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

A. 510(k) Number:
k063662

B. Purpose for Submission:
New device

C. Measurand:
N-terminal pro-brain natriuretic peptide (NT-proBNP)

D. Type of Test:
Quantitative, immunochromatographic fluorescence immunoassay

E. Applicant:
Response Biomedical Corporation

F. Proprietary and Established Names:
RAMP NT-proBNP Assay

G. Regulatory Information:

1. Regulation section:
   21 CFR §862.1117

2. Classification:
   Class II

3. Product code:
   NBC - B-type natriuretic peptide test system

4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See indications for use below.
2. Indication(s) for use:
   The RAMP NT-proBNP Assay is a quantitative immunochromatographic test indicated for use as an in vitro diagnostic product used with the RAMP Reader to measure N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in EDTA whole blood. Measurement of NT-proBNP aids in the diagnosis and assessment of severity in individuals suspected of having congestive heart failure and may aid in the risk stratification of patients with heart failure.

3. Special conditions for use statement(s):
   Prescription use only
4. Special instrument requirements:
   RAMP® Clinical Reader (k033747)

I. Device Description:

The RAMP NT-proBNP Assay consists of test cartridges, assay tips, sample buffer vials for preparation of the sample, a transfer device, a lot card, and the package insert. The RAMP® Clinical Reader is required but provided separately. External quality control materials are suggested but not provided.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Roche Diagnostics Elecsys NT-proBNP Assay
2. Predicate 510(k) number(s):
   k022516, k032646, k051382
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The RAMP NT-proBNP Assay is a quantitative immunochromatographic test indicated for use as an in vitro diagnostic product used with the RAMP Reader to measure N-terminal pro-brain natriuretic peptide (NT-proBNP) levels in EDTA whole blood.	Immunoassay for the in vitro quantitative determination of N-terminal pro-brain natriuretic peptide in human serum and plasma.
Measuring Range	34 -22,000 pg/mL	5-35,000 pg/mL

Differences		
Item	Device	Predicate
Indication for Use	Measurement of NT-proBNP aids in the diagnosis and assessment of severity in individuals suspected of having congestive heart failure (CHF) and may aid in the risk stratification of patients with acute coronary syndrome and heart failure.	Elecsys proBNP is used as an aid in the diagnosis of individuals suspected of having congestive heart failure. The test is further indicated for the risk stratification of patients with acute coronary syndrome and congestive heart failure. The test may also serve as an aid in the assessment of increased risk of cardiovascular

Differences		
Item	Device	Predicate
		events and mortality in patients at risk for heart failure who have stable coronary disease.
Test Principle	Immunochromatographic fluorescence immunoassay	Electrochemiluminescent Immunoassay
Antibodies Used	One sheep polyclonal and one mouse monoclonal antibody, recognizing epitopes located in the N-terminal part (1-76) of proBNP (1-108)	Two sheep polyclonal antibodies, recognizing epitopes located in the N-terminal part (1-76) of proBNP (1-108)
Site of Use	Central laboratory and point-of-care facilities	Same
Specimen Type	Whole blood (EDTA)	Serum and Plasma (lithium and sodium heparin, EDTA)
Instrument	RAMP Reader	Elecsys 1010, Elecsys 2010 and MODULAR analytics E 170 family of analyzers
Test Time	15 minutes	18 minutes
Standard Curve	Lot specific values provided on Lot Card	Generate with each reagent lot
Limit of Detection	34 pg/mL	5 pg/mL
Assay Cutoff	125 pg/mL for patients younger than 75 years and 450 pg/mL for patients ≥75 years	125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years and older

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

- CLSI EP5-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition"
- CLSI EP7-A "Interference Testing in Clinical Chemistry; Approved Guideline"
- CLSI EP9-A "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline"
- "Class II Special Control Guidance Document for B-Type Natriuretic Peptide Pre-Market Notifications; Final Guidance for Industry and FDA Reviewers"

L. Test Principle:

The RAMP NT-proBNP Assay is a quantitative immunochromatographic test for the determination of NT-proBNP levels in EDTA whole blood. Diluted EDTA whole blood is added to the sample well of the Test Cartridge which houses the immunochromatographic test strip. The red blood cells are retained in the sample pad, and the separated plasma migrates along the strip. Fluorescent-dyed latex particles

coated with anti- NT-proBNP antibodies bind to the NT-proBNP present in the sample. As the sample migrates along the strip, NT-proBNP bound particles are immobilized at the detection zone, and additional particles are immobilized at the internal control zone.

The RAMP Clinical Reader then measures the amount of fluorescence emitted by the complexes bound at the detection zone and at the internal control zone. Using a ratio between the two fluorescence values, a quantitative reading is calculated.

