SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k111452

B. Purpose for Submission:

New device

C. Measurand:

Soluble ST2

D. Type of Test:

Quantitative sandwich monoclonal ELISA

E. Applicant:

Critical Diagnostics

F. Proprietary and Established Names:

1. Presage ST2 Assay Kit
2. Presage ST2 Assay Kit Controls

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OYG, ST2 Assay	Class II	21 CFR 862.1117, Test, Natriuretic Peptide	Clinical Chemistry (75)
JJX, Quality Control Material	Class I, reserved	21 CFR 862.1660 Quality Control Material	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

The Critical Diagnostics Presage® ST2 Assay kit is an *in vitro* diagnostic device that quantitatively measures ST2 in serum or plasma by enzyme-linked immunosorbant assay (ELISA) in a microtiter plate format. The Presage® ST2 Assay is indicated to be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure.

The Presage® ST2 Assay Kit Controls, Level 1 and Level 2, are designed to be used for monitoring the performance of test procedures on the Critical Diagnostics Presage® ST2 Assay kit.

3. Special conditions for use statement(s):

   For prescription use only.

   The Presage ST2 Assay is intended to be used only in patients with chronic heart failure and should not be used for diagnosis of heart failure.

4. Special instrument requirements:

   Microtiter plate shaker capable of shaking plate at 750 rpm.

   96-well microplate reader capable of reading at 450 nm.

I. Device Description:

1. The Presage® ST2 Assay kit is provided in microplate configuration (96 tests). The content of the kit includes:

Item	Qty	Description
Plate	1 plate	Ready-to-use microtiter plate coated with mouse monoclonal anti-human ST2 monoclonal antibodies (96 wells in 12x8 strips).
Standard	2 vials	Recombinant human ST2 Standard Calibrator (lyophilized) (8 ng/vial which corresponds to a range of standards of 3.1 to 200.0 ng/ml when used with specimens diluted 1:50)
Standard Diluent		ST2 Standard Diluent (fetal bovine serum, tissue culture grade) (13 ml)
Biotinylated Antibody	1 bottle	Anti ST2 Biotinylated Antibody Reagent in phosphate buffered saline (13 ml)
Sample Diluent	1 bottle	ST2 Sample Diluent (50 ml) (MOPS buffered)
Tracer Concentrate	1 bottle	100X Streptavidin-HRP Conjugate Concentrate (100X) (0.2 ml)
Tracer Diluent	1 bottle	Streptavidin-HRP Conjugate Diluent (MOPS buffer) (13 ml)
Wash Concentrate	1 bottle	20X Wash Buffer (potassium phosphate buffer with NaCl and Tween 20) (50 ml)
TMB Reagent	1 bottle	Tetramethylbenzidine Reagent (11 ml)
Stop Solution	1 bottle	Stop Solution of diluted HCl (11 ml)

2. Presage ST2 Assay Controls are packaged separately and contain:

   Two levels of controls are provided in a sealed, lyophilized vial format. The base matrix of the controls is delipidized human serum. The lower control (C1) is formulated with a target ST2 concentration between 18.8 ng/ml and 35.0 ng/ml. The higher control (C2) is formulated with a target ST2 concentration between 65.3 ng/ml and 105.0 ng/ml.

   The sponsor stated in the labeling that all donor units of serum used in the preparation of Controls were tested by FDA-approved method and found negative for HIV (HIV I/II antibody), HBV (HBsAg) and HCV (antibody).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BG Medicine Galectin-3 Assay

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

   k093758

3. Comparison with predicate:

Characteristic	**New device** **Presage ST2 Assay**	**Predicate Device** **Galectin-3** (k093758)
Intended Use	For the in vitro quantitative determination of soluble ST2 protein in human serum and plasma.	Quantitative measurement of galectin-3 in serum or EDTA-plasma.
Indication for Use	The assay is used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure.	same
Assay Type	Sandwich ELISA	same
Analyte	ST2	Galectin-3
Antibody	Mouse monoclonal antibody pair	Rat and mouse monoclonal antibody pair
Detection Method	Spectrophotometry Absorbance at 450 nm	same
Reference standards	Critical Diagnostics Presage ST2 Assay Control materials	BGM Galectin-3 Quality Control materials
Reportable Range	3.1-200.0 ng/ml	1.4 - 94.8 ng/ml

Characteristic	New device Presage ST2 Assay	Predicate Device **Galectin-3** (k093758)
Analytical Sensitivity	1.8 ng/ml (Limit of Detection) 2.4 ng/ml (Limit of Quantitation)	1.13 ng/ml (Limit of Detection) 1.32 ng/ml (Limit of Quantitation)
Sample Volume	0.020 ml	0.030 ml
Sample Type	Serum or plasma (EDTA or heparin plasma)	EDTA plasma and serum
Hook Effect	No high dose effect up to 200.0 ng/ml	No high dose effect up to 500 ng/ml
Calibration Interval	Calibration performed with each analysis	same

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

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline
- CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach: Approved Guideline
- CLSI EP7-A: Interference Testing in Clinical Chemistry; Approved Guideline
- CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
- CLSI EP25-P: Evaluation of Stability of In Vitro Diagnostic Products; Proposed Guideline
- CLSI C28-A3: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition

L. Test Principle:

ST2 (for growth STimulation expressed gene 2; also known as IL1RL1, or Interleukin-1 Receptor-Like 1) is a member of the interleukin-1 receptor family. The ST2 protein has two isoforms directly implicated in the progression of cardiac disease: soluble ST2 and a cell membrane-bound isoform, ST2L. When soluble ST2 levels are low, ST2's ligand, IL-33, is available to bind to ST2L and has a cardioprotective effect resulting in preserved cardiac function. However, when soluble ST2 levels are high, soluble ST2 competitively binds to IL-33, making it less likely to bind to ST2L and thereby making IL-33 unavailable for cardioprotective signaling. As a result, the heart is subjected to greater stress in the presence of high levels of soluble ST2, leading to cellular death and tissue fibrosis, reduced cardiac function, and increasing the rate of disease progression (Kakkar & Lee 2008; Seki et al. 2009).

The Critical Diagnostics Presage ST2 Assay is a quantitative sandwich monoclonal ELISA in

a 96-well microtiter plate format for measurement of soluble ST2 in serum or plasma (EDTA or heparin). A mouse monoclonal anti-human ST2 antibody is coated onto the surface of the microtiter plate wells and acts as the capture antibody to bind ST2 molecules in solution. A second mouse monoclonal anti-human ST2 antibody is biotinylated and functions as the tracer antibody for detecting ST2 molecules that have bound to the capture antibody. After binding of Streptavidin-HRP to the tracer antibody, a substrate is added which yields a blue color that absorbs at 450 nm. The absorbance is proportional to the ST2 levels in the specimens. The test results of the specimens are read from the standard curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Study Protocol:
Assay precision was evaluated according to CLSI EP5-A2 Guidelines at two CLIA-certified clinical laboratories as well as the sponsor's internal lab, for a total of 3 sites. EDTA plasma pools at 3 different ST2 concentrations, one in the normal reference range (low), one near the 35 ng/ml cutpoint (medium) and one at an elevated concentration (high) were prepared from plasma purchased from a commercial vender. A set of 20 aliquots of each pool was prepared and shipped to each testing site so that all 3 sites analyzed the same test materials and all used assay materials from the same lot of kits. All 3 sites followed the same test protocol performing 2 runs per day with 4 replicates of each test sample per run for 20 consecutive days.

Result Summary:
Results of within-run and total precision were summarized in the below tables.

Precision Analysis Results from Site 1

Test Pool	N	Mean ST2 (ng/ml)	Within Run		Between Runs		Between Days		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
Low	152	20.2	0.95	4.7%	1.40	7.0%	0.98	4.9%	1.96	9.7%
Medium	160	32.1	1.73	5.4%	1.94	6.0%	0.95	3.0%	2.76	8.6%
High	160	76.1	3.63	4.8%	4.84	6.4%	1.71	2.2%	6.05	8.0%

Precision Analysis Results from Site 2

Test Pool	N	Mean ST2 (ng/ml)	Within Run		Between Runs		Between Days		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
Low	160	21.6	0.73	3.4%	0.40	1.8%	0.72	3.3%	1.10	5.1%
Medium	160	36.7	0.98	2.7%	0.91	2.5%	0.83	2.3%	1.57	4.3%
High	160	87.6	1.72	2.0%	0.95	1.1%	3.08	3.5%	3.66	4.2%

Precision Analysis Results from Site 3

Test Pool	N	Mean ST2 (ng/ml)	Within Run		Between Runs		Between Days		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
Low	152	20.0	1.44	7.2%	1.81	9.0%	0.64	3.2%	2.40	12.0%
Medium	152	30.2	2.36	7.8%	1.64	5.4%	1.83	6.1%	3.40	11.3%
High	144	79.0	3.34	4.2%	5.71	7.2%	3.84	4.9%	7.65	9.7%

b. Linearity/assay reportable range:

Study Protocol:
Linearity was evaluated following CLSI guideline EP6-A. A direct dilution series of 11 test samples was prepared by mixing the high patient plasma pool and low patient plasma pool obtained from a commercial source at different ratios. The high patient pool was prepared at a ST2 concentration slightly greater than the 200.0 ng/ml upper limit of the standard curve and the low concentration pool had a mean concentration of 3.2 ng/ml, a value just above the proposed lower range of 3.1 ng/mL. Each test sample was measured in replicates of 4.

Result Summary:
Data from the linearity analysis demonstrated that the tested replicates were highly consistent with CV values of <5% across the entire concentration range and linear with coefficient of $R^2 = 0.999$, liner regression of Y=0.99 X-0.97

Conclusion:
The result support the sponsor claimed measuring range of 3.1 to 200.0 ng/ml, which is the full dynamic range of the assay calibration curve.

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

Traceability and value assignment:

Calibrators

Calibrators (Standard included in each assay kit, provided lyophilized at 400 ng/vial) are prepared using purified recombinant ST2 protein. Concentration is determined by Bradford protein assay and confirmed using the Presage ST2 Assay. The calibrator is normalized to a "gold standard" stock of the initial preparation of recombinant ST2 protein that was used as the antigen to generate hybridomas of the ST2 antibody.

Controls

Controls are produced at 2 target concentrations using recombinant ST2 protein in delipidized human serum matrix. The lower control (C1) is formulated with a target ST2 concentration between 18.8 ng/ml and 30.0 ng/ml and corresponds to a normal concentration range. The higher control (C2) is formulated with a target ST2 concentration between 65.0 ng/ml and 85.0 ng/ml and corresponds to the concentration range of patients with severe cardiac disease and worse prognosis. Lot-specific ranges are assigned by testing a minimum of 3 vials per lot, each vial in duplicate, over 3 days. The average of all control values for

each level is calculated and reported on the Certificate of Analysis along with the ± 10% performance range.

Stability:

The Presage ST2 Assay Kit and Presage ST2 Controls were evaluated for storage stability at various temperatures and for successively longer periods of time, up to 12 months. Stability study protocol and the acceptance criteria have been reviewed and found to be acceptable.

- *Presage ST2 Assay Kit*

  Shelf-life Stability:

  Real-time testing supports a shelf life of 12 months when stored at 2-8°. However, exposure to elevated temperatures, 37°C or above, for even a brief time (1 day) or a prolonged period (⩾4 months) of exposure to room temperature results in compromised performance of the product as evaluated by the characteristics of the standard curve, specifically the decrease in standard curve slope over time. Consequently, the sponsor stated in the labeling that care should be taken to ensure that the product is always maintained at refrigerated temperatures until ready to use, including the use of cold packs during shipment.

  Open-Vial Stability:

  Not applicable.

- *Presage ST2 Control*

  Shelf-life Stability:

  Testing has demonstrated a closed bottle shelf-life of 1 year, stored at 2-8ºC.

  Open-Vial Stability:

  Testing has demonstrated an open bottle stability of 7 days after reconstitution, capped at 2-8ºC

- *Test Sample*

  Stability of samples stored at different conditions was evaluated using 12 plasma samples collected from the ER department of a hospital. Baseline ST2 plasma concentrations were determined immediately after blood collection, and at the same time the plasma samples were aliquoted into 1.5 ml plastic tubes which were stored at the specified temperatures for various amount of time. % Recovery from the baseline were calculated for each sample and plotted against time of storage. The sponsor's acceptance criterion for analyte stability was that the mean % recovery of the 12 plasma samples is >=90%.

  Analyte freeze/thaw stability was evaluated at 0, 5, 10 and 15 cycles using 5

commercial EDTA plasma samples. Linear regression analysis showed that none of the samples tested resulted in a slope that significantly deviated from the baseline over the course of 15 freeze/thaw cycles and had an average CV of 4%. This analysis confirms that ST2 is not significantly affected by sample freeze/thaw cycles.

Based on the testing results, the sponsor made the following recommendation in the labeling:

Storage conditions	**Sample stability**
-20°C and -80°C	18 months
4°C	7 days
20°C	48 hours

The sponsor also stated that "ST2 in EDTA-plasma is not significantly affected by sample freeze/thaw cycles and has been shown to be stable for 15 freeze-thaw cycles."

d. Detection limit:

- Analytical sensitivity was determined per CLSI EP17-A.

  For LoB determination, calibrator diluent (fetal bovine serum), was measured over 4 days, with 16 replicates per day giving 64 determinations in total. LoB was calculated as $LoB = \mu_B + 1.645\ \sigma_B$ where μB and σ_B are the mean and standard deviation values respectively.

  For LoD determination, 60 replicates of 4 different low ST2 concentration human plasma samples (were measured in sets of 15 replicates each over 4 consecutive days. LoD was calculated as $LoD = LoB + 1.645\sigma_S$

  The same sample measurements used for the LoD determination were used to estimate bias and imprecision. LoQ was calculated as LoQ = bias +2xSDs

  The resulting values from this analysis are summarized in the following table.

Parameter	ST2 Value (ng/ml)
Limit of Blank (LoB)	0.5
Limit of Detection (LoD)	1.8
Limit of Quantitation (LoQ)	2.4

- The sponsor claimed that the assay has a measuring range from 3.1 to 200 ng/mL.

e. Analytical specificity:

- Interference

Interference testing was performed on 5 most common endogenous substances i.e. total protein (BSA); triglycerides; hemoglobin; cholesterol; and bilirubin as well as 49 common therapeutic substances.

Endogenous Substances

Three human EDTA plasma pools with low (~15 ng/ml); medium (~25 ng/ml); and high (~100 ng/ml) concentrations of ST2 were used in this study. Potentially interfering test substances were added at low and high levels to each plasma pool and assayed in sets of 8 replicates. The sponsor defines no significant interference as <10% difference between the spiked and the control (solvent only) samples. No significant interference was observed on any of the substances/concentrations tested as summarized in the below table.

Endogenous Substances	Concentration tested (mg/dL)	
Total Protein (BSA)	1500	6000
Hemoglobin	100	200
Bilirubin	10	30
Cholesterol (total)	150	500
Triglycerides (total)	150	3000

Based on these result, the sponsor concluded in the labeling that no significant interference was observed from: Bilirubin < 30 mg/dl, Hemoglobin < 200 mg/dl, Triglycerides < 3000 mg/dl, Cholesterol < 500 mg/dl, Total protein (BSA) <6000 mg/dl.

Therapeutic Substances

A plasma sample pooled from 5 normal individuals was used in the testing of 49 common therapeutic substances. Each substance was tested at two concentrations per CLSI EP7-A2 recommendations or at 1x and 5x normal therapeutic levels if the substances are not described in CLSI EP7-A2. The mean of 8 replicate measurements was used to calculate the deviation between the spiked and the solvent only plasma samples. The sponsor defines no significant interference as <10% difference between the spiked and the control samples. Among the 49 commonly used drugs tested, 6 substances had nominal >10% deviations from the controls and are tested further using plasma pools with ST concentrations at low (~15 ng/ml), medium (~35 ng/ml) and high (~90 ng/ml). None of the compounds tested showed an effect of >=10% change from the control in any of the plasma pools.

Based on these result, the sponsor concluded in the labeling that no significant interference was observed from the commonly used therapeutic substances as listed in the below Table.

Acetaminophen	Cinnarizine	Heparin sodium	Propranolol hydrochloride

Acetylsalicyclic acid	Cyclosporin A	Hydralazine hydrochloride	Quinidine
Allopurinol	Digitoxin	Hydrochlorothiazide	Simvastatin
Ambroxol	Digoxin	Indomethacin	Spironolactone
Amiodarone	Diltiazem	Lisinopril	Sulfamethoxazole
Amlodipine besylate	Dopamine	Nicotine	Theophylline
Ampicillin	Dipyridamole	Nifedipine	L-Thyroxine
Ascorbic Acid	Enalapril Maleate	Nitrofurantoin	Trimethoprim
Atenolol	Eplerenone	Oxytetracycline	Verapamil hydrochloride
Bivalirudin	Erythromycin	Phenytoin	Warfarin
Caffeine	Fluvastatin	Pravastatin Sodium	
Captopril	Furosemide	Probenecid	
Chloramphenicol	Glyburide	Procainamide hydrochloride	

- Cross-Reactivity

  Study Protocol:
  ST2 is recognized to be structurally and genetically related to the interleukin-1 receptor class of molecules but has not been shown to exhibit significant sequence homology (Tominaga et al. 1989). To confirm that the ST2 assay does not cross react with proteins from this class of receptors, a representative set of three (3) molecules was tested, including: 1) human interleukin-1 soluble receptor type I (IL-1 sR-1); 2) recombinant human IL-Iα; 3) recombinant human IL-Iß". Each molecule was tested at equimolar and 5x molar concentrations of ST2. Evaluations at each test concentration were performed in replicates of 8.

  Results and Conclusion:
  Based on the results summarized in the below table, the sponsor concluded that none of the three molecules tested, at concentrations 1x and 5x the measured ST2 concentration, exhibited measurable cross reactivity. A summary of the cross reactivity test is presented in the below table.

	substance concentration (pg/ml)	Ave ST2 (ng/ml)	SD	CV	% deviation
solvent (PBS)	0	18.9	0.9	4.6%	
IL-1 sR-1	18,700	19.3	0.7	3.4%	2.0%
	98,500	18.5	0.6	3.0%	2.1%
IL-1 alpha	6,120	19.0	0.4	2.1%	0.4%
	30,600	19.1	0.7	3.6%	0.6%
IL-1 beta	5,780	19.7	0.5	2.7%	3.7%
	28,900	19.8	0.5	2.6%	4.3%

f. Assay cut-off:

See Clinical Cutoff in 3 c below.

2. Comparison studies:

a. Method comparison with predicate device:

See clinical studies in 3 c below

b. Matrix comparison:

Study Protocol:

The Presage ST2 assay has been validated for use with plasma or serum samples. Matched sets of native samples from 40 donors were obtained from healthy donors and hospital emergency department patients. The reported ST2 values for each sample and for each matrix were obtained from single measurements.

Results of linear regressions:

Matrix Y	Matrix X	Slope (95% CI)	Intercept (95% CI)	R^2	N	ST2 range (ng/mL)
Lithium heparin plasma	Serum	0.96 (0.94 to 0.98)	0.32 (-0.58 to 1.2)	0.9965	40	3.1-200.0
K3-EDTA plasma	Serum	1.01 (0.99-1.03)	-0.27 (-1.2 to 0.6)	0.9969		

3. Clinical studies:

a. Clinical Sensitivity:

See 3 (c) below

b. Clinical specificity:

See 3 (c) below

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

The ST2 analysis cutpoint of 35 ng/mL was selected by choosing a Presage ST2 Assay concentration value above the 90th and below the 95th percentile of the Reference Group (See section M [5] Expected values/Reference range below for further details).

The prognostic performance of ST2 at 35 ng/mL cutoff was evaluated in the following study:

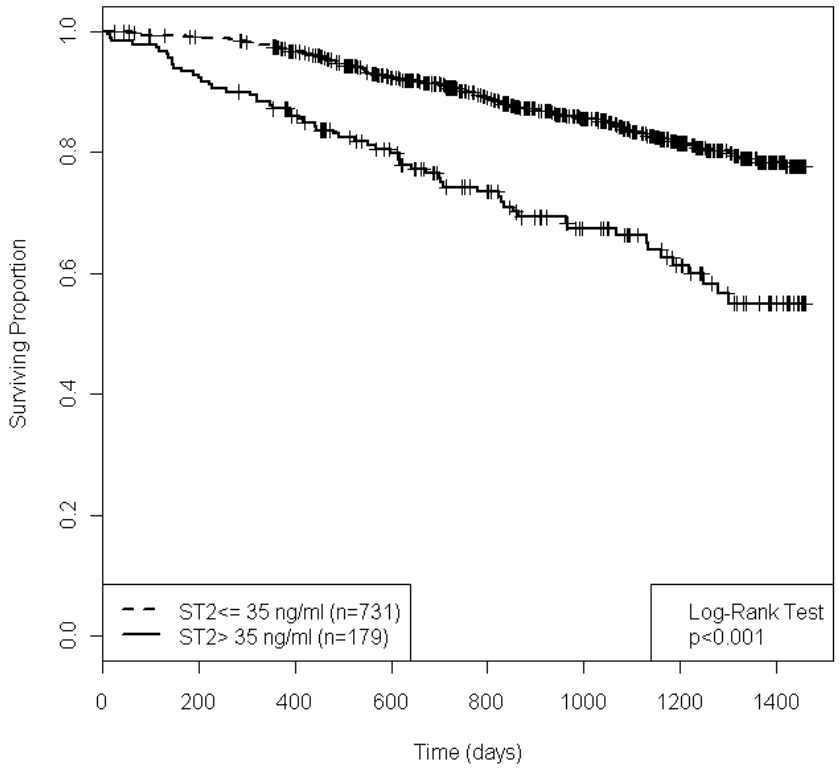
***Description of study cohort*:**

The intended use of the Presage® ST2 Assay as an aid in assessing the prognosis of patients diagnosed with chronic heart failure was established in an analysis of the HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise TraiNing) study. HF-ACTION was a multicenter, randomized, controlled, blinded trial of 2,331 patients with chronic heart failure that compared patients in the exercise training group vs. patients in the usual care group. Patients were randomized from April 2003 through February 2007 within the United States, Canada, and France. A complete description of the design of HF-ACTION has been published previously (Whellan et al. 2007). Plasma samples from a total of 912 patients from this cohort were available for biomarker analysis. Sensitivity analysis was performed comparing the set of 912 HF-ACTION subjects having evaluable ST2 values with all other HF-ACTION participants, and it was found that the clinical validation results based on the evaluable set of subjects were robust and representative of the larger study population.

***Results*:**

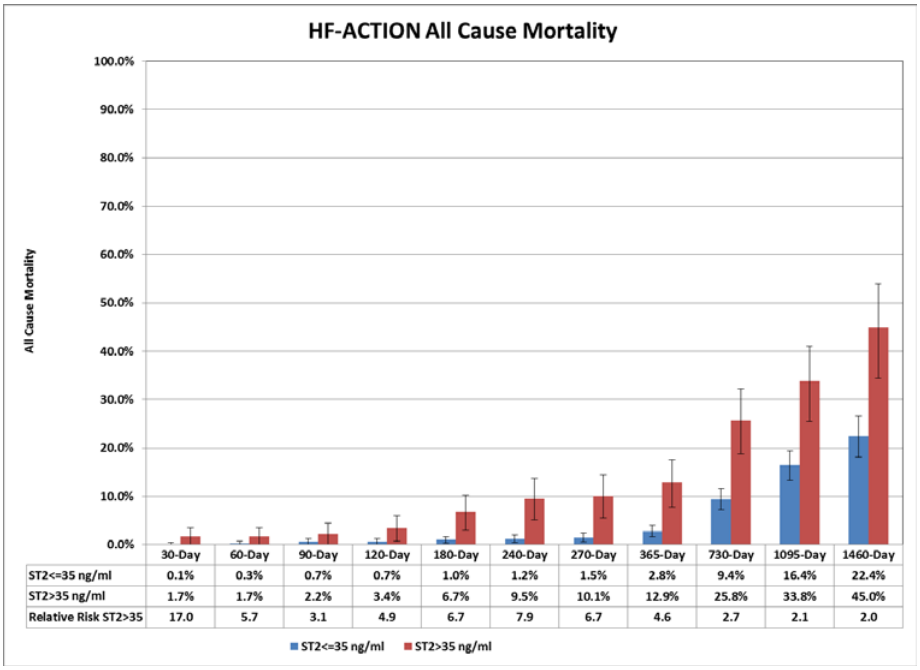
- A Kaplan-Meier analysis using the cutpoint of 35 ng/ml in the HF-ACTION Study is presented in Figure 1. Mortality risk is higher in patients with ST2 >35 ng/ml (log rank p<0.001).

Figure 1. Kaplan-Meier Analysis
ST2 Value >35 ng/ml As a Predictor of All Cause Mortality in the HF-ACTION study.



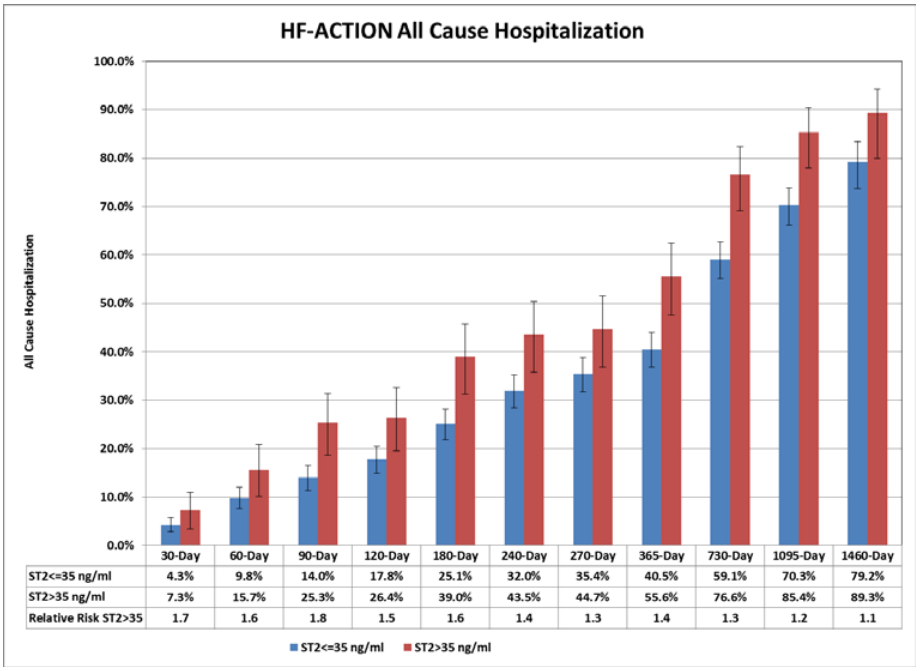
- ST2 is also prognostic for clinical outcomes in addition to all-cause mortality. Figures 2 through 4 summarize a quantitative analysis of ST2 at 35 ng/ml as a predictor of all-cause mortality, all-cause hospitalization, cardiovascular mortality, and cardiovascular hospitalization, respectively.

Figure 2: ST2 Relationship to Time of All Cause Mortality Event



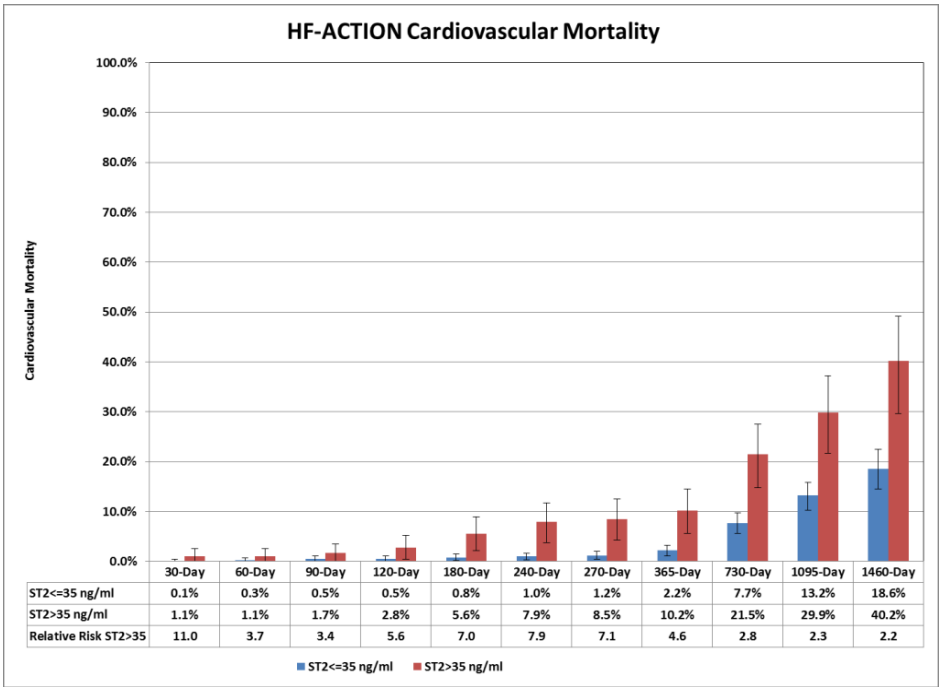
	30-Day	60-Day	90-Day	120-Day	180-Day	240-Day	270-Day	365-Day	730-Day	1095-Day	1460-Day
ST2<=35 ng/ml	0.1%	0.3%	0.7%	0.7%	1.0%	1.2%	1.5%	2.8%	9.4%	16.4%	22.4%
ST2>35 ng/ml	1.7%	1.7%	2.2%	3.4%	6.7%	9.5%	10.1%	12.9%	25.8%	33.8%	45.0%
Relative Risk ST2>35	17.0	5.7	3.1	4.9	6.7	7.9	6.7	4.6	2.7	2.1	2.0

Figure 3: ST2 Relationship to Time to All Cause Hospitalization Event



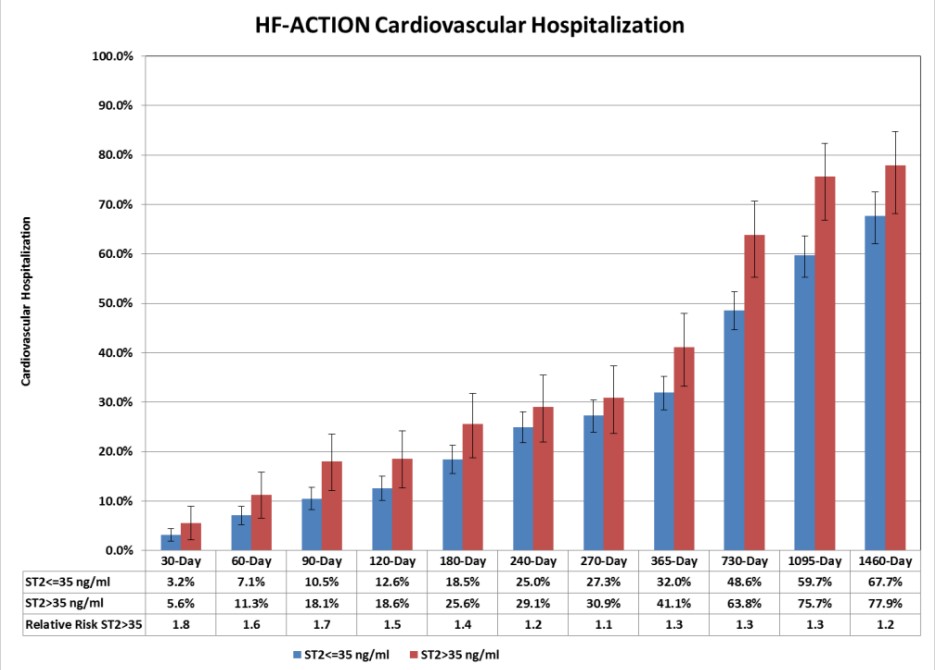
	30-Day	60-Day	90-Day	120-Day	180-Day	240-Day	270-Day	365-Day	730-Day	1095-Day	1460-Day
ST2<=35 ng/ml	4.3%	9.8%	14.0%	17.8%	25.1%	32.0%	35.4%	40.5%	59.1%	70.3%	79.2%
ST2>35 ng/ml	7.3%	15.7%	25.3%	26.4%	39.0%	43.5%	44.7%	55.6%	76.6%	85.4%	89.3%
Relative Risk ST2>35	1.7	1.6	1.8	1.5	1.6	1.4	1.3	1.4	1.3	1.2	1.1

Figure 4: ST2 Relationship to Time to CVD Mortality Event



	30-Day	60-Day	90-Day	120-Day	180-Day	240-Day	270-Day	365-Day	730-Day	1095-Day	1460-Day
ST2<=35 ng/ml	0.1%	0.3%	0.5%	0.5%	0.8%	1.0%	1.2%	2.2%	7.7%	13.2%	18.6%
ST2>35 ng/ml	1.1%	1.1%	1.7%	2.8%	5.6%	7.9%	8.5%	10.2%	21.5%	29.9%	40.2%
Relative Risk ST2>35	11.0	3.7	3.4	5.6	7.0	7.9	7.1	4.6	2.8	2.3	2.2

Figure 5: ST2 Relationship to Time to CVD Hospitalization Event



	30-Day	60-Day	90-Day	120-Day	180-Day	240-Day	270-Day	365-Day	730-Day	1095-Day	1460-Day
ST2<=35 ng/ml	3.2%	7.1%	10.5%	12.6%	18.5%	25.0%	27.3%	32.0%	48.6%	59.7%	67.7%
ST2>35 ng/ml	5.6%	11.3%	18.1%	18.6%	25.6%	29.1%	30.9%	41.1%	63.8%	75.7%	77.9%
Relative Risk ST2>35	1.8	1.6	1.7	1.5	1.4	1.2	1.1	1.3	1.3	1.3	1.2

As illustrated in Figures 2-5 absolute event rates in patient with ST2 >35 ng/ml for the endpoints of all-cause mortality, all-cause hospitalization, CVD mortality, and CVD hospitalization are higher than for those patients with ST2 ≤35 ng/ml. These results are summarized in the below Table. Duration of follow-up in this study is 4

years.

Outcome	ST2 ⩽35 ng/ml	ST2 >35 ng/ml
All-Cause Mortality within 1 year	2.7%	12.8%
All-Cause Mortality within study	15.2%	33.5%
All-Cause Hospitalization within study	65.0%	79.9%
CVD Mortality within study	12.2%	28.5%
CVD Hospitalization within study	53.9%	72.21%

* Study follow up period is 4 years.

- *Multivariate Covariate Risk-Adjusted Analysis*

  Using Cox proportional hazards models of ST2 as a dichotomous marker at 35 ng/ml shows that ST2 is a statistically significant predictor of all clinical endpoints assessed in this study i.e. all-cause mortality within 1 year, all-cause mortality within study, all-cause hospitalization within study, death due to CVD within study, and hospitalization due to CVD within study. Results from Multivariate Covariate Risk-Adjusted Analysis are summarized in the below table. Variables in this adjusted model include age, gender, NYHA class, eGFR, LVEF, diabetes status, hypertension status and smoking status. Importantly the prognostic utility of the Presage ST2 Assay is not adversely influenced by the common confounders; age, gender and renal function (eGFR).

  Table. Summary of Risk-Adjusted Cox Proportional Hazards Prediction Models for ST2 at 35 ng/ml

Outcome	HR (95% CI)	P
All-Cause Mortality within 1 year	3.20 (1.66-6.19)	<0.001
All-Cause Mortality within study	1.76 (1.26-2.46)	<0.001
All-Cause Hospitalization within study	1.35 (1.10-1.65)	<0.043
CVD Mortality within study	1.87 (1.29-2.69)	<0.001
CVD Hospitalization within study	1.33 (1.06-1.66)	0.0128

* Study follow up period is 4 years. Variables adjusted include age, gender, NYHA class, eGFR, LVEF, diabetes status, hypertension status and smoking status.

- Use of ST2 in Conjunction with Natriuretic Peptides

  ST2 and natriuretic peptides, such as NT-proBNP, are measures of separate and distinct biological processes. ST2 and natriuretic peptides provide independent and complementary prognostic information. Table below summarizes an analysis of ST2, at 35 ng/ml, and NT-proBNP, at the study median value of 852 pg/ml, for assessment of risk of mortality within the study follow-up period.

Table. Additive Value of ST2 and NT-proBNP

Category	Death Rate	HR (95% CI)	p
ST2 ≤35 ng/ml NT-proBNP ≤Median	9.0%	1	NA
ST2 ≤35 ng/ml NT-proBNP >Median	23.3%	2.87 (1.9 – 4.32)	<0.001
ST2 >35 ng/ml NT-proBNP ≤Median	22.2%	2.70 (1.25 – 5.84)	0.0115
ST2 >35 ng/ml NT-proBNP >Median	38.9%	5.59 (3.61 – 8.66)	<0.001

4. Clinical cut-off**:**

The ST2 analysis cutpoint of 35 ng/mL was selected by choosing a Presage ST2 Assay concentration value above the 90th and below the 95th percentile of the Reference Group (See section M [5] Expected values/Reference range below for further details).

5. Expected values/Reference range:

Soluble ST2 concentrations representative of the general population were determined from 490 individuals (245 women and 245 men) in a reference group of donors from two sources selected for this purpose. Donor group one was comprised of 240 individuals from a reference laboratory. Donor group two was comprised of 250 individuals recruited by a commercial vender. All donors were self declared to be healthy with no history of heart disease. No other inclusion or exclusion criteria were applied. Demographic information collected on these donors is summarized in the Tables below. In summary this population included apparently healthy individuals, selected without regard to diabetes, hypertension, pulmonary disease, and renal insufficiency status.

Reference Group: Males and Females

Presage® ST2 Assay (ng/ml)	Reference Group - All						
Metric	**All**	**<45**	**45-54**	**55-64**	**65-74**	**>=75**	**<75**
Mean	20.9	21.7	18.8	20.7	20.4	22.8	20.8
SD	9.3	9.7	8.4	8.3	9.3	10.2	9.3
Median	18.8	20.0	17.4	18.8	18.9	19.3	18.8
90th percentile	34.2	35.4	30.0	32.5	33.9	37.4	34.2
% > 35 ng/ml	9.0	10.8	6.5	6.2	6.8	12.5	8.8
N	490	249	93	65	59	24	466

Reference Group: Males

Presage® ST2 Assay (ng/ml)	Reference Group - Males Only						
Metric	**All**	**<45**	**45-54**	**55-64**	**65-74**	**>=75**	**<75**
Mean	24.9	25.9	22.8	23.6	23.9	28.6	24.8
SD	10.1	10.4	9.9	9.1	10.1	9.1	10.1
Median	23.6	24.5	20.7	22.1	21.9	29.5	23.4
90th percentile	37.1	37.7	35.5	34.8	35.5	39.4	36.9
% > 35 ng/ml	16.3	19.4	14.0	9.4	13.3	18.2	16.2
N	245	129	43	32	30	11	234

Reference Group: Females

Presage® ST2 Assay (ng/ml)	Reference Group - Females Only						
Metric	**All**	**<45**	**45-54**	**55-64**	**65-74**	**>=75**	**<75**
Mean	16.9	17.1	15.4	17.8	16.8	18.0	16.8
SD	6.2	6.3	4.7	6.4	6.8	8.7	6.1
Median	16.2	16.0	14.9	17.4	17.5	15.6	16.4
90th percentile	23.7	23.7	21.8	24.4	22.8	20.2	23.7
% > 35 ng/ml	1.6	1.7	-	3.0	-	7.7	1.3
N	245	120	50	33	29	13	232

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.