REF #: 12836

US IVD BGM Galectin-3® Kit

Enzyme-linked Immunosorbent Assay for the Quantitative Determination of Galectin-3 in Human Serum and Plasma

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Table of Contents

BGM Galectin-3®
(Galectin-3 Assay)

Intended Use

BGM Galectin-3® is an *in vitro* diagnostic device that quantitatively measures galectin-3 in serum or plasma by enzyme linked immunosorbent assay (ELISA) on a microtiter plate platform, to be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure (HF).

Summary and Explanation of the Test

Galectin-3 is a structurally unique member of a family of beta-galactoside-binding lectins. Expression of galectin-3 has been associated with the epithelium and inflammatory cells including macrophages, neutrophils and mast cells. Galectin-3 has been implicated in a variety of biological processes important in heart failure including myofibroblast proliferation, fibrogenesis, tissue repair, cardiac remodeling and inflammation.

Principle of the Test Procedure

BGM Galectin-3 is a microtiter plate-based ELISA for the quantitative determination of galectin-3 levels in human serum and plasma.

BGM Galectin-3 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 specimens are introduced in duplicate into the wells and incubated for 60 minutes. During this incubation, the galectin-3 present in the standards and specimens binds to the capture antibody coated onto the well surface. Subsequent washing removes all unbound material introduced with the samples, including 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 tetramethylbenzidine (TMB) 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, which is read at an absorbance of 450 nm.

The absorbance is proportional to the galectin-3 levels in the specimens. The test results of the specimens are read from the standard curve.

Kit Contents

Each BGM Galectin-3 kit (box) contains the following:

Qty	Name	Description	Abbreviation
1 plate	Plate	Ready-to-use microtiter plate coated with rat monoclonal anti-mouse galectin-3 monoclonal antibody	(P)
1 bottle	Assay Buffer*	Phosphate buffered saline with 1% bovine serum albumin (55 mL)	(AB)
1 bottle	TMB substrate	Tetramethylbenzidine (15 mL)	(TS)
1 bottle	Stop solution	0.5M sulfuric acid (10 mL)	(ST)
2 bottles	Wash concentrate*	0.5M Tris buffered saline (2 x 50 mL; 10X concentrate)	(WC)
1 bottle	Tracer concentrate*	Horseradish peroxidase (HRP) labeled mouse monoclonal anti-human galectin-3 monoclonal antibody (0.45 mL)	(TC)
2 vials	Standard	Recombinant human galectin-3, 12 ng per vial (lyophilized), 10 ng/mL reconstituted and diluted	(S1)
2 vials	Low Quality Control (QC) †	Low QC material, Recombinant human galectin-3 in protein matrix (lyophilized)	(C1)
2 vials	High Quality Control (QC) †	High QC material, Recombinant human galectin-3 in protein matrix (lyophilized)	(C2)
2	Plate seals	Adhesive plastic plate seals	

** Contains ProClin® as a preservative.*

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

Storage Instructions

Store the assay kit at 2-8°C upon receipt. Expiration dates of the reagents are printed on the labels. Return all kit components to 2-8°C immediately after use. Reconstituted or diluted kit components may be stored for a maximum of 10 days at 2-8°C.

Note: The expiration dates of the reagents can only be assured if the reagents are stored properly, and, in case of repeated use of one reagent, the reagent is not contaminated by the first handling.

Do not expose assay reagents to strong light (e.g. direct sunlight) during storage or use.

Specimen Collection and Storage

- BGM Galectin-3 is validated for use with the following matrices:

Matrix	Tube Type
Serum	no anticoagulant, no gel barrier
Plasma	EDTA

- Citrated plasma is not suitable for use with BGM Galectin-3.
- Other tube types and anticoagulants have not been evaluated.
- Blood should be collected using standard venous blood collection techniques and equipment.

  Centrifugation and separation of the serum or plasma from the cellular components should occur as soon as possible following collection.
- The recommended specimen volume for BGM Galectin-3 is 30 µL which is sufficient volume for duplicate measurements.
- Specimens with visible hemolysis *(i.e. specimens with visible pink or red color)* should not be used as falsely elevated galectin-3 levels will occur.
- If necessary, serum (no anticoagulant, no gel barrier) or EDTA-plasma may be stored for future analysis. Endogenous human galectin-3 has been tested and shown to be stable under the following conditions:

Storage Condition (temperature)	Specimen Stability
22-28°C	22 days
2-8°C	22 days (EDTA-plasma) 1 month (serum[a])
-20°C	12 months
-70°C	24 months

a no anticoagulant, no gel barrier

Galectin-3 in human serum (no anticoagulant, no gel barrier) and EDTA-plasma has been shown to be stable for 9 freeze-thaw cycles after storage at -20°C or -70°C.

Warnings and Precautions

- **For *in vitro* diagnostic use.**
- For use by healthcare professionals.
- Follow the instructions carefully when performing tests; failure to follow the instructions may result in inaccurate results.
- Results should be interpreted along with clinical findings and other laboratory test results.
- Levels of galectin-3 in blood may be increased in patients with certain forms of advanced cancer and other conditions associated with organ fibrosis. BGM Galectin-3 results should be interpreted with caution in such patients.
- BGM Galectin-3 is not indicated for detection, diagnosis, prognosis, or any uses associated with any type of cancer, conditions associated with organ fibrosis, or any other condition not noted under Intended Use.
- Handle all serum and plasma specimens as potentially biohazardous material. Follow universal precautions and handle specimens as potentially contaminated and as if capable of transmitting an infectious agent.
- Dispose of waste in accordance with local requirements. A Safety Data Sheet (SDS) is included in each kit.
- Avoid contact of assay reagents or specimens with skin, eyes, or mucous membranes. In the event of contact with skin or eyes, wash immediately with water. Consult SDS included in the kit for more information.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Reagents contain the active ingredient 5-chloro-2-methyl-thiazol-3-one and 2-methylthiazol-3-one, as a biocide preservative.

Materials Required But Not Provided

- Deionized water
- Adjustable pipettes (for pipetting 30, 50, 100, 125, 270, 300, 900 µL volumes), 8 Channel Adjustable Multi-Pipette (for pipetting 50, 100 µL volumes) and appropriate tips
- Multi-channel pipette reservoirs (polypropylene)
- One of the following transfer vessel(s) to prepare sample dilutions and to facilitate transfer (pipetting) of samples into the BGM Galectin-3 antibody-coated plate:
    - Disposable borosilicate glass or polypropylene or other low protein-binding plastic test tubes or vials for dilution of standard, tracer, TMB-substrate, controls and patient specimens
    - Non-binding 96-well U-bottom microtiter plate for use as a transfer plate
- Mechanical microplate washer as defined in Table 1 *or suitable* wash buffer bottle for manual plate washing

Table 1: Minimum Recommended Plate Washer Requirements for BGM Galectin-3[*]

Plate Washer Feature	Minimum Recommended Requirements for Use in BGM Galectin-3
Plate Size	Support 96 well flat-bottom plate in 1x8 strips
Number of cycles	Support at least 4 cycles/wash
Dispense Volume	Support dispense volume of 400 µL/ well
Accuracy	< 5%
Precision	< 5% CV
Residual Volume/Well	< 3 µL
Soak Time	Support soak time interval of 15 seconds
Fluid reservoir capacity	Support at least 500 mL

** Each laboratory should ensure proper validation of their equipment and software for use with BGM Galectin-3.*

- Absorbent paper towels for plate blotting after washes
- Microtiter plate reader capable of reading at 450 nm and other characteristics as defined in Table 2:

Table 2: Minimum Recommended Plate Reader Requirements for BGM Galectin-3*

Plate Reader Feature	Minimum Recommended Requirements for Use with BGM Galectin-3
Detection method	Absorbance
Wavelength range	Able to measure at 450 nm
Band width	≤ 10 nm
Dynamic range	0.000 to 3.000 OD
Resolution	0.001 OD
Accuracy	±1.0% or 0.010 from 0 to 2.0 OD; ≤ 2.0% to 3.0 OD
Reproducibility	±1.0% or 0.010 from 0 to 2.0 OD; ≤ 1.5% to 3.0 OD
Linearity	±1.0% from 0 to 2.0 OD; ±2.0% to 3.0 OD
Plate type	Compatible with 96 well standard flat-bottom microplates
Read method	Endpoint
Read temperature	Able to achieve the above performance at 20–25°C

** Each laboratory should ensure proper validation of their equipment and software for use with BGM Galectin-3.*

- Appropriate software to perform curve fitting of the standard (calibration) curve. Note that many microtiter plate readers have curve-fitting software built-in, otherwise separate curve-fitting software should be obtained. It is important that the curve-fitting software includes one of the curve-fitting methods that is suitable for use with the BGM Galectin-3 assay, which include:
    - Third-order polynomial (cubic) with least squares optimization (*avoid use of "cubic-spline" curve fit methods*)
    - Four-parameter logistic (4PL)
    - Five-parameter logistic (5PL)

  In addition, use of a $1/Y^2$ weighting scheme, if available, is recommended for all three curve fitting methods as curve fitting results tend to be slightly improved

through the use of this particular weighting scheme. However, reliable results can be obtained with or without the use of a $1/Y^2$ weighting scheme.

