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

A. 510(k) Number:
k093960

B. Purpose for Submission:
New device

C. Measurand:
Cystatin C

D. Type of Test:
Quantitative fluorescent immunoassay

E. Applicant:
Tosoh Bioscience, Inc.

F. Proprietary and Established Names:
ST AIA-PACK Cystatin C
ST AIA-PACK Cystatin C CALIBRATOR SET

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NDY	Class II	21 CFR § 862.1225	Clinical Chemistry (75)
JIT	Class II	21 CFR § 862.1150	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   ST AIA-PACK Cystatin C is designed for *in vitro* diagnostic use only for the quantitative measurement of cystatin C in human serum, heparinized plasma or EDTA plasma on TOSOH AIA System Analyzers. Cystatin C measurement is used as an aid in the diagnosis and treatment of renal disease.

   The ST AIA-PACK Cystatin C CALIBRATOR SET is intended for *in vitro* diagnostic use only for the calibration of the ST AIA-PACK Cystatin C assay.

2. Indication(s) for use:
   See intended use above

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   TOSOH AIA-1800

I. Device Description:

The ST AIA-PACK Cystatin C consists of the following components:
ST AIA-PACK Cystatin C test cups which contain 12 magnetic beads coated with anti-cystatin C mouse monoclonal antibody and lyophilized anti-cystatin C mouse monoclonal antibody conjugated to bovine alkaline phosphatase with preservative.

The AIA-PACK Cystatin C Calibrator Set is sold separately.

AIA-PACK Cystatin C Calibrator Set: All calibrators are liquid and ready to use. The calibrators contain bovine serum albumin with a preservative. The calibrators, unlike the patient samples, are not diluted prior to being assayed and contain the following concentrations of cystatin C: Calibrator 1 (0 mg/L), Calibrator 2 (0.01 mg/L), Calibrator 3 (0.04 mg/L), Calibrator 4 (0.08 mg/L), Calibrator 5 (0.16 mg/L) and Calibrator 6 (0.35 mg/L).

All human source materials used in the preparation of kit components were tested and found to be negative for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies by FDA approved methods.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Dade Behring N Latex Cystatin C Test Kit

2. Predicate 510(k) number(s):
   k041878

3. Comparison with predicate:

Item	Device	N Latex Cystatin C Test Kit
Similarities		
Intended use	The quantitative measurement of cystatin C to be used as an aid in the diagnosis and treatment of renal disease.	Same
Differences		
Methodology	Quantitative fluorescent immunoassay	Quantitative particle-enhanced immunonephelometry
Sample types	Serum, heparinized plasma and EDTA plasma	Serum or heparinized plasma
Calibrators	6 calibrators	Multiple point

Item	Device	N Latex Cystatin C Test Kit
		calibration
Calibration frequency	90 days	14 days
Control materials	No control material provided – sponsor recommends the use of commercially available control materials	2 levels of control material

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

- Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition (CLSI EP5-A2)
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)
- Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (CLSI EP9-A2)
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A)
- Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline (CLSI EP21-A)
- Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline (C28-A3)

L. Test Principle:

The ST AIA-PACK Cystatin C is a two-site quantitative fluorescent immunoassay which is performed entirely in the ST AIA-PACK Cystatin C test cups. Cystatin C present in the test sample is bound with monoclonal antibody immobilized on magnetic beads and enzyme-labeled monoclonal antibody. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the cystatin C concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The precision study was performed according to the CLSI guideline EP5-A2. The first study was performed on three levels of unaltered serum and heparinized plasma (Na-heparin) specimens using one AIA-1800 instrument, one lot of reagents, one operator and one calibration cycle for a total number of 80 per sample. The results of the study are summarized below:

Sample type	n	Mean (mg/L)	Within Run		Total	
			Pooled SD (mg/L)	%CV	Pooled SD (mg/L)	%CV
Serum	80	0.70	0.023	3.3	0.026	3.7
	80	2.15	0.043	2.0	0.056	2.6
	80	4.69	0.175	3.7	0.160	3.4
Heparinized plasma	80	0.69	0.018	2.5	0.022	3.1
	80	2.08	0.057	2.7	0.063	3.0
	80	4.69	0.154	3.3	0.160	3.4

A second precision study was performed on a set of unaltered serum, heparinized plasma (Na-heparin) and EDTA plasma specimens (three levels of each) using two AIA-1800 instruments, two lots of reagents, three operators and one calibration cycle for a total number of 160 per sample. The results of the study are summarized below:

Sample type	N	Mean (mg/L)	Within Run		Total	
			Pooled SD (mg/L)	%CV	Pooled SD (mg/L)	%CV
Serum	160	1.15	0.017	1.5	0.036	3.1
	160	1.75	0.037	2.1	0.050	2.9
	160	5.11	0.108	2.1	0.180	3.5
Heparinized plasma	160	1.10	0.019	1.7	0.042	3.8
	160	1.77	0.037	2.1	0.055	3.1
	160	4.93	0.076	1.5	0.151	3.1
EDTA plasma	160	0.88	0.013	1.5	0.045	5.1
	160	1.65	0.039	2.4	0.060	3.6
	160	4.87	0.099	2.0	0.163	3.3

b. Linearity/assay reportable range:

The linearity study was performed according to the CLSI guideline EP6-A. Low and high serum, heparinized plasma and EDTA plasma specimens were selected. The low specimen was prepared by 1:10 dilution of a higher serum, heparinized plasma or EDTA plasma specimen using the sample dilution solution. The high specimen was unaltered serum, heparinized plasma or EDTA plasma.

The intermediate concentrations were made by mixing the high and low samples at constant intervals. A total of 11 specimens each for serum, heparinized plasma and EDTA plasma ranging from 0.1 – 8.0 mg/L were assayed on the AIA-1800 in 4 replicates. The data was analyzed using linear regression as well as second and third order non-linear fitted polynomial regression. The third order model fit the data better than the linear model. However, the difference between the linear and non-linear model (within the claimed interval) was within ± 10 %. Furthermore, the % recovery and the

coefficient of variability at each dilution were within ± 10 %.

Therefore, the sponsor determined the linearity of the proposed assay to be from 0.1 to 8.0 mg/L which is the same as the reportable range.

Dilution Studies:
To determine recovery following the recommended dilution ratio for samples that read above the measuring range, 5 unaltered serum, 5 heparinized plasma, and 5 EDTA plasma samples were spiked with cystatin C (ranging from approximately 8 to 40 mg/L) and were automatically diluted onboard the instrument (as recommended in the package insert). All samples fell within ± 10 % of the expected value.

Recovery Studies:
Three serum, 3 heparinized plasma, and 3 EDTA plasma with different concentrations of cystatin C were spiked with 3 different levels of cystatin C. Each sample was assayed in triplicate before and after spiking. The percent recovery was calculated (measured value/expected value) x 100. The percent recovery for all samples fell within ± 10 % of the expected value.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no international recognized reference standard for cystatin C. The calibrators are traceable to internal cystatin C standards.

The calibrators are value assigned using the mean of multiple replicates of each calibrator on multiple analyzers using multiple ST AIA-PACK Cystatin C assays. The protocols for value assignment and validation of value assignment were reviewed and found to be acceptable.

Real-time stability studies were performed. The closed vial stability of the calibrators was determined to be 12 months when stored at 8 to 12 ºC. The open vial stability of the calibrators was determined to be 1 day when stored at 8 to 12 ºC.

Calibrations were determined to be stable for 90 days.

d. *Detection limit:*
The detection limit studies were developed with reference to the CLSI Guideline EP17-A. To define the limit of the blank (LoB), a blank sample was measured in 60 replicates. A non-parametric principle based on ordered values was used since values less than zero are not reported. LoB was determined to be 0.0011mg/L.

LoD: To determine the LoD, the standard deviation of sample measurements (SDs) was obtained from 10 measurements of 6 low level samples. Pooled CV and SD were calculated for the 6 samples. LoD was calculated using the

formula: LoD = LoB + cβ x SDs. LoD was determined to be 0.0012 mg/L where cβ was derived from the 95th percentile of the standard Gaussian distribution (and the correction factor).

LoQ: The test results from the LoD study were used to calculate the bias and imprecision for that level of the analyte. Pooled SDs and total bias were calculated. The TE was then determined using the formula: TE= Bias +/- 2SDs. The TE was determined to be 0.0004 mg/L. The goal for the TE was set as 0.005 mg/L. Since the TE was lower than the goal, the LoD was equal to the LoQ. LoQ was determined to be 0.0012 mg/L.

Since the linearity of the proposed assay was demonstrated to be from 0.1 to 8.0 mg/L the sponsor claims 0.1 to 8 mg/L as the reportable range of the assay.

e. Analytical specificity:

Interference studies were performed on serum, heparinized plasma and EDTA plasma samples. Test samples (with approximately 0.6, 2 and 4 mg/L cystatin C concentrations) were spiked with the potentially interfering substances and analyzed in triplicate at each level. The sponsor defined interference as recovery outside of 10% of known specimen concentrations. The following potential interfering compounds were evaluated and found not to interfere with the assay:

- Hemoglobin (up to 470 mg/dL)
- Conjugated bilirubin (up to 18 mg/dL)
- Free bilirubin (up to 19 mg/dL)
- Triglyceride (up to 1600 mg/dL)
- Human albumin (up to 5g/dL)
- Ascorbic acid (up to 20 mg/dL)
- Rheumatoid factor (up to 1300 IU/mL)

Hook Effect:

The sponsor demonstrated that no hook effect was observed with cystatin C concentrations up to 121 mg/L.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The methods comparison study was developed with reference to the CLSI Guideline EP9-A2. A total of 140 well-characterized serum specimens were assayed in singleton utilizing the proposed device and the predicate device. A combination of fresh and frozen specimens was utilized for this study. The results of the linear regression including the 95% confidence interval (CI) are

summarized below:

	Slope (95% CI)	Intercept (95% CI)
Deming:	0.984 (0.972 to 0.996)	0.072 (0.037 to 0.106)
Regular:	0.981 (0.969 to 0.993)	0.077 (0.043 to 0.111)

Correlation Coefficient (R): 1.00
Result Range: 0.26 to 7.99 mg/L

b. Matrix comparison:
A total of 143 serum and EDTA plasma specimens were assayed in singleton utilizing the proposed device. The results of the regression analysis, including the 95% confidence interval (CI) are summarized below:

	Slope (95% CI)	Intercept (95% CI)
Deming:	0.987 (0.980 to 0.993)	0.018 (0.001 to 0.036)
Regular:	0.986 (0.980 to 0.993)	0.020 (0.002 to 0.037)

Correlation Coefficient (R): 1.00
Result Range 0.10 to 7.87 mg/L

A total of 117 serum and heparinized (sodium heparin) plasma specimens were assayed in singleton utilizing the proposed device. The results of the regression analysis, including the 95% confidence interval (CI) are summarized below:

	Slope (95% CI)	Intercept (95% CI)
Deming:	0.981 (0.972 to 0.990)	0.015 (-0.005 to 0.035)
Regular:	0.980 (0.971 to 0.989)	0.017 (-0.003 to 0.037)

Correlation Coefficient (R): 1.00
Result Range 0.10 to 7.92 mg/L

The sponsor claims that EDTA plasma and sodium heparin plasma are acceptable anti-coagulants to be used with the 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:
   A total of 149 well-characterized serum specimens were assayed utilizing the proposed device on the AIA-1800 analyzer. The specimens were collected from apparently healthy and ambulatory males and females between the ages of 19 and 73 years. The specimens were representative of a United States population. The reference range specimens had a normal distribution and the central 95% range was defined as the reference interval. The reference range for ST AIA-PACK Cystatin C is 0.50 to 1.16 mg/L.

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.