Every test cartridge has an internal standard zone that is scanned as part of the test protocol. This area is used to determine that sufficient sample was applied to the test device, that unbound fluorescent label was properly washed from the detection zone, and the device was inserted and read properly by the instrument. This control also prevents a used cartridge from being re-run by the reader. Antibody quality, system function and assay timing are checked on each assay run. An unacceptable result from the control displays a warning message on the instrument indicating that the test should be repeated.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Within-day precision of the upper end of the claimed assay range was determined by testing ten replicates of each EDTA anti-coagulated whole blood samples (some endogenous, some spiked). The ten replicates were tested by a single operator on a single day. The mean and coefficient of variation (%CV) are shown in the table below:

RAMP NT-proBNP: Within-day precision in EDTA whole blood

Mean (pg/mL)	2306	4051	5889	8445	19504
Std Dev	103	180	256	455	582
CV (%)	4.5	4.4	4.3	5.4	3.0

Another study examined the precision at the lower end of the assay range. NT-proBNP Spiked EDTA anti-coagulated whole blood samples ranging from 73 to 2336 pg/mL were tested five to ten times by a single operator on a single day. The mean and coefficient of variation (%CV) are shown in the table below:

RAMP NT-proBNP: Within-day precision in EDTA whole blood

Mean (pg/mL)	73	113	161	375	625	1243	1836	2336
Std Dev	12	14	11	29	57	58	62	204
CV (%)	16.6	12.1	6.6	7.8	9.2	4.7	3.4	8.7

Between-day precision of the RAMP NT-proBNP assay was determined by one operator assaying duplicates of three concentrations of control material twice each day over a 10 day period:

RAMP NT-proBNP: Between-day precision

	Within-Run		Total	
Mean Control Conc (pg/mL)	SD	% CV	SD	% CV
140	13.2	9.4 %	14.4	10.3 %
449	28.5	6.4 %	44	9.8 %
1675	92.4	5.5 %	149.7	8.9 %

Point Of Care Reproducibility:
One operator at three different clinical sites and two operators at a fourth site tested one of two commercially available control materials in triplicate according to the instructions in the package insert.

RAMP NT-proBNP: Within Day Precision at Clinical Sites

Sample 1: Observed Mean = 285 pg/mL						
Site :	1	2	2	3	4	
Operator:	A	C	D	F	J	
Mean (pg/mL)	**277**	**343**	**328**	**248**	**229**	
SD	26	33	51	22	27	
CV	**9.3%**	**9.5%**	**15.5%**	**8.8%**	**11.8%**	
Sample 2: Observed Mean = 6781 pg/mL						
Site :	1	2	3	3	4	4
Operator:	B	E	G	H	K	L
Mean (pg/mL)	**6961**	**8241**	**6387**	**7357**	**5792**	**5948**
SD	238	549	282	30	716	150
CV	**3.4%**	**6.7%**	**4.4%**	**0.4%**	**12.4%**	**2.5%**

Inter-day precision was evaluated through routine quality control testing results throughout the clinical trial at each clinical site. Sites 1, 2 and 3 performed a single control level each week during the three month trial. Site 4 performed a single level of control on each of 4 RAMP Readers five times across three months. The CVs obtained by each site for each level of control evaluated are presented below:

RAMP NT-proBNP: Inter-day Precision at Clinical Sites

	Level 1				Level 2			
	n =	**Mean**	**SD**	**% CV**	**n =**	**Mean**	**SD**	**% CV**
Site 1	7	310	44	14.0	4	8197	801	9.8
Site 2	7	265	26	9.7	7	6724	355	5.3
Site 3	6	241	29	12.2	7	5759	199	3.5
Site 4	12	255	21	8.4	8	6646	392	5.9

b. *Linearity/assay reportable range:*
The claimed assay range is 34 - 22, 000 pg/mL. A linearity study was conducted to compare observed versus expected values obtained with the RAMP NT-proBNP assay using a normal donor EDTA anticoagulated whole blood spiked with NT-proBNP to obtain values across the assay range. This sample was serially diluted six times with the same donor blood. Samples tested ranged from 343 – 21,921 pg/mL and recovery ranged from 101% to 120%; average percent recovery for all samples was 108%. A Passing-Bablok regression analysis was performed, yielding the following: y = 1.06x -1.4 pg/mL, R = 1.00.

To assess the linearity of the assay around the lower end of the assay range, an EDTA anticoagulated whole blood sample was spiked with a low level of NT-proBNP and diluted with the same donor blood. Test samples ranged from 33 – 264 pg/mL and recovery ranged from 103% to 110%. A Passing-Bablok regression analysis was performed, yielding the following: y = 1.06x – 2.0 pg/mL, R = 1.00.

Hook effect was evaluated using 6 samples containing NT-pro-BNP concentrations ranging from 52- 350,000 pg/mL. The sponsor claimed that there is no hook effect up to 350,000 pg/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The assay is traceable to a purified synthetic NT-pro-BNP material. The assay is calibrated by inserting a lot-specific Lot Card into the reader. The procedure and acceptance criteria used to calibrate the assay lot card was reviewed and found acceptable.