For additional information, please contact Technical Support at 1-877-665-0077, option 2 or via email at tsupport@bg-medicine.com.

- Additional plate seals, if desired, can be procured (Corning 3095)

Quality Control

BGM Galectin-3 Controls (supplied with kit)

Description and Intended use: The BGM Galectin-3 assay kit includes two lyophilized quality control materials (C1 and C2) intended to monitor the performance of the BGM Galectin-3 assay. The lower control (C1) is formulated with a target galectin-3 concentration of approximately 17 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 a target galectin-3 concentration of approximately 70 ng/mL 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.

Assigned values: The BGM Galectin-3 Controls are assayed materials which are supplied with assigned values and QC ranges that are printed on the vials and the accompanying Value Assignment Sheet. Lot-specific ranges have been assigned with multiple operators over multiple days and are based on the mean galectin-3 values ± 3 standard deviations (mean ± 3 SD). These values and associated ranges are provided as guidelines to the end user. It is recommended that each clinical laboratory confirm the suitability of the assigned ranges or establish their own ranges based on their own test system and criteria.

Composition and operating instructions: The controls are comprised of galectin-3 in a protein matrix to closely simulate the composition of patient specimens. Controls should be prepared and analyzed with the same procedure as patient samples (i.e. 1:10 dilution in Assay Buffer and duplicate measurement) to ensure adequate assay performance including preparatory and operational steps.

Frequency for QC: It is recommended that both QC levels (C1 and C2) be prepared and analyzed for each analytical run of the BGM Galectin-3 assay. In addition, QC materials should be used in accordance with local, state and/or federal regulations or accreditation requirements.

Additional quality control (optional for users)

It is recommended that clinical laboratories consider additional quality control (QC) materials to enhance their overall quality control strategy. Labs may use patient pools with galectin-3 concentrations at specific clinical decision levels or cut-points to supplement the BGM Galectin-3 controls that are supplied with the kit. Users are referred to CLSI C24-A3 for additional information.

BGM Galectin-3 Test Procedure

(Refer to flow chart in Figure 2)

Follow the instructions carefully when performing tests; failure to follow the instructions may result in inaccurate results.

Preparation of Reagents

1. **Prior to use, allow all kit components to equilibrate to 20-25**°C **for a <u>minimum</u> of 30 minutes.** Note: Return all kit components to storage at 2-8°C after use.
2. **Plate (P):** After opening the pouch, number the top surface of each strip with permanent ink in case they are unintentionally released from the plate frame. *Note: Plates are designed for partial use. If partial use is desired, unneeded strips must be removed from the frame and returned to the plate pouch for storage. Do not remove the desiccant from the pouch. Securely reseal the plate pouch and return to storage for a maximum of 14 days at 2-8°C. When the first partial plate assay is complete, save the plate frame for subsequent partial run(s).*
3. **Wash Concentrate (WC):** Each WC bottle contains 10x concentrated wash buffer. Using deionized water, prepare a 1:10 dilution of the WC. The entire fill volume may be diluted and stored or smaller aliquots may be prepared for a given day's experiment. If a plate washer is to be used to perform washes, ensure that adequate wash volume is prepared to account for priming the plate washer (check manufacturer's recommendation). Diluted buffer may be stored for a maximum of 10 days at 2-8°C.

Detailed Assay Procedure

Note: All samples (patient specimens, controls and calibrators) must be diluted prior to the start of the testing procedure. Sample dilution should never be performed in the wells of the antibody-coated plate. In order to allow samples to react with the coated antibody for approximately the same duration, sample transfer should be completed across the plate in five minutes or less. In order to facilitate rapid sample transfer, use of transfer vessel(s) and a multi-channel pipette is recommended.

1. Reconstitute and Dilute Galectin-3 Standard (S1)

BGM Galectin-3 is calibrated with a set of seven standards that are prepared by serial dilution of the standard (S1) that is supplied with each kit. The calibration range is 0.156 ng/mL to 10.0 ng/mL. NOTE: the concentration of the lyophilized standard provided is 12 ng/vial. Once reconstituted and diluted per the instructions which follow, the concentration of the standard becomes 10 ng/mL. The 10 ng/mL standard is also the first level standard (S1) used to generate the standard curve.

Open vacuum sealed vial slowly to avoid loss of material due to aerosol formation. *Note: To avoid potential cross-contamination, write "S1" on the stopper with permanent ink.* Before use, reconstitute one vial of the galectin-3 standard (S1) with 300 µL of deionized water, immediately followed by dilution with 900 µL of Assay Buffer. Allow the vial to stand for a minimum of 15 minutes at 20-25°C with periodic vortexing and gentle inversion ensuring full dissolution of the lyophilized material. Complete dissolution of the standard is critical. Verify dissolution is complete prior to use. After use, remaining reconstituted standard may be stored for a maximum of 10 days at 2-8°C, if reuse is desired.

2. Reconstitute Galectin-3 Controls (C1 and C2)

The controls are comprised of a protein matrix spiked with recombinant human galectin-3. The BGM Galectin-3 Controls are supplied with assigned QC ranges that are printed on the vials and in the accompanying Value Assignment Sheet. Ranges are lot-specific and the user must confirm the appropriate range with each new lot of a BGM Galectin-3 kit.

Open vacuum sealed vials slowly to avoid loss of material due to aerosol formation. *Note: To avoid potential cross-contamination, write "C1 or "C2", as applicable on the appropriate stopper of each vial with permanent ink.* Reconstitute one vial of C1 and C2 with 250 µL deionized water. Allow the vials to stand for a minimum of 15 minutes at 20-25°C with periodic vortexing and gentle inversion ensuring that the reconstitution water wets the entire surface area inside the vial. Verify complete dissolution prior to use. After use, remaining reconstituted controls may be stored for a maximum of 10 days at 2-8°C, if reuse is desired.

3. Define a Plate Map

Designate microtiter plate wells for each of the controls, test specimens, diluted standards and blank. All samples should be tested in duplicate (i.e. blank, diluted standards, controls and test specimens).

4. Prepare Diluted Specimens and Blank

While the standard and controls are reconstituting, dilute each test specimen 10-fold (1:10) using the Assay Buffer (AB). Final dilution volume should be sufficient for duplicate measurement. Therefore, it is recommended that a minimum of 30 µL of the specimen be used for the dilution (i.e. 30 µL specimen + 270 µL AB). An assay blank should be prepared using just Assay Buffer (AB).

Dilutions must be performed externally in a separate transfer vessel (i.e. off-line and not in the BGM Galectin-3 antibody-coated plate). Recommended transfer vessels are a non-binding 96-well U-bottom microtiter plate ("transfer plate") or disposable test tubes composed of borosilicate glass, polypropylene or other low protein-binding plastic. If a transfer plate is used, ensure plate is clean by inspecting for dust particles prior to use and make sample dilutions in the corresponding wells per the plate map defined in step 3 above. Mix each dilution by performing multiple aspiration and dispense cycles with the pipette (if transfer plate is used), or by vortexing or inversion (if test tubes are used).

Note: BGM Galectin-3 is designed to analyze samples (patient specimens and controls) that are diluted 10-fold (1:10) in Assay Buffer prior to analysis. This provides the proper

sample to reagent ratio that yields optimal results within the measurement range up to 94.8 ng/mL. **Patient specimens that yield galectin-3 results greater than 94.8 ng/mL should NOT be further diluted.**

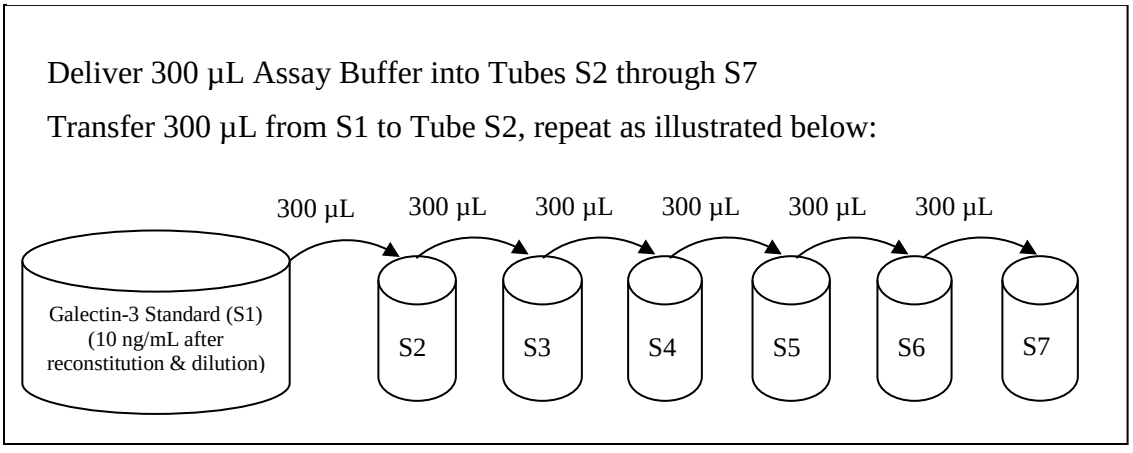
5. Prepare Diluted Controls

Dilute each reconstituted Control (C1 and C2) 10-fold (1:10) using the Assay Buffer (AB) in transfer vessels (i.e., in designated wells of transfer plate, or disposable test tubes composed of borosilicate glass, polypropylene or other low protein-binding plastic). Mix each dilution by pipette aspiration, vortexing or inversion. Final dilution volume should be sufficient for duplicate measurement. It is recommended that a minimum of 30 µL of the reconstituted control be used for the dilution (i.e. 30 µL C1 or C2 + 270 µL AB). Dilutions must be performed externally (i.e. off-line and not in the BGM Galectin-3 antibody-coated plate). After use, remaining reconstituted or diluted (1:10) control material may be stored for a maximum of 10 days at 2-8°C, if reuse is desired.

6. Prepare Set of Diluted Standards

Dilute the standards immediately before use. Label 6 disposable test tubes with numbers S2 to S7 according to the dilution scheme illustrated in Figure 1. Pipette 300 µL Assay Buffer (AB) into each labeled tube. Next, pipette 300 µL Galectin-3 Standard (S1) to tube S2 and mix gently by pipette aspiration, vortexing or inversion. Then, transfer 300 µL from tube S2 to tube S3 and mix gently, transfer 300 µL from tube S3 to S4, etc. If using a transfer plate, pipette 300uL of S1 through S7 into the corresponding wells of the transfer plate per the plate map defined in step 3 above. After use, remaining reconstituted or diluted standard material may be stored for a maximum of 10 days at 2-8°C, if reuse is desired.

Figure 1: Serial Dilution Scheme for Preparation of Standards



7. Prepare Samples for Transfer

Samples are transferred from the transfer vessel (i.e. transfer plate or test tubes) using a multi-channel pipette. If a transfer plate was used as the transfer vessel, the samples are already prepared for transfer to the BGM Galectin-3 antibody-coated plate using adjustable or multi-channel pipette. If test tubes or vials were used as the transfer vessel, arrange all test tubes in a suitable rack corresponding to the sample order per the plate map defined in step 3 above so samples can be readily transferred to the BGM Galectin-3 antibody-coated plate using a multi-channel pipette.

8. Transfer Samples

Transfer should be completed within 5 minutes, regardless of method.

Transfer 100 µL of each sample (blank, diluted standards, diluted controls, and diluted test specimens) to duplicate wells of the BGM Galectin-3 antibody-coated plate using a multi-channel pipette and according to the plate map defined in step 3 above.

9. Seal and Incubate

Cover the wells with a clean plate seal and incubate for 1 hour ± 5 minutes at 20-25°C **without shaking.** The **incubation time** at this step **is critical**. The plate should incubate for **1 hour ± 5 minutes**. ***Use of a timer is strongly recommended.***

10. Remove Seal and Wash

Carefully remove the plate seal and wash wells with diluted wash buffer.

Mechanical washer: 400 µL per well, 4 cycles. Dispensed wash should remain in wells a minimum of 15 seconds before aspiration step. After the fourth wash, empty wells by tapping on an absorbent paper towel. Inspect wells for any remaining wash and repeat tapping on absorbent paper towel if necessary.

Note: Prior to mechanical washing, ensure wash/aspirator tips have been adjusted to be close to the bottom of the wells but not touching or scratching the surface. If the mechanical washer model does not have the ability to adjust the washer wash/aspirator tip height, an additional wash cycle may be added if blank wells are inconsistent or the absorbance reading is too high.

Manual wash: Empty wells, add 300 µL wash buffer per well with a wash bottle and soak for 15 seconds; empty wells by tapping on an absorbent paper towel. Repeat 3 more times for a total of 4 wash cycles**.**

11. Prepare Diluted Tracer

Dilute the Tracer Concentrate 1:30 with the Assay Buffer according to the dilution scheme shown in Table 3. After use, remaining diluted Tracer material may be stored for a maximum of 10 days at 2-8°C, if reuse is desired.

Note: This step may be performed while the plate is being washed.

Table 3: Recommended Dilution Scheme for Tracer Concentrate (30x)*

Number of Strips	Assay Buffer (µL)	Tracer Concentrate (µL)	Number of Strips	Assay Buffer (µL)	Tracer Concentrate (µL)
1	928	32	7	6496	224
2	1856	64	8	7424	256
3	2784	96	9	8352	288
4	3712	128	10	9280	320
5	4640	160	11	10208	352
6	5568	192	12	11020	380

12. Add Diluted Tracer

Pipette 100 µL diluted tracer solution to each well.

13. Seal and Incubate

Cover the wells with a clean plate seal and incubate 1 hour at 20-25°C **without shaking**.

14. Remove Seal and Wash

Carefully remove plate seal and wash wells with diluted wash buffer.

Mechanical washer: 400 µL per well, 4 cycles. Dispensed wash should remain in wells a minimum of 15 seconds before aspiration step. After the fourth wash, empty wells by tapping on an absorbent paper towel. Inspect wells for any remaining wash and repeat tapping on absorbent paper towel if necessary.

Manual wash: Empty wells, add 300 µL wash buffer per well with a wash bottle and soak for 15 seconds; empty wells by tapping on an absorbent paper towel. Repeat 3 more times for a total of 4 wash cycles.

15. Add TMB Substrate and Incubate in the Dark

Pipette 100 µL TMB-substrate (TS) to each well and incubate the plate for 20 minutes at 20-25°C in the dark. *Note: Avoid pipetting directly from the TS bottle. Pour volume needed into intermediate 15 mL conical tube to measure volume needed, then transfer to the reservoir.*

16. Add Stop Solution

Pipette **50 µL** stop solution (ST) to each well. Mix well by drawing up and down using a clean pipette tip, or by gently tapping the side of the plate. The contents of the well will turn from blue to yellow. ***Note: Other steps have required 100 µL; this step requires only 50 µL.***

17. Remove Bubbles

Check for and remove any bubbles from the liquid surface of each well. Remove any dirt or liquid from the well exterior. *Note: Bubbles may be removed by using a clean pipette tip to gently touch and burst the air bubble; be certain to use a clean tip for each well.*

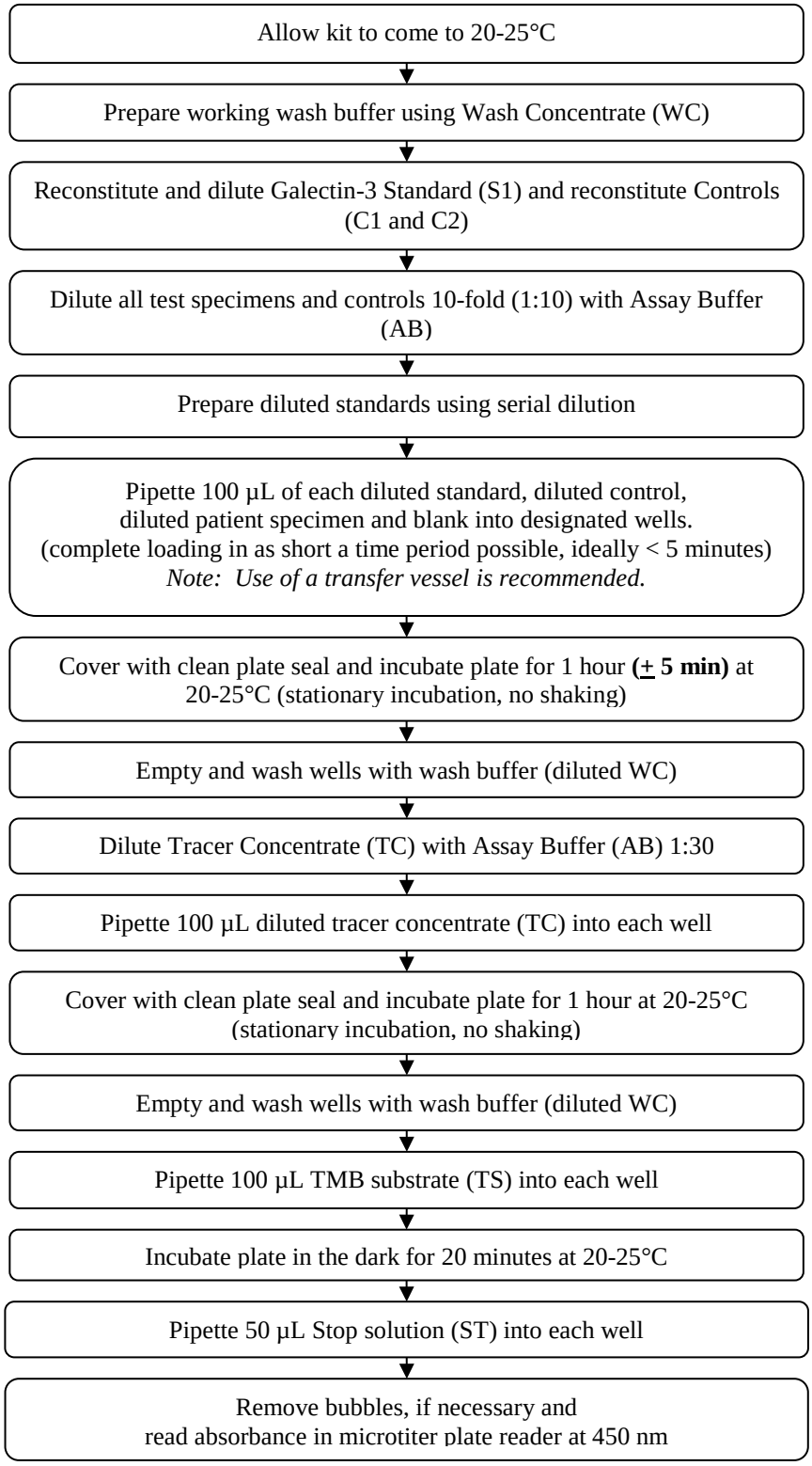
18. Measure Absorbance

Measure the absorbance of each well in a microtiter plate reader at 450 nm within 30 minutes of adding the stop solution.

Procedural Notes

- All components in the kit must be at 20-25°C prior to use.
- Do not pool reagents from different lots.
- All samples should be assayed in duplicate, including blank, diluted standards, diluted controls and all diluted patient specimens.
- Controls (C1 and C2) should be treated exactly like patient specimens (i.e. diluted 1:10, assayed in duplicate, checked for agreement).
- Do not use any reconstituted material without visually verifying complete dissolution before sampling.
- To avoid potential cross-contamination, do not mix up stoppers for the controls and standard. Labeling stoppers is recommended.
- It is critical to keep the loading time of diluted standards, controls and test specimens into the BGM Galectin-3 plate within 5 minutes to reduce within plate variability. Use of a transfer plate and a multi-channel pipette are strongly advised.
- Use clean, dedicated reagent trays and pipette tips for dispensing the conjugate and substrate reagents.
- After each loading step, check for and remove any bubbles from the liquid surface of each well. *Note: Bubbles may be removed by using a clean pipette tip to gently touch and burst the air bubble; be certain to use a clean tip for each well.*
- Do not use a shaker during plate incubation periods.
- Do not reuse plate seals. If partial use is desired, plate seals may be cut.
- If partial plate use is desired, retain plate frame for reuse.
- Discard all consumed reagent upon completion of procedure in compliance with local biohazardous waste regulations.
- Exposure to acids will inactivate the conjugate.

Figure 2: Overview of the BGM Galectin-3 Test Procedure



Calculation of Results

BGM Galectin-3 is based on traditional spectrophotometry and a multi-point standard (calibration) curve. After completing the assay steps, the absorbance of each specimen is read at 450 nm using the microplate reader. The absorbance is proportional to the concentration of galectin-3 in the specimens. Galectin-3 concentrations in the specimens and controls are based on the relationship of the absorbance of the specimens compared to that of the standards, which have a known concentration of galectin-3 and should be assigned using the following procedure:

- Verify that the average absorbance of the blank is less than the average absorbance of the lowest standard. If the absorbance of the blank exceeds the absorbance of the lowest standard (e.g. the low standard absorbance is negative when the blank absorbance is subtracted), the entire plate should be repeated.
- Subtract the average absorbance of the blank from each individual replicate absorbance of the standards, controls and test specimens.
- Calculate the average absorbance for each of the blank-corrected standards. Use the average of the blank-corrected absorbance for each standard to generate the standard curve using either 4-parameter logistic (4PL), 5-parameter logistic (5PL) or third order polynomial (cubic) curve fitting with least squares optimization *(note: avoid use of "Cubic-Spline" curve fit methods).* See 'Materials Required but Not Provided' section for more details. ***Standard curves must be created using all seven (7) standard levels.***
- Calculate concentrations for each of the duplicate measurements of unknown test specimens and controls based upon the selected curve fit equation using the corresponding blank-corrected absorbances. Multiply the measured concentration of specimens and controls by 10 (dilution factor of specimens and controls).
- Calculate the average, standard deviation, and coefficient of variation (CV) of the assigned concentration for each set of duplicate controls and test specimens.
- The coefficient of variation (CV) of the duplicate measurements of controls and test specimens should be within 20%. Specimens with duplicate CVs greater than 20% should be re-analyzed. If either of the controls has a duplicate CV greater than 20%, the entire plate is rejected and all specimens should be re-analyzed.
- Verify that the average concentration of the duplicate measurement for each control is within the corresponding acceptable range. If the average concentration of either control is out of the acceptable range, the assay should be repeated.
- Report the average concentration of the duplicate measurement of each test specimen as the galectin-3 concentration.

For reference, a representative standard curve is shown in Figure 3 and representative values for the absorbance of each diluted standard are shown in Table 4. *Note: The figure and table are shown for informational purposes only and should not be used to derive test results.*

Figure 3: A Representative Standard Curve

Note: The standard curve in Figure 3 is for illustration only.

*Do **not** use to derive test results. A new standard curve should be established for each assay.*

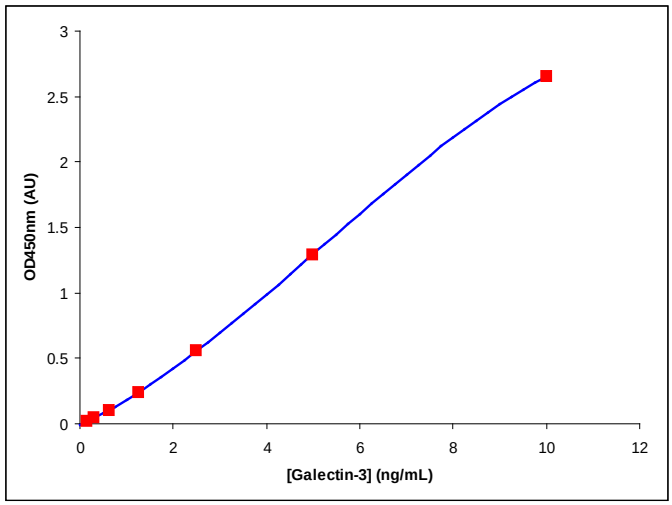


Table 4: A Representative Standard Curve and Typical Absorbance Values at 450 nm

*Note: The standard curve data in Table 4 is for illustration only. Do **not** use data in Table 4 to derive test results. A new standard curve should be established for each assay.*

Dilution	Galectin-3 Concentration (ng/mL)	$Abs_{450\ nm}$
1	10.0	2.690
2	5.0	1.326
3	2.5	0.595
4	1.25	0.269
5	0.625	0.131
6	0.313	0.082
7	0.156	0.054
Blank	0	0.033

Measuring Range of the Assay

The BGM Galectin-3 measuring range is 1.4 to 94.8 ng/mL with clinical specimens. The assay is calibrated with seven standards spanning the range of approximately 0.1 to 10.0 ng/mL. Each test sample (i.e. control or patient specimen) is pre-diluted 1:10 prior to assay allowing the measurement to occur within the range bracketed by the calibrators.

Limitations

- BGM Galectin-3 is intended to be used only in patients with chronic heart failure and should not be used for diagnosis of heart failure.
- There is a possibility that factors such as technical or procedural errors, as well as additional substances in blood specimens that are not listed below, may interfere with the test and cause erroneous results.
- Hemolysis is known to interfere with measurement of galectin-3 using the BGM Galectin-3 assay. Specimens with visible hemolysis should not be used because falsely elevated galectin-3 results may occur.
- Presence of human anti-mouse antibodies (HAMA) or rheumatoid factor (RF) may interfere with the BGM Galectin-3 assay, which could cause falsely elevated results. The BGM Galectin-3 assay should not be used in patients with known HAMA or RF. BGM Galectin-3 results should be interpreted with caution in patients with a history of therapeutic use of murine monoclonal antibodies (IgG) or their fragments, or who have known autoimmune disorders.
- Specimens with high levels of gamma globulins ($\geq$ 2.5 g/dL) may cause false elevation in results. Galectin-3 results from patients with diseases associated with hyperglobulinemia, such as multiple myeloma, should be interpreted with caution.
- DO NOT further dilute patient samples with galectin-3 levels greater than the upper limit of the measurement range (94.8 ng/mL). Recovery of samples that are not diluted at the recommended 1:10 dilution may yield variable results. Report as "galectin-3 value exceeds the upper limit of the measurement range" or utilize language consistent with laboratory or institutional policies.
- Spike/recovery experiments should be avoided to assess the accuracy of BGM Galectin-3 as recovery of exogenously spiked recombinant human galectin-3 does not adequately represent the behavior of endogenous native galectin-3 in clinical specimens.
- Blood samples should be collected in serum tubes (no anticoagulant, no gel barrier) or EDTA plasma tubes only. Citrated plasma is not suitable for use with BGM Galectin-3. Other tube types and anticoagulants have not been evaluated for use with BGM Galectin-3. It is the responsibility of the user to evaluate other tube types.

Clinical Studies and Interpretation of Results

To validate the clinical effectiveness of the BGM Galectin-3 assay, galectin-3 levels were measured in a set of 895 banked EDTA-plasma samples from 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. Sensitivity analysis was performed comparing the set of 895 HF-ACTION subjects having evaluable galectin-3 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. The median follow-up time was approximately 30 months. Participants were categorized at baseline based on the following risk categories:

- galectin-3 greater than 25.9 ng/mL
- galectin-3 greater than 17.8 ng/mL and less than or equal to 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 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 (Table 5, Table 7, Table 9 and Table 12). 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. Figure 4 displays Kaplan Meier curves for the composite endpoint of all-cause mortality and all-cause hospitalization, by baseline galectin-3 category. Figure 5, Figure 6, and Figure 7 display 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 timepoints of 6, 12, 24 and 36 months after baseline.

All-Cause Mortality and All-Cause Hospitalization

Table 5: Hazard Ratios for All-Cause Mortality and All-Cause Hospitalization Events for HF Subjects in the Clinical Validation Study

	Galectin-3 Category		
	≤ 17.8 ng/mL	**> 17.8 and ≤ 25.9 ng/mL**	**> 25.9 ng/mL**
Number of Subjects	647	170	78
Hazard Ratio (95% confidence interval, p value) adjusted for baseline risk factors (age, gender, NYHA functional classification, LVEF, diabetes status, and smoking status)	1.0	1.35 (1.10-1.65, p= 0.004)	1.46 (1.11-1.92, p= 0.006)

Abbreviations: LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.
The reference category is the ≤ 17.8 ng/mL galectin-3 category.

Figure 4: Kaplan-Meier Curves for the Composite Endpoint of All-Cause Mortality and All-Cause Hospitalization, for HF Subjects in the Clinical Validation Study, by Baseline Galectin-3 Level

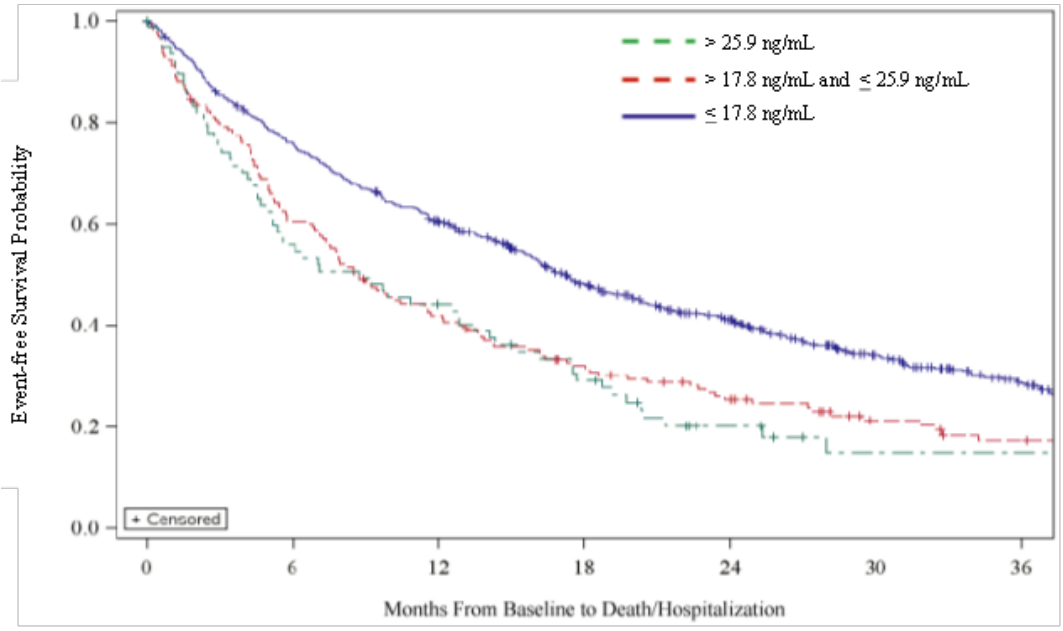


Figure 5: Cumulative Probability of Event for the Composite Endpoint of <u>All-Cause Mortality and All-Cause Hospitalization</u>, at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study

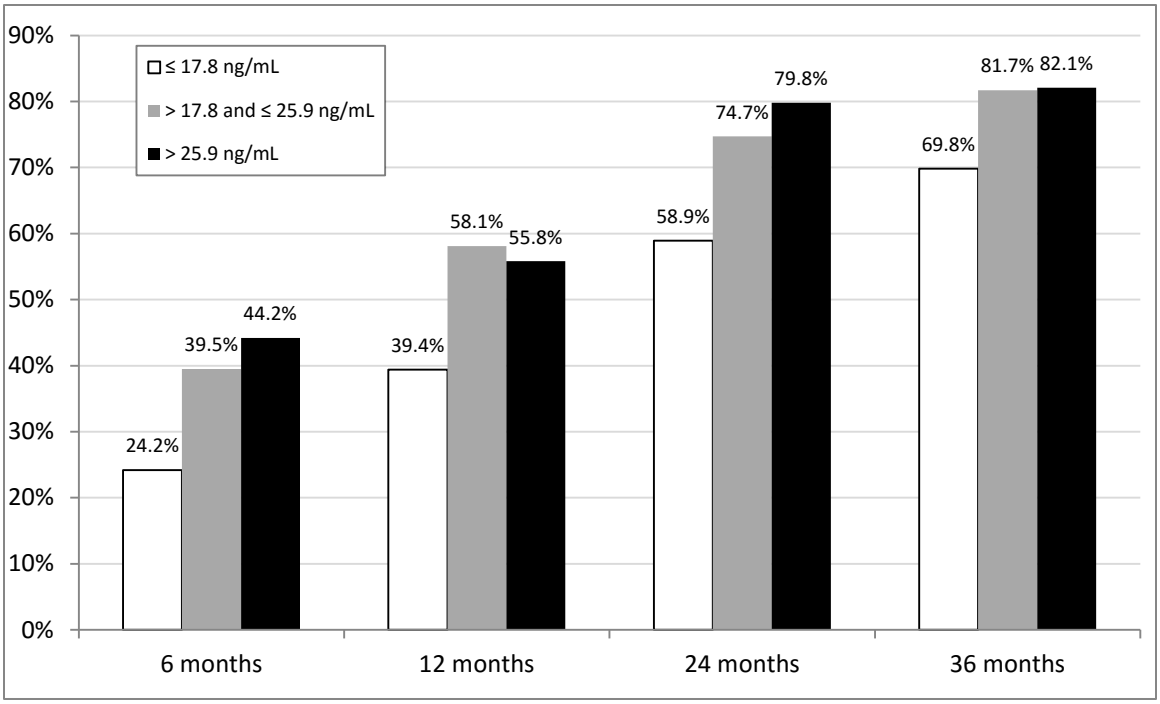


Table 6: Cumulative Probability (with 95% Confidence Intervals) of Event for the Composite Endpoint of <u>All-Cause Mortality and All-Cause Hospitalization</u>, at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study

	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 and ≤ 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)

Cardiovascular Mortality

Table 7: Hazard Ratios for Cardiovascular Mortality Events for HF Subjects in the Clinical Validation Study

	Galectin-3 Category		
	≤ 17.8 ng/mL	**> 17.8 and ≤ 25.9 ng/mL**	**> 25.9 ng/mL**
Number of Subjects	647	170	78
Hazard Ratio (95% confidence interval, p value) adjusted for baseline risk factors (age, gender, NYHA functional classification, LVEF, diabetes status, and smoking status)	1.0	1.91 (1.28-2.86, p= 0.002)	2.33 (1.43-3.80, p < 0.001)

Abbreviations: LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.
The reference category is the ≤ 17.8 ng/mL galectin-3 category.

Figure 6: Cumulative Probability of Event for the Endpoint of Cardiovascular Mortality, at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study

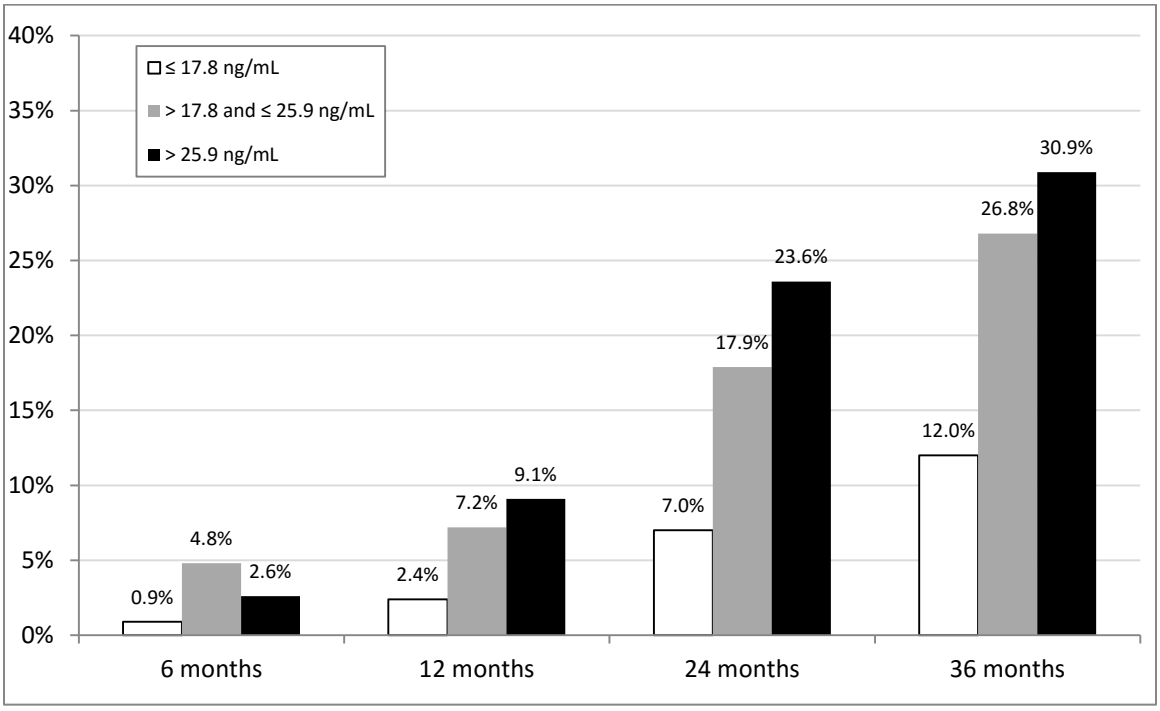


Table 8: **Cumulative Probability (with 95% Confidence Intervals) of Event for the Cardiovascular Mortality, at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study**

	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 and ≤ 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

Table 9: Hazard Ratios for Cardiovascular Mortality and Heart Failure-Related Hospitalization Events for HF Subjects in the Clinical Validation Study

	Galectin-3 Category		
	≤ 17.8 ng/mL	**> 17.8 and ≤ 25.9 ng/mL**	**> 25.9 ng/mL**
Number of Subjects	647	170	78
Hazard Ratio (95% confidence interval, p value) adjusted for baseline risk factors (age, gender, NYHA functional classification, LVEF, diabetes status, and smoking status)	1.0	1.51 (1.14-2.00, p= 0.004)	1.70 (1.19-2.42, p= 0.004)

Abbreviations: LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.
The reference category is the ≤ 17.8 ng/mL galectin-3 category.

Figure 7: 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 HF Subjects in the Clinical Validation Study

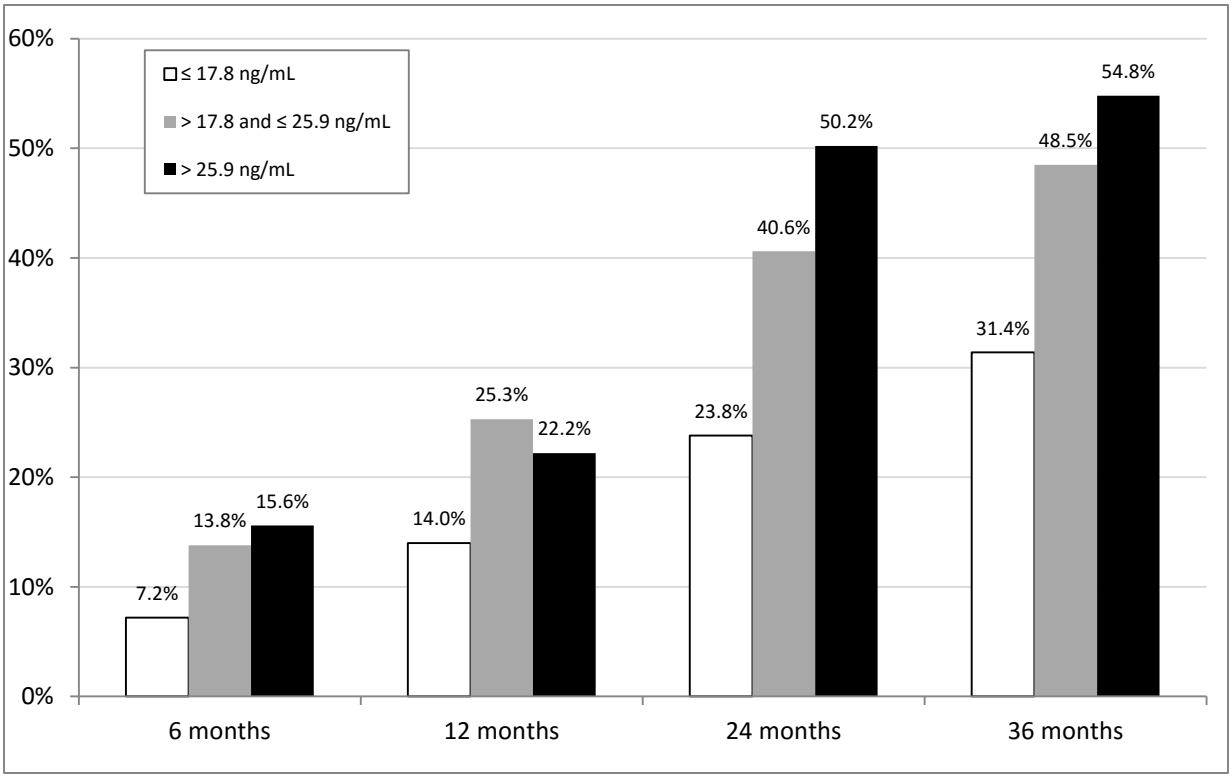


Table 10: Cumulative Probability (with 95% Confidence Intervals) of Event for Cardiovascular Mortality and Heart Failure-Related Hospitalization, at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study

	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 and ≤ 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

Figure 8: Kaplan-Meier Curves for the Endpoint of <u>All-Cause Mortality</u>, for HF Subjects in the Clinical Validation Study, by Baseline Galectin-3 Level

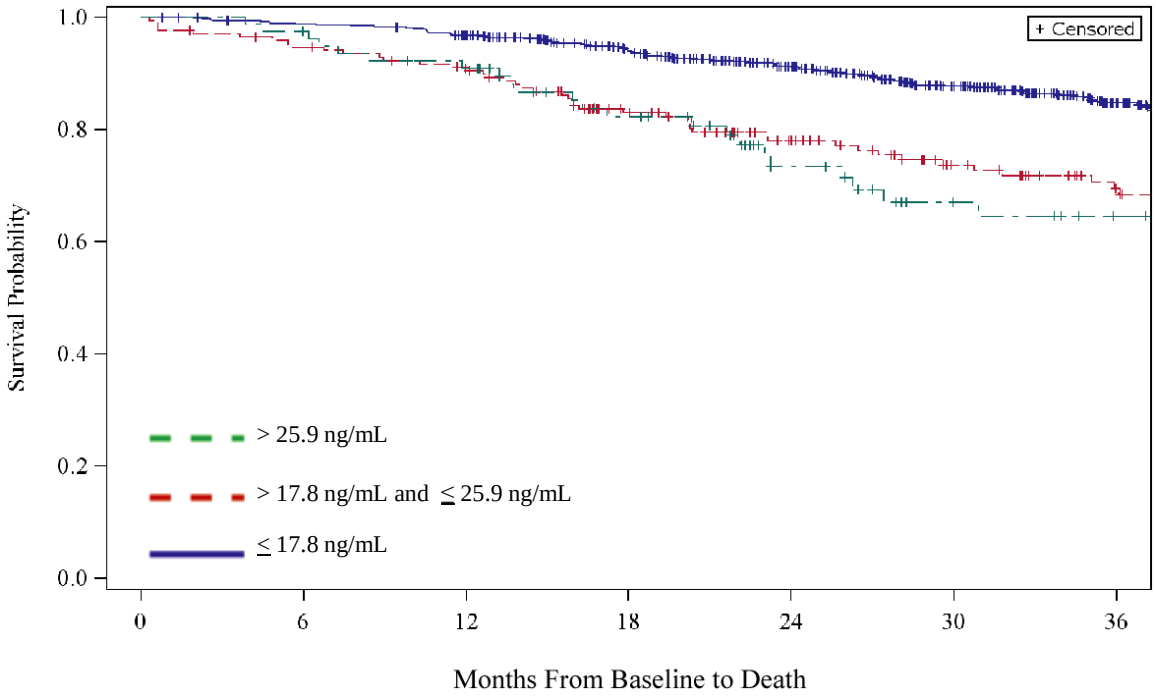


Table 11: Cumulative Probability (with 95% Confidence Intervals) of Event for the Endpoint of <u>All-Cause Mortality</u>, at Various Time Points and By Baseline Galectin-3 Level, for HF Subjects in the Clinical Validation Study

	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 and ≤ 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)

Table 12: Hazard Ratios for <u>All-Cause Mortality Events</u> for HF Subjects in the Clinical Validation Study

	Galectin-3 Category		
	≤ 17.8 ng/mL	**> 17.8 and ≤ 25.9 ng/mL**	**> 25.9 ng/mL**
Number of Subjects	647	170	78
Hazard Ratio (95% confidence interval, p value) adjusted for baseline risk factors (age, gender, NYHA functional classification, LVEF, diabetes status, and smoking status)	1.0	1.84 (1.28-2.64, p= 0.001)	2.06 (1.31-3.23, p= 0.002)

Abbreviations: LVEF = left ventricular ejection fraction; NYHA = New York Heart Association. The reference category is the ≤ 17.8 ng/mL galectin-3 category.

Interpretation

The BGM Galectin-3 assay results should be interpreted in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure.

Patients with chronic heart failure with galectin-3 levels over 17.8 ng/mL were found to have a higher risk of adverse outcomes including mortality or hospitalization compared to patients with levels below 17.8 ng/mL. Galectin-3 levels between 17.8 ng/mL and 25.9 ng/mL should be interpreted with caution because these values lie within the reference range.

Interpretation Relative to Natriuretic Peptides

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.

Table **13** illustrates this for N-terminal pro B-type natriuretic peptide (NT-proBNP) in the clinical validation study by evaluating primary endpoint event rates by categories of galectin-3 and NT-proBNP.

Table 13: Event Rates at 6, 12, 24 and 36 Months for the Composite Endpoint of <u>All-Cause Mortality and All-Cause Hospitalization</u>, by Galectin-3 Category and NT-proBNP level, for HF

Subjects in the Clinical Validation Study. The median value for NT-proBNP in the Clinical Validation Study was 848 pg/mL.

	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%

Performance Characteristics

Precision

Precision of BGM Galectin-3 was assessed in an evaluation according to the CLSI EP5-A2 guideline. Six (6) EDTA-plasma pools spanning a range of galectin-3 concentrations were analyzed in duplicate with two (2) runs per day over twenty (20) days using one (1) reagent lot, two (2) operators and one (1) microtiter plate reader. Estimates of within-run, run-to-run, day-to-day and total precision were calculated and met acceptance criteria. Results are summarized in Table 14.

Table 14: Precision of BGM Galectin-3

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

Table 14 shows the results of the precision evaluation with EDTA-plasma pools. An additional experiment was performed using serum pools which yielded similar results. Additional test pools at multiple galectin-3 concentrations were also tested and yielded similar results.

Clinical Laboratory Precision

Precision was also evaluated at three (3) CLIA-certified clinical laboratories according the CLSI EP5-A2 guideline. The study included testing of EDTA-plasma pools spanning three (3) galectin-3 concentrations, across two (2) reagent lots, using three (3) different models of microtiter plate readers, and a total of four (4) different operators. Total testing days were 17, 18 and 20 days across the three sites, yielding 110 unique analytical runs. Results from each of the CLIA laboratories were within acceptable limits. Within run and total imprecision estimates are summarized in Table 15.

Table 15: Clinical Laboratory Precision - Within Run and Total Imprecision

CLIA Lab	# days, # runs	Galectin-3 mean (ng/mL)	Within Run		Total	
			SD	CV%	SD	CV%
A	20 days, 40 runs	6.0	0.30	5.0	0.46	7.7
		20.1	0.59	2.9	1.20	6.0
		68.3	2.71	4.0	9.97	14.6
B	17 days, 34 runs	6.7	0.36	5.4	0.63	9.4
		21.6	0.56	2.6	1.55	7.2
		75.5	1.71	2.3	12.75	16.9
C	18 days, 36 runs	6.3	0.46	7.3	0.59	9.4
		21.2	0.71	3.3	1.19	5.6
		71.5	2.16	3.0	6.25	8.7

Detection Limit

The limit of detection and limit of quantitation of BGM Galectin-3 were established according to the recommendation of the CLSI EP17-A guideline. 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 LoD = LoB + c_β SDs, where SDs is the pooled standard deviations from four (4) serum samples with different levels of galectin-3, each of which was measured in sixteen (16) replicates (for a total of sixty-four (64) measurements) and c_β 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 of the serum samples closest to while above the LoD, which is 1.32 ng/mL. For this sample, the coefficient of variation (CV) for the galectin-3 measurement was 10.4%.

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

Note: The LoQ does not represent the lower end of the measuring range and should not be used for that purpose. The measuring range is 1.4 to 94.8 ng/mL as reported in the Measuring Range and Linearity sections of this package insert.

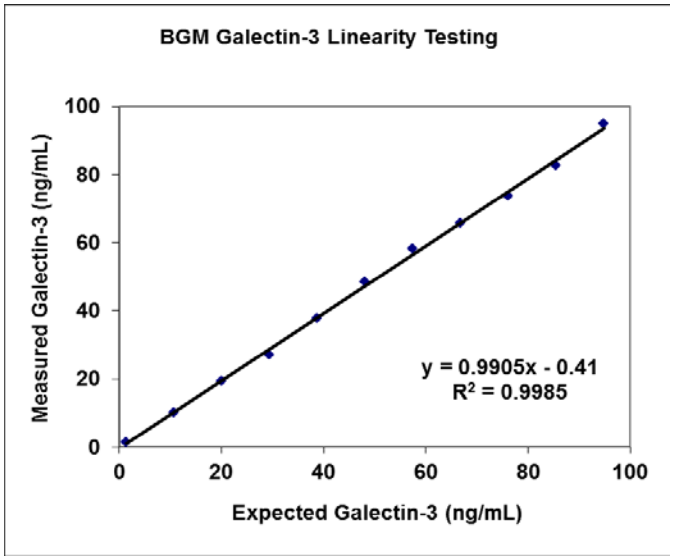
Cross Reactivity

BGM Galectin-3 displayed no significant cross-reactivity when tested in the presence of the following compounds: galectin-1, galectin-2, galectin-4, galectin-7, galectin-8, galectin-9, galectin-12, collagen I and collagen III, all at a concentration of 500 ng/mL. The mean % cross-reactivity of the above potential cross-reactants is at or below 0.3%.

Linearity

The linearity of BGM Galectin-3 was established according to the recommendations of the Clinical and Laboratory Standards Institute Evaluation Protocol 6 (CLSI EP6-A guideline). Samples were prepared to span a clinically-meaningful measurement range of galectin-3 concentrations. Linearity of BGM Galectin-3 was demonstrated between 1.4 and 94.8 ng/mL. These linearity data are shown Figure 9.

Figure 9: Linearity of BGM Galectin-3



Interfering Substances

BGM 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, using an interference acceptance limit of +/-10%. 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 in the assay based on the interference acceptance limit (+/- 10%). Purified hemoglobin (up to 500 mg/dL) does not show significant interference in BGM Galectin-3; however, packed blood cell lysate does show interference.

Human anti-mouse antibodies (HAMA) and rheumatoid factor (RF) cause significant positive interference and rheumatoid factor (RF) greater than 50 IU/mL causes significant positive interference with BGM Galectin-3. High levels of gamma globulins ($\geq$ 2.5 g/dL) may cause false elevation in galectin-3 levels. This information is summarized in Table 16.

Table 16: Endogenous Interference Summary

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 $\geq$ 2.5 g/dL

BGM Galectin-3 measurements were not significantly affected when tested in the presence of thirty-four (34) common pharmaceutical substances; including HF drugs (refer to Table 17). All analytes fall within the interference acceptance limit of +/- 10%.

Table 17: Common Drugs That Did Not Show 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	

High Dose Hook Effect

There is no high dose hook effect at galectin-3 levels up to 500 ng/mL.

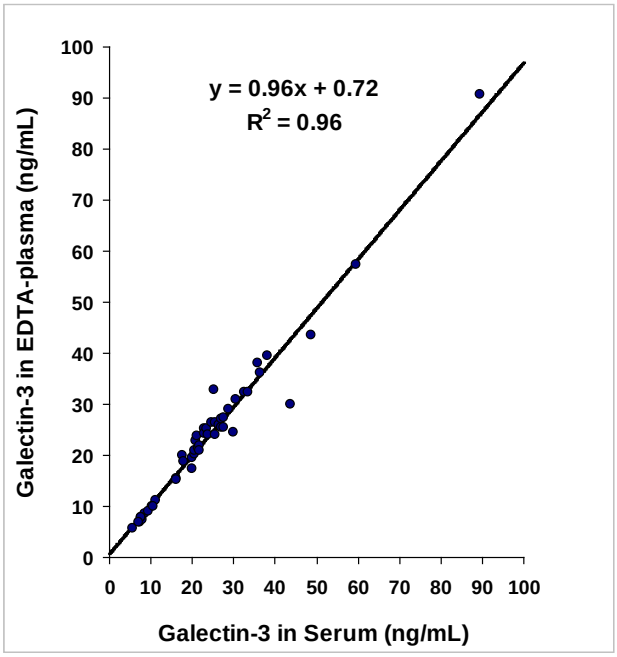
Dilution Parallelism

Dilution parallelism was evaluated by analyzing ten (10) clinical specimens with endogenous native galectin-3 concentrations from 21.6 ng/mL to 88.5 ng/mL at 1:20, 1:40, 1:80 and 1:160 dilutions. The grand mean recovery was 97.6%. For patient samples, dilute ten-fold (1:10) prior to measurement according to the instructions provided in the Procedure Section. Dilutions other than ten-fold are not recommended.

Sample Matrices

The BGM Galectin-3 assay has been validated for use with plasma and serum. The equivalence of serum (no anticoagulant, no gel barrier) and EDTA-plasma sample matrices were demonstrated in a study of forty-nine (49) matched serum and EDTA-plasma samples with values spanning the measurement range. The regression equation is shown in the x/y scatter plot in Figure 10.

Figure 10: **BGM Galectin-3 Serum and EDTA-Plasma Regression**



Reference Range Study

A reference distribution for galectin-3 was determined through an observational study. Galectin-3 levels were measured in 1,099 banked plasma samples from a 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. Blood plasma samples were collected from study participant into tubes containing EDTA. The blood was processed and blood plasma was subsequently frozen at -20°C or colder. Table 18 summarizes the galectin-3 distribution results. The 97.5th percentile of the galectin-3 distribution from this reference population is 26.2 ng/mL.

Each laboratory should establish a reference range that is representative of the patient population to be evaluated. Additionally, each laboratory should consider their current practice in the evaluation of heart failure patients at each institution.

Table 18: Distribution of Galectin-3 Levels in Subjects without Known Heart Disease

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

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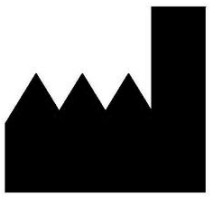

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