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

A. 510(k) Number:

k093758

B. Purpose for Submission:

New device

C. Measurand:

Galectin-3

D. Type of Test:

Quantitative enzyme-linked immunosorbant assay (ELISA)

E. Applicant:

BG Medicine, Inc.

F. Proprietary and Established Names:

BG Medicine, Inc. Galectin-3 Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1117, Test, Natriuretic Peptide

2. Classification:

   Class II

3. Product code:

   OSX, Galectin-3 in vitro diagnostic assay

4. Panel:

   75, Chemistry

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The BGM Galectin-3 Assay is an *in vitro* diagnostic device that quantitatively measures galectin-3 in serum or plasma by enzyme-linked immunosorbant assay (ELISA) on a microtiter plate platform. BGM Galectin-3 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 (HF).

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   96-well microtiter plate reader capable of reading at 450 nm

I. Device Description:

The Galectin-3 Assay is an *in vitro* diagnostic device that contains the microtiter plate, reagents, controls and standards required to perform analyses on serum or EDTA plasma samples. The Galectin-3 Assay contents are listed in the table below:

Quantity	Name	Description
1 plate	Plate	Ready-to-use microtiter plate coated with rat monoclonal anti- mouse galectin-3 monoclonal antibody
1 bottle	Assay diluent	Phosphate buffered saline with 1% bovine serum albumin
1 bottle	TMB substrate	Tetramethyl benzidine (15 mL)
1 bottle	Stop solution	0.5 M sulfuric acid (10 mL)
2 bottles	Wash buffer concentrate	0.5M tris buffered saline (2 x 50 mL); (10X concentrate)
1 bottle	Tracer concentrate	Horseradish peroxidase (HRP) labeled mouse monoclonal anti- human galectin-3 monoclonal antibody (0.45 mL)
2 vials	Standard	Recombinant human galectin-3 12 ng per vial (lyophilized)
2 vials	Low Quality Control (QC)*	Low QC material. Recombinant human galectin-3 in protein matrix (lyophilized)
2 vials	High Quality	High QC material, assayed. Recombinant human

	Control (QC)*	galectin-3 in protein matrix (lyophilized)
2	Plate seals	Adhesive plastic plate seals

Contains processed human plasma tested negative or nonreactive for anti-HIV-1/2, anti-HCV and HBsAg when tested by an FDA approved method.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Triage B-Type Natriuretic Peptide (BNP) Test

2. Predicate 510(k) number(s):
   k080269

3. Comparison with predicate:

Item	Device	Predicate
Indications for Use	indicated for use in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure	aid in the diagnosis and assessment of severity of heart failure, an aid in the risk stratification of patients with heart failure, and an aid in the risk stratification of patients with acute coronary syndromes.
Sample type	Serum and plasma	Whole blood and plasma
Instrumentation	Microtiter plate reader	Triage® Meter
Technology	ELISA	Lateral flow immunoassay
Cut-off	galectin-3 risk categories: • galectin-3 greater than 25.9 ng/mL • galectin-3 between 17.8 and 25.9 ng/mL •galectin-3 less than or equal to 17.8 ng/mL	100 pg/mL
Assay range	1.4 to 94.8 ng/mL	5-5000 pg/mL

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 C28-A2: How to Define and Determine Reference Intervals in the Clinical Laboratory

L. Test Principle:

BGM Galectin-3 is a microtiter plate-based ELISA for the quantitative determination of galectin-3 levels in human serum and plasma. The Galectin-3 Assay utilizes two monoclonal antibodies against galectin-3. One rat monoclonal anti-mouse galectin-3 antibody is coated onto the surface of the wells in a microtiter plate and serves as the capture antibody to bind galectin-3 molecules in samples, while the other mouse monoclonal anti-human galectin-3 antibody is provided in solution and functions as the tracer antibody for detecting galectin-3 molecules bound to the capture antibody. The microtiter plate is ready to use.

Standards, quality control materials, and patient samples are introduced into the wells and incubated for 60 minutes. During this incubation, the galectin-3 present in the standards and samples is bound to the capture antibody coated onto the well surface. A subsequent wash step removes any unbound galectin-3.

The tracer antibody, a horseradish peroxidase (HRP) labeled anti-galectin-3 antibody, is then introduced into the well and incubated for 60 minutes. During this time, an antibody-antigen-antibody complex is formed.

After a wash step to remove any unbound tracer antibody, the tetramethyl benzidine substrate is added, yielding a blue color in the presence of HRP. The color development is stopped after 20 minutes by the addition of sulfuric acid, changing the color to yellow that can be read at an absorbance of 450 nm. The test results of the samples are read from the calibration curve. The absorbance is proportional to the galectin-3 levels in the samples.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision of the Galectin-3 Asay was assessed in an evaluation according to CLSI EP5-A2 Guidelines. EDTA plasma pools spanning a range of galectin-3 concentrations were analyzed in duplicate with 2 runs per day over 20 days using one reagent lot, two operators and one microtiter plate reader. Estimates of within-run, run-to-run, day-to-day and total precision were calculated. The results are summarized in the table below:

Test specimen (n)	Mean (ng/mL)	Within-run		Run to run		Day to day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1 (160)	6.1	0.3	5.7	0.6	10.5	0.0	0.0	0.7	12.0
2 (168)	17.6	0.4	2.1	0.7	3.8	0.5	2.8	0.9	5.1
3 (160)	20.7	0.7	3.4	1.4	6.7	0.3	1.7	1.6	7.7
4 (168)	26.3	0.6	2.2	0.8	3.0	0.5	2.1	1.1	4.2
5 (168)	46.2	1.1	2.4	1.6	3.6	0.5	1.1	2.0	4.4
6 (160)	72.2	2.4	3.3	4.3	6.0	2.9	4.0	5.7	8.0

Additional precision studies were performed at three CLIA-certified clinical laboratories. Three EDTA plasma pools spanning a range of galectin-3 concentrations were analyzed in quadruplicate with 2 runs over 20 days for Site 1, 17 days for Site 2 and 18 days for Site 3 using two reagent lots and 3 different microtiter plate readers (one at each site) and a total of four operators. Pooled human plasma was divided and spiked to yield 3 concentrations spanning the measurement range. The results are summarized in the table below:

Site	Test specimen	n	Mean (ng/mL)	SD (Total)	CV% (Total)
Site 1	Low	160	5.96	0.46	7.66
	Medium	160	20.12	1.20	5.96
	High	156	68.32	9.97	14.59
Site 2	Low	132	6.72	0.63	9.36
	Medium	136	21.60	1.55	7.17
	High	136	75.50	12.75	16.89
Site 3	Low	140	6.28	0.59	9.46
	Medium	144	21.22	1.19	5.60
	High	140	71.47	6.25	8.75

b. *Linearity/assay reportable range:*

Linearity was calculated based on CLSI guideline EP6-A, Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach. The claimed measurement range of the assay is 1.4 to 94.8 ng/mL. Low and high serum samples were mixed in different fractional parts to create an 11-level dilution series of samples with equally spaced concentrations of galectin-3 ranging from 1.4 to 94.8 ng/mL. Samples were tested in duplicate. Linearity of BGM Galectin-3 was demonstrated and supported the claimed measurement range. The regression equation for the linear regression studies is $y = 0.9905x - 0.41$. The percent recoveries for each dilution level ranged from 95.5 to 101.5 %.

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

The Galectin-3 kit includes two lyophilized quality control materials (C1 and C2). The controls are assayed materials that are supplied with target values and QC ranges. Lot-specific target values and ranges have been assigned with multiple operators over multiple days. The lower control (C1) is formulated with a target galectin-3 concentration of 18.4 ng/mL and is designed to monitor the performance of patient specimens near the clinical cutoff level of 17.8 ng/mL and specimens in the lower or normal range corresponding to the lower end of the standard curve. The higher control (C2) is formulated with approximately 69 ng/mL galectin-3 and is designed to monitor the performance of the assay for patient specimens with higher galectin-3 values and the upper end of the standard curve. The controls are comprised of recombinant galectin-3 in a protein matrix. The controls are stable for 12 months. After use, remaining reconstituted controls may be stored for a maximum of 10 days at 2-8°C.

Galectin-3 is calibrated with a set of seven standards that are prepared by the user by serially diluting a reconstituted vial of the standard (S1). The seven standards once prepared are stable for a maximum of 10 days at 2-8°C . Two vials of lyophilized standard (S1) are supplied with each kit. The calibrators (standard S1) are stable for 12 months un-opened. After reconstitution, remaining standard may be stored for a maximum of 10 days at 2-8°C.

The calibrator (SI) is prepared from recombinant human galectin-3 protein expressed in *E. coli.* A stock solution of galectin-3 is prepared and the concentration is determined spectrophotometrically in-house. From the stock solution, 10 working calibrators are prepared and used to derive a calibration curve for value assignment of the lyophilized calibrator materials (standard S1) which are supplied with assay kit.

d. Detection limit:

LoB, LoD and LoQ were determined according to the CLSI guideline EP 17-A. The limit of blank (LoB) was determined as the 95th percentile value of forty-eight (48) replicate measurements of the BGM Galectin-3 Assay Buffer. The limit of detection (LoD) was determined as LoD = LoB + cβ SDs, where SDs is the pooled standard deviation from four (4) serum samples with different levels of galectin-3, each of which was measured in thirty-two (32) replicates and ß is the 95th percentile of the standard Gaussian distribution corrected for the degree of freedom. The Limit of quantitation (LoQ) was specified as the lowest galectin-3 concentration that is above the LoD and corresponds to a coefficient of variation (CV) of no more than 10.4% in the four serum samples.

Limit of Blank (LoB): LoB = 0.86 ng/mL

Limit of Detection (LoD): LoD = 1.13 ng/mL

Limit of Quantitation (LoQ): LoQ = 1.32 ng/mL

The lower limit of the measuring range was set at 1.4 ng/mL based on the linearity studies (see section M[b] above).

e. Analytical specificity:

Galectin-3 was evaluated for the effects of potential interfering substances, both endogenous and exogenous, according to the recommendations of the CLSI EP7-A guideline. In the studies, potential interferents were added to EDTA plasma samples with levels of galectin-3 at approximately 12, 22 and 40 ng/mL. Conjugated bilirubin (up to 16.8 mg/dL), unconjugated bilirubin (up to 40.3 mg/dL), albumin (BSA, up to 12 g/dL), triglycerides (up to 3000 mg/dL), cholesterol (up to 747 mg/dL), and creatinine (up to 5 mg/dL) do not show any significant interference with the assay (defined by the sponsor as ≥10 % interference). Purified hemoglobin (up to 500 mg/dL) does not show significant interference in BGM Galectin-3; however, packed blood cell lysate does show interference ≥ 10%. Human anti-mouse antibodies (HAMA) and rheumatoid factor (RF) above 50 IU/mL cause significant positive interference with BGM Galectin-3. High levels of gamma globulins (> 2.5 g/dL) may cause false elevation in galectin-3 levels. The following table is included in the labeling:

Potential interfering substance	Result of interference study based on an interference acceptance limit of +/- 10%
Conjugated bilirubin	No significant interference up to 16.8 mg/dL
Unconjugated bilirubin	No significant interference up to 40.3 mg/dL
Albumin	No significant interference up to 12 g/dL
Triglycerides	No significant interference up to 3000 mg/dL
Cholesterol	No significant interference up to 747 mg/dL
Creatinine	No significant interference up to 5 mg/dL
Purified hemoglobin	No significant interference up to 500 mg/dL
Whole blood lysate	Hemolyzed specimens should not be used with BGM Galectin-3™
Human anti-mouse antibodies (HAMA)	Specimens from patients with HAMA should not be used with BGM Galectin-3™
Rheumatoid Factor (RF)	Interference seen at levels > 50 IU/mL
Gamma globulins	Interference seen at levels ≥ 2.5 g/dL

BGM Galectin-3 measurements were not significantly affected (defined by the sponsor as ≥10 % interference) when tested in the presence of 34 common pharmaceutical substances; including heart failure drugs.

Common Drugs That Did Not Show Significant Interference with BGM Galectin-3:

Acetaminophen	Carvedilol	Dopamine	Lisinopril	Quinidine
Acetylsalicylic acid	Captopril	Enalaprilat	Losartan	Ramipril
Amlodipine	Chloramphenicol	Furosemide	Lovastatin	Spironolactone
Ampicillin	Diclofenac	Hydrochlorothiazide	Methyldopa	Theophylline
Ascorbic Acid	Digoxin	Ibuprofen	Metoprolol	Verapamil
Atenolol	Diltiazem	Indomethacin	Naproxen	Warfarin
Caffeine	Disopyramide	Lidocaine	Nifedipine	

Studies were performed which demonstrated that there is no high dose hook effect at galectin-3 levels up to 500 ng/mL. High concentrations of galectin-3 were spiked into different matrices (serum and EDTA plasma) and analyzed in duplicate. At spiked concentrations of 250 and 500 ng/mL, readings were outside the optical range of the plate reader indicating values greater than the measuring range of the assay.

f. Assay cut-off:

See Clinical Cutoff in M (4) below

2. Comparison studies:

a. Method comparison with predicate device:

See clinical studies in M (3) and M (4) below

b. Matrix comparison:

The BGM Galectin-3 assay has been validated for use with EDTA plasma or serum samples. The equivalence of these sample matrices were demonstrated in a study of forty-nine (49) matched natural serum and EDTA-plasma samples with values spanning the measurement range. The samples had values ranging from 5.45 to 90.61 ng/mL. The regression equation for the study was $y = 0.96x + 0.72$.

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):

Cutoff values were determined in an independent, controlled multi-center clinical study in Europe enrolling NYHA I, II, III and IV subjects (see section M [4] Clinical Cutoff below for further details).

To validate the clinical effectiveness of the cut-off values for the BGM Galectin-3 assay, galectin-3 levels were measured in a set of 895 banked EDTA-plasma samples from chronic heart failure participants in the United States and Canada in a controlled multi-center clinical study, the Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training (HF-ACTION) study. The HF-ACTION study involved 2,331 chronic HF patients with left ventricular dysfunction and with NYHA class II, III or IV symptoms. The average age of the 895 participants whose galectin-3 levels were assessed in the clinical validation study was 58 years, 29% were female, and 36% were non-white. A statistical analysis (sensitivity analysis) was performed comparing the set of 895 HF-ACTION subjects having evaluable galectin-3 values with all other HF-ACTION participants (1436), and it was found that

the clinical validation results based on the evaluable set of 895 subjects were robust and representative of the larger study population. In this sensitivity analysis, galectin-3 values were imputed conservatively for the 1436 remaining patients in the dataset based on the probability of the assay categorizing a patient into a high or low risk group. The difference in survival curves for the risk groups remained statistically significant, indicating that the results on the evaluable subset (895) were robust and representative of the entire study population. The median follow-up time was approximately 30 months. Chronic heart failure participants were categorized based on galectin-3 risk categories defined below:

The derived galectin-3-dependent risk categories are as follows:

• galectin-3 greater than 25.9 ng/mL

• galectin-3 between 17.8 and 25.9 ng/mL

• galectin-3 less than or equal to 17.8 ng/mL

For the clinical validation study, Cox regression models were used to evaluate the association of baseline galectin-3 levels in 895 chronic HF patients with the endpoints of: (i) composite of all-cause mortality and all-cause hospitalization, (ii) cardiovascular mortality, (iii) composite of cardiovascular mortality and heart failure-related hospitalization, and (iv) all-cause mortality. Galectin-3 levels were found to be significantly associated with increased risk of each of these endpoints in Cox regression models. Galectin-3 remained significantly associated with increased risk upon adjustment for baseline risk factors of age, gender, NYHA functional classification, left ventricular ejection fraction, diabetes status, and smoking status. The following figures and tables display Kaplan Meier curves for the composite endpoint of all-cause mortality or all-cause hospitalization, by baseline galectin-3 category and cumulative probabilities for events for the endpoints of the composite of all-cause mortality and all-cause hospitalization, cardiovascular mortality, and the composite of cardiovascular mortality and heart failure-related hospitalization in the clinical validation study, by baseline galectin-3 category at time points of 6, 12, 24 and 36 months after baseline.

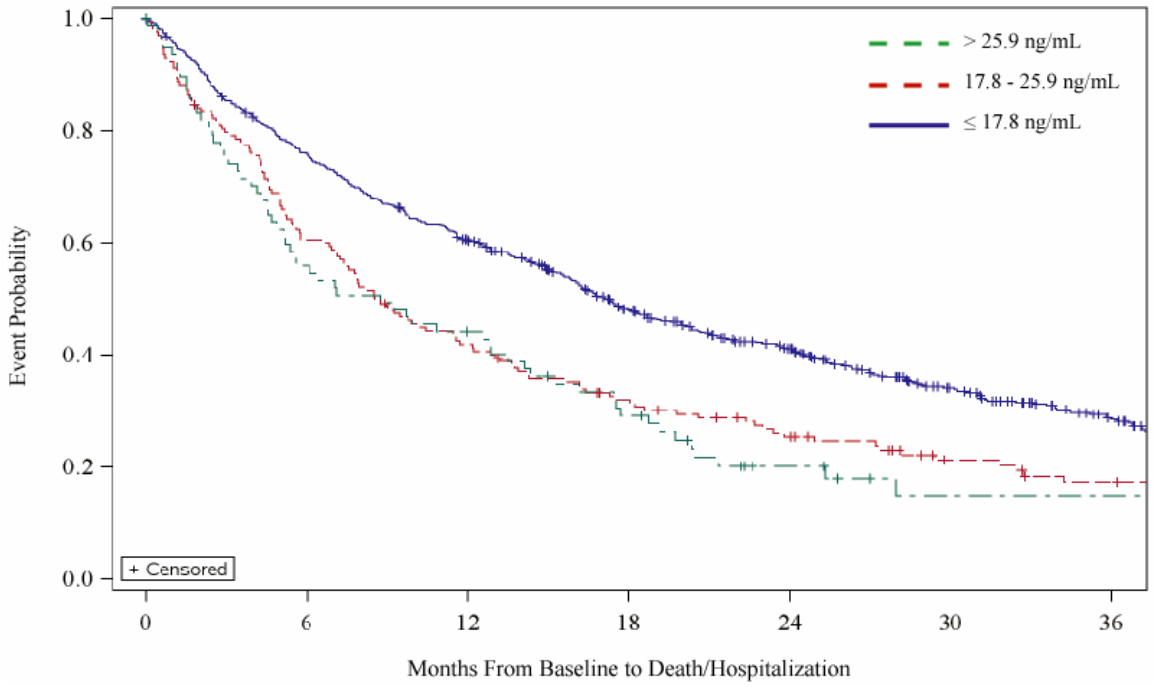
All Cause Mortality and All-Cause Hospitalization

Hazard Ratios for <u>All-Cause Mortality and All-Cause Hospitalization Events</u> for Chronic HF Subjects in the Clinical Validation Study

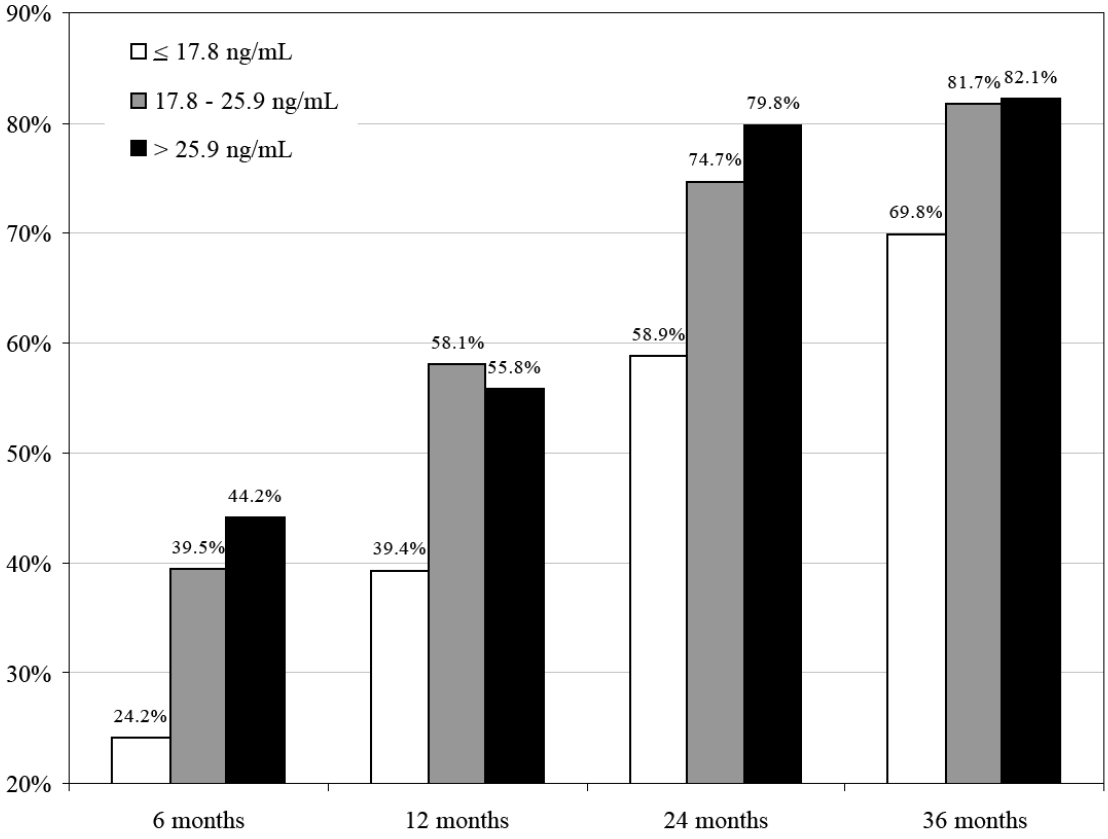
	Hazard Ratio (95% CI, p value)		
Galectin-3 Category	≤ 17.8 ng/mL	17.8-25.9 ng/mL	> 25.9 ng/mL
Number of Subjects	647	170	78
Galectin-3*	1.0	1.35 (1.10-1.65, p= 0.004)	1.46 (1.11-1.92, p= 0.006)

**adjusted for baseline risk factors: age, gender, NYHA functional classification, left ventricular ejection fraction, diabetes status, and smoking status*

Kaplan-Meier Curves for the Composite Endpoint of <u>All-Cause Mortality or All-Cause Hospitalization</u>, for chronic HF Subjects in the Clinical Validation Study, by Baseline Galectin-3 Level



Cumulative Probability of Event for the Composite Endpoint of <u>All-Cause Mortality and All-Cause Hospitalization</u>, at Various Time Points and By Baseline Galectin-3 Level, for chronic HF Subjects in the Clinical Validation Study



Cumulative Probability (with 95% Confidence Intervals) of Event for the Composite Endpoint of <u>All-Cause Mortality and All-Cause Hospitalization</u> , at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study

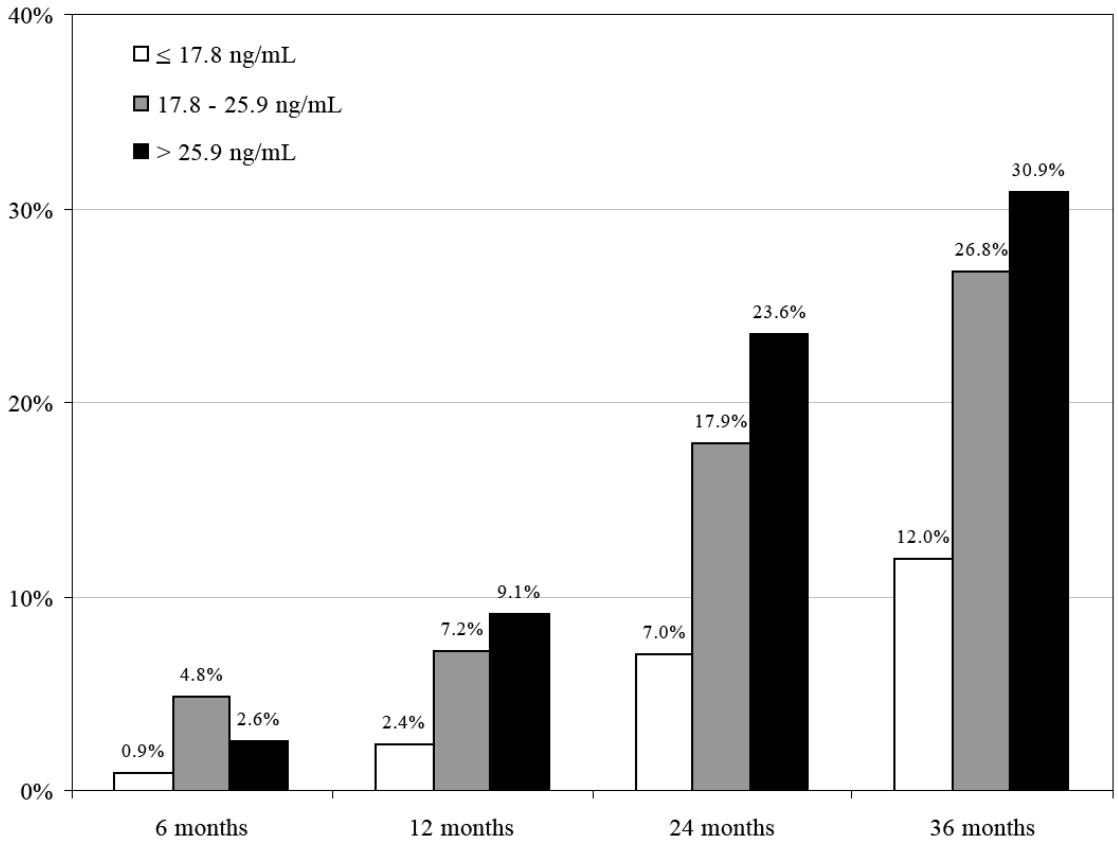
	Cumulative Probability of All-Cause Mortality and All-Cause Hospitalization (95% CI) by Galectin-3 Category and Time Point (in percent)			
Galectin-3 Category	**6 months**	**12 months**	**24 months**	**36 months**
≤ 17.8 ng/mL	24.2% (21.1%-27.7%)	39.4 (35.7-43.3)	58.9 (55.0-62.9)	69.8 (65.8-73.7)
17.8-25.9 ng/mL	39.5 (32.5-47.3)	58.1 (50.8-65.7)	74.7 (67.7-81.1)	81.7 (74.9-87.6)
> 25.9 ng/mL	44.2 (33.9-55.9)	55.8 (45.2-67.1)	79.8 (69.9-88.2)	82.1 (72.0-90.0)

Hazard Ratios for Cardiovascular Mortality Events for chronic HF Subjects in the Clinical Validation Study

	Hazard Ratio (95% CI, p value)		
Galectin-3 Category	≤ 17.8 ng/mL	17.8-25.9 ng/mL	> 25.9 ng/mL
Number of Subjects	647	170	78
Galectin-3*	1.0	1.91 (1.28-2.86, p= 0.002)	2.33 (1.43-3.80, p < 0.001)

**adjusted for baseline risk factors: age, gender, NYHA functional classification, left ventricular ejection fraction, diabetes status, and smoking status*

Cumulative Probability of Event for the Endpoint of Cardiovascular Mortality, at Various Time Points and By Baseline Galectin-3 Level, for chronic HF Subjects in the Clinical Validation Study



Cumulative Probability (with 95% Confidence Intervals) of Event for the Cardiovascular Mortality , at Various Time Points and By Baseline Galectin-3 Level, for chronic HF Subjects in the Clinical Validation Study

	Cumulative Probability of Cardiovascular Mortality (95% CI) by Galectin-3 Category and Time Point (in percent)			
Galectin-3 Category	**6 months**	**12 months**	**24 months**	**36 months**
≤ 17.8 ng/mL	0.9% (0.4%-2.1%)	2.4(1.4-3.9)	7.0(5.2-9.3)	12.0(9.4-15.2)
17.8-25.9 ng/mL	4.8(2.4-9.3)	7.2(4.1-12.3)	17.9(12.7-24.9)	26.8(20.0-35.5)
> 25.9 ng/mL	2.6(0.6-9.9)	9.1(4.4-18.1)	23.6(15.0-36.0)	30.9(20.4-45.0)

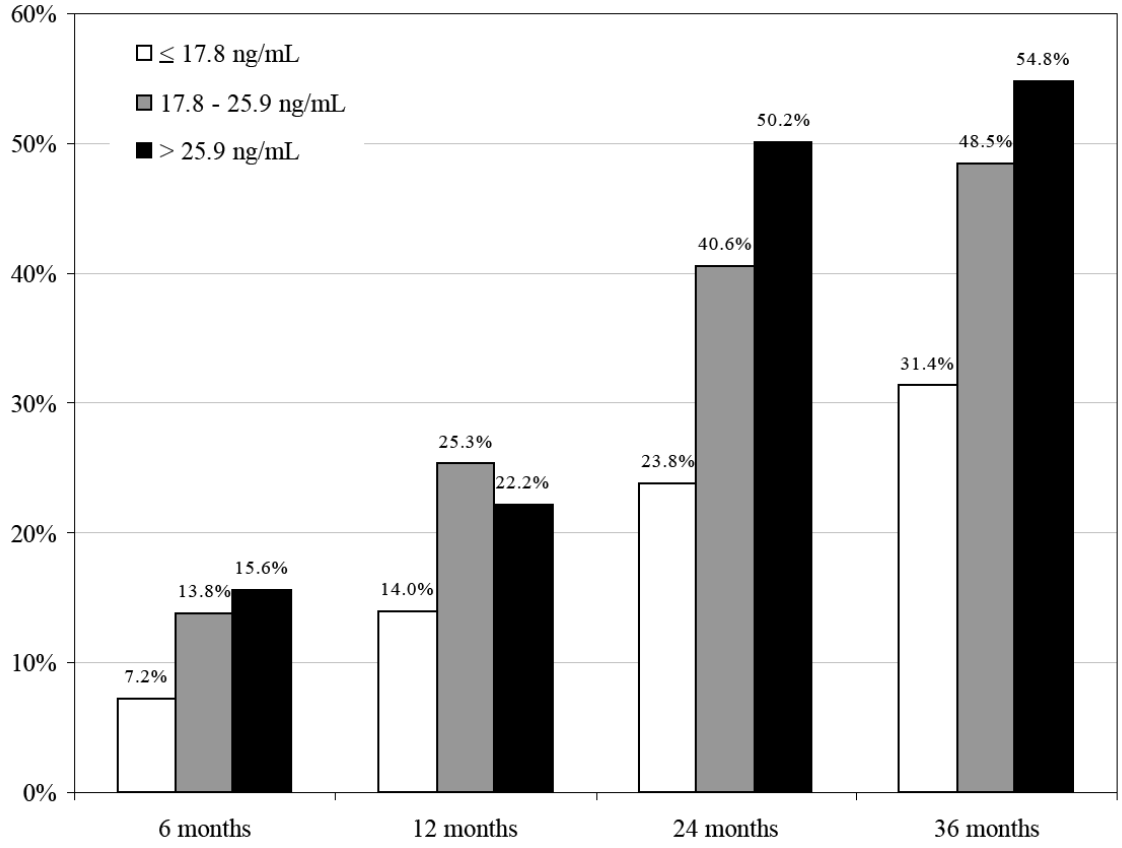
Cardiovascular Mortality and Heart Failure Related Hospitalization

Hazard Ratios for Cardiovascular Mortality and Heart Failure-Related Hospitalization Events for chronic HF Subjects in the Clinical Validation Study

	Hazard Ratio (95% CI, p value)		
Galectin-3 Category	≤ 17.8 ng/mL	17.8-25.9 ng/mL	> 25.9 ng/mL
Number of Subjects	647	170	78
Galectin-3*	1.0	1.51 (1.14-2.00, p= 0.004)	1.70 (1.19-2.42, p= 0.004)

**adjusted for baseline risk factors: age, gender, NYHA functional classification, left ventricular ejection fraction, diabetes status, and smoking status*

Cumulative Probability of Event for the Composite Endpoint of Cardiovascular Mortality and Heart Failure-Related Hospitalization, at Various Time Points and By Baseline Galectin-3 Level, for chronic HF Subjects in the Clinical Validation Study

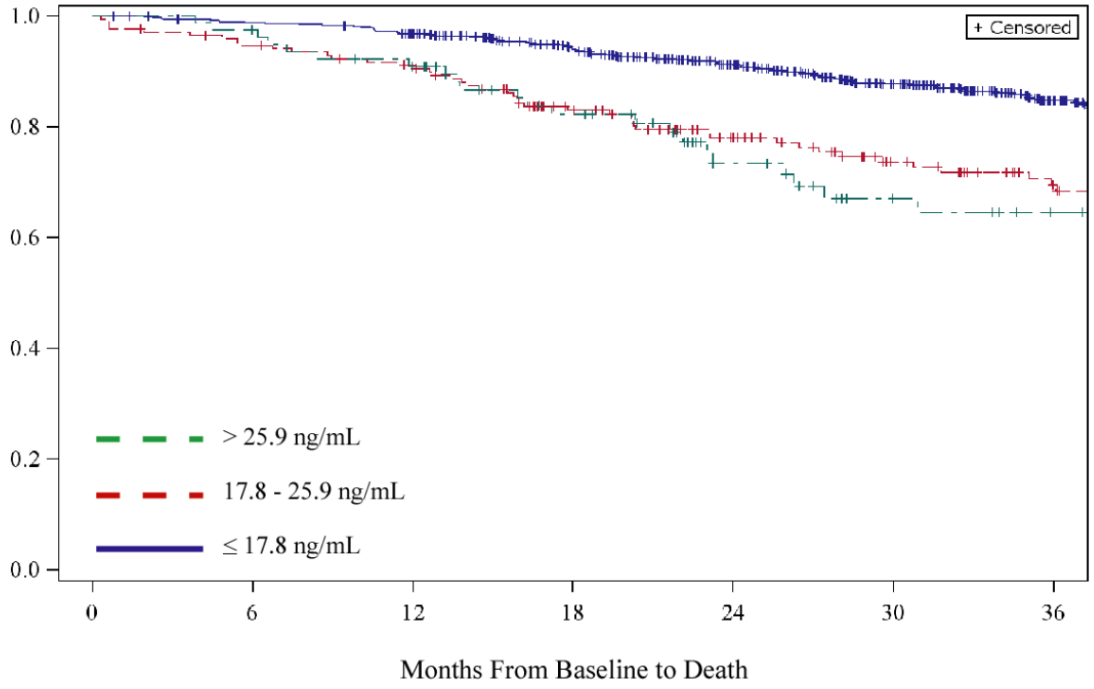


Cumulative Probability (with 95% Confidence Intervals) of Event for Cardiovascular Mortality and Heart Failure-Related Hospitalization , at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study

	Cumulative Probability of Cardiovascular Mortality and Heart Failure-Related Hospitalization (95% CI) by Galectin-3 Category and Time Point (in percent)			
Galectin-3 Category	**6 months**	**12 months**	**24 months**	**36 months**
≤ 17.8 ng/mL	7.2% (5.4%-9.5%)	14.0 (11.5-17.0)	23.8 (20.6-27.4)	31.4 (27.6-35.6)
17.8-25.9 ng/mL	13.8 (9.4-20.1)	25.3 (19.4-32.7)	40.6 (33.4-48.7)	48.5 (40.5-57.2)
> 25.9 ng/mL	15.6 (9.2-25.8)	22.2 (14.4-33.2)	50.2 (38.9-62.8)	54.8 (42.9-67.6)