External quality control materials are not provided but the manufacturer cautions operators to follow federal state and local requirements to assess quality control.

Sample stability: The sponsor presented a study that supports the claim that whole blood specimens collected in EDTA may be stored at 2 – 8 °C for up to 2 days before testing. The manufacturer does not recommend freezing the sample.

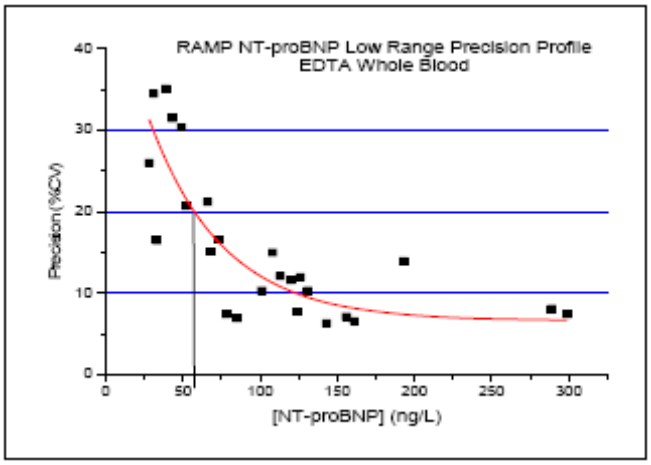
Reagent stability: The sponsor presented a study that supports the instruction to use unpouched cartridges and assay tips within 60 minutes of opening.

d. *Detection limit:*

Limit of Blank (LoB) was calculated as the 95^{th} percentile from forty replicates of a blank sample run using the RAMP NT-proBNP Assay and was determined to be 27.2 pg/mL

The Limit of Detection (LoD) was calculated using the method described in NCCLS guideline EP17-A; the LoD for the RAMP NT-proBNP Assay is 34.5 pg/mL.

An estimate of functional sensitivity for the RAMP NT-proBNP Assay was determined based on 10 replicate measurements of 25 clinical and in-house donor EDTA blood samples in the range of 28 to 299 pg/mL NT-proBNP. The functional sensitivity at 20% in blood is 57 pg/mL NT-proBNP.



e. *Analytical specificity:*
Potential cross-reactivity of other substances with NT-proBNP was evaluated by spiking different concentrations of potential cross-reactants into EDTA blood with NT-proBNP added. Different blood samples were used for each potential cross-reactant. Less than one percent cross-reactivity (calculated as the apparent NT-proBNP concentration divided by the concentration of the cross-reactant and multiplied by 100) was seen for the following substances:

Material Tested	Conc.	Material Tested	Conc.	Material Tested	Conc.
ANP_{28}	3.1 µg/mL	$preproANP_{104-123}$	1 ng/mL	Endothelin	20 pg/mL
BNP_{32}	3.5 µg/mL	Aldosterone	0.6 ng/mL	Arg-Vasopressi	1 ng/mL
CNP_{22}	2.2 µg/mL	Angiotensin I	0.6 ng/mL	Renin	50 ng/mL
$preproANP_{26-55}$	3.5 µg/mL	Angiotensin I	0.6 ng/mL	Andrenomedull	1 ng/mL

Material Tested	Conc.	Material Tested	Conc.	Material Tested	Conc.
preproANP$_{56-92}$	1 ng/mL	Angiotensin I	1 ng/mL	Urodilatin	3.5 µg/mL

Potentially interfering substances were evaluated by spiking different concentrations of potential interferents into EDTA blood with NT-proBNP added. Interference was defined as a difference of ≥ 10% difference between the unspiked and interferent-spiked samples. No evidence of interference was observed for hemoglobin, triglyceride, bilirubin, cholesterol, or heparin at levels of very high physiological concentrations of 2 g/dL, 4 g/dL, 35 mg/dL, 500 mg/dL, and 104 IU/mL, respectively.

Common prescription and over-the-counter compounds, as well as medications often prescribed in a heart failure patient population were tested to characterize their effect, if any, on the RAMP NT-proBNP Assay as described in CLSI EP7-A. Maximum concentrations of potential interferents were determined as equivalent concentrations of up to three times the maximum recommended therapeutic dose as suggested in EP7-A or the concentration reported in other manufacturer's NT-proBNP assay package inserts, whichever was higher. A second concentration, one half of the determined maximum concentration, was also tested. Each concentration of potential interferent was spiked into blood at two medically relevant decision concentrations of NT-proBNP targeting 125 and 450 pg/mL. Independent blood samples were used for each potential interferent, and all testing was performed in duplicate. Interference was evaluated by calculating the NT-proBNP concentration of potential interferent-spiked blood, expressed as a percentage of the NT-proBNP concentration of unspiked (no potential interferent) blood. The sponsor defined no interference as ≤ 10% difference for the average of all concentrations tested. A list of the substances tested is found in the package insert.

The sponsor provided a study that demonstrated that an additive in the sