalicylic Acid	1000 mg/L
Ampicillin – Na	1000 mg/L
Ascorbic Acid	300 mg/L
Biotin	3510 ng/mL
Calcium-dobesilate	200 mg/L
Cefoxitin	6600 mg/L
Cyclosporine	5 mg/L
Doxycycline	50 mg/L
Ibuprofen	500 mg/L
Levodopa	20 mg/L

Methyldopa	22.5 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Rifampicin	60 mg/L
Sodium Heparin	10 U/mL
Theophylline (1, 3-dimethylxanthine)	100 mg/L

Interference was observed at the concentrations shown below for the following substances:

Substance	Interferent Level	Analyte Level	Interference (95% CI)
Rheumatoid factor	1200 IU/mL	0.79 mg/L	0.19 mg/L (0.16 mg/L, 0.22 mg/L)
Total Protein	15 g/dL	0.79 mg/L	0.27 mg/L (0.26 mg/L, 0.28 mg/L)
Total Protein	15 g/dL	3.95 mg/L	18.5% (18%, 18.9%)

The following limitations are listed in the labeling:

- Samples with elevated total protein may cause falsely elevated results.
- In a very rare cases anomalous values for cystatin C may be obtained from samples taken from patients who have been treated with rabbit antibodies or have developed anti-rabbit antibodies [1].
- Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays [2].

[1] Kricka L. J. Human anti-animal antibody interferences in immunological assays. Clin Chem 1999; 45 (7): 942-956.
[2] Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988; 34 (1): 27-33.

4. Assay Reportable Range:

   0.30 to 10.00 mg/L.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability

   The Cystatin C assay on Alinity c system is aligned to the certified reference material ERM-DA471/IFCC. The Cystatin C Calibrators are manufactured gravimetrically and are referenced to European Reference Material ERM-DA471/IFCC at each concentration.

Sample Stability

The sponsor has provided information to support the following sample stability claims for serum and lithium heparin plasma tubes:

- 2 days at room temperature (15 to 25ºC)
- 7 days at 2-8ºC
- 30 days at -20ºC

Avoid more than 2 freeze-thaw cycles.

Reagent On-board Stability

The sponsor has provided information to support that the Cystatin C reagent is stable for 45 days on-board.

Calibration Stability

The sponsor has provided information to support that the calibration is stable for approximately 45 days for the same reagent lot. Re-calibration is required with each change in reagent lot.

6. Detection Limit:

Limit of blank (LoB), limit of detection (LoD), and limit of Quantification (LoQ) studies for the Cystatin C assay were conducted following the recommendations from CLSI EP17-A2 guideline using two instruments, three lots of calibrators, three lots of reagents and three lots of controls for a total of six instrument/reagent/calibrator/control combinations.

Limit of Blank (LoB)

The LoB was determined by analyzing four blank samples. Each sample was measured in replicates of five per day, over three days for a total of 60 determinations per instrument/reagent/calibrator/control combination and the LoB was determined non-parametrically as the 95^{th} percentile of the 60 determinations. The maximum LoB value obtained across the instrument/reagent/calibrator/control combination is reported as the LoB for the candidate device.

Limit of Detection (LoD)

The LoD was determined by analyzing four human serum pools with low concentration of cystatin C. Each sample was measured in replicates of 5 each day, over three days for a total of 60 determinations per instrument/reagent/calibrator/control combination. The LoD was determined using the parametric approach described in EP17-A2. The maximum LoD value obtained across the instrument/reagent/calibrator/control combination is reported as the LoD for the candidate device.

Limit of Quantification (LoQ)

The LoQ was determined by analyzing two different sample panels. Each sample panel had eleven serum samples with low cystatin C concentrations. Each level of sample was measured in replicates of ten per day, over three days per instrument/reagent/calibrator/control combination. The LoQ was determined as the lowest concentration of analyte concentration with total error (TE) less than or equal to 25%.

The results of the studies are shown below:

LoB (mg/L)	LoD (mg/L)	LoQ (mg/L)	Observed Total Error at LoQ
0.03	0.05	0.30	23.2%

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was conducted following recommendations from CLSI EP09c 3rd Edition guideline to compare the Cystatin C assay to a comparator device. A total of 161 native serum samples were tested using one lot of Cystatin C assay reagent and one lot of calibrator on one Alinity c system and one predicate device test system. Each sample was tested in replicates of 2 using both methods. The first replicate result from the candidate device was compared to the mean result from the predicate device. A Passing-Bablok regression analysis was performed using the first replicate result from the candidate device and the mean result from the predicate device. The results are summarized in the table below:

Analyte (Units)	Matrix	N	Sample Concentration Range by Comparator (mg/L)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient r (95% CI)
Cystatin C	Serum	161	0.60 -8.20	1.03 (1.02,1.05)	-0.07 (-0.10, 0.04)	1.00 (1.00, 1.00)

2. Matrix Comparison:

A matrix comparison study was conducted following the CLSI EP35 1st Edition guideline to demonstrate the equivalence between vacutainer serum separator tube (SST), lithium heparin, lithium heparin separator tube, sodium heparin, K_2EDTA, K_3EDTA (test tube) and serum plasma tube (reference tube) using two reagent lots. Matched samples were tested on the Cystatin C assay using the same reagent lot. The first replicate of the candidate tube type was compared against the mean of the serum tube results. Passing-Bablok regression analysis was conducted for each tube type relative to the comparator tube type (serum). The representative results of the analysis, including the slope with 95% confidence interval (CI), the intercept with 95% CI, and the correlation coefficient, are shown below.

Matrix	N	Test Range (serum) (mg/L)	Slope (95% CI)	Intercept (95% CI) (mg/L)	R
Serum Gel Separator vs Serum	50	0.6 – 9.315	1.031 (1.023 – 1.048)	-0.0238 (-0.0398 – 0.01512)	1.000
Plasma Li-Heparin vs Serum	50	0.6 – 9.315	1.067 (1.063 – 1.071)	-0.0459 (-0.05199 – -0.03981)	1.000
Plasma Li-Heparin gel vs Serum	50	0.6 – 9.315	1.067 (1.055 – 1.099)	-0.05667 (-0.07818 – -0.04311)	1.000
Plasma Na-Heparin vs Serum	50	0.6 – 9.315	1.033 (1.029 – 1.040)	-0.02569 (-0.03229 – 0.0201)	1.000
Plasma K_2EDTA vs Serum	50	0.6 – 9.315	1.016 (1.000 – 1.027)	-0.01846 (-0.02953 – -0.0050)	1.000
Plasma K_3EDTA vs Serum	50	0.6 – 9.315	0.969 (0.9615 – 0.9752)	-0.01312 (-0.02237 - -0.0004)	1.000

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

The reference range study was performed based on the recommendations from the CLSI EP28-A3c guideline. The testing was conducted on apparently healthy individuals from a US population, including 105 females and 145 males, with an estimated glomerular filtration rate (eGFR) > 80 (mL/min/1.73 m2) and an age range from 18 to 69 years. The samples were tested using 2 instruments, 2 reagent lots and 1 calibrator lot. The reference range was determined using a non-parametric approach from the 2.5% to the 97.5% percentile and gave a cystatin C reference range of 0.59 mg/L to 1.28 mg/L.

The sponsor recommends that "each laboratory determine its own reference range based upon its particular locale and population characteristics."

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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