All Cause Mortality

Kaplan-Meier Curves for the Endpoint of <u>All-Cause Mortality</u>, for chronic HF Subjects in the Clinical Validation Study, by Baseline Galectin-3 Level



Cumulative Probability (with 95% Confidence Intervals) of Event for the Endpoint of <u>All-Cause Mortality</u> , at Various Time Points and By Baseline Galectin-3 Level, for chronic HF Subjects in the Clinical Validation Study

	Cumulative Probability of All-Cause Mortality Event (95% CI) by Galectin-3 Category and Time Point (in percent)			
Galectin-3 Category	**6 months**	**12 months**	**24 months**	**36 months**
≤ 17.8 ng/mL	1.2% (0.6%-2.5%)	3.3 (2.1-5.0)	8.7 (6.7-11.3)	15.3 (12.4-18.8)
17.8-25.9 ng/mL	5.3 (2.8-10.0)	8.9 (5.5-14.4)	22.0 (16.3-29.4)	30.5 (23.4-39.1)
> 25.9 ng/mL	2.6 (0.6-9.9)	9.1 (4.4-18.1)	26.6 (17.5-39.1)	35.5 (24.5-49.5)

Hazard Ratios for <u>All-Cause Mortality Events</u> for chronic HF Subjects in the Clinical Validation Study

	Hazard Ratio (95% CI, p value)		
Galectin-3 Category	≤ 17.8 ng/mL	17.8-25.9 ng/mL	> 25.9 ng/mL
Number of Subjects	647	170	78
Galectin-3*	1.0	1.84 (1.28-2.64, p= 0.001)	2.06 (1.31-3.23, p= 0.002)

**adjusted for baseline risk factors: age, gender, NYHA functional classification, left ventricular ejection fraction, diabetes status, and smoking status.*

4. <u>Clinical cut-off</u>**:**

To determine cutoff values for the BGM Galectin-3 assay in HF, galectin-3 levels were measured in 582 banked EDTA-plasma samples from an independent, controlled multi-center clinical study conducted in Europe enrolling NYHA class II, III and IV subjects. The average age of patients was 71 years, and 38% were female. The median follow-up time was greater than 18 months, and the primary end point was the composite of all-cause mortality and hospitalization for HF. Two cutoff values were derived that in turn defined three ordered galectin-3 categories. The derived galectin-3-dependent risk categories are as follows:

- galectin-3 greater than 25.9 ng/mL
- galectin-3 between 17.8 and 25.9 ng/mL
- galectin-3 less than or equal to 17.8 ng/mL

Galectin-3 and natriuretic peptides are measures of separate and distinct biological processes. Each marker provides independent and complementary information on the prognosis of patients with chronic heart failure.

The following additional interpretive information is provided in the labeling which provides information on the use of galectin-3 and NT-proBNP together.

Event Rates at 6, 12, 24 and 36 Months for the Composite Endpoint of All-Cause Mortality and All-Cause Hospitalization, by Galectin-3 Category and NT-proBNP level, for HF Subjects in the Clinical Validation Study:

	Galectin-3 ≤ 17.8 ng/mL and NT-proBNP ≤ median	Galectin-3 ≤ 17.8 ng/mL and NT-proBNP > median *or* Galectin-3 > 17.8 ng/mL and NT-proBNP ≤ median	Galectin-3 > 17.8 ng/mL and NT-proBNP > median
Event rate at 6 months	19.4%	31.8%	42.7%
Event rate at 12 months	32.0%	50.0%	58.0%
Event rate at 24 months	55.3%	71.1%	85.7%
Event rate at 36 months	76.1%	85.8%	93.0%

5. Expected values/Reference range:

Subjects included in the reference range study were drawn from a large ongoing prospective observational study recruiting subjects without previous cardiac disease. Galectin-3 levels were measured in 1,099 banked plasma samples (EDTA) from the population of apparently healthy subjects without known heart disease but that otherwise resemble, by age and gender distribution, the HF patient population. Specimens were from women between the ages of 60 and 80 years (n=575) and men between the ages of 55 and 80 (n=524). This reference population comprised individuals of different ethnic background, as follows: Black or African-American (n=307, 27.9%), Caucasian (n=691, 62.9%), Hispanic (n=42, 3.8%), Asian or Pacific Islander (n=30, 2.7%), and not specified (n=29, 2.6%). All subjects had detectable galectin-3 levels (min-max, 3.2 - 94.6 ng/mL) within the measuring range of BGM Galectin-3 (1.4 to 94.8 ng/mL). The table below summarizes the galectin-3 distribution results. The 97.5th percentile of the galectin-3 distribution from this reference population is 26.2 ng/mL. Samples with values above 26.2 ng/mL were in the upper 2.5th percentile.

Percentile	Galectin-3 (ng/mL)
2.5th	5.4
5th	6.3
25th	9.7
50th	12.4
75th	15.6
90th	19.0
95th	22.1
97.5th	26.2

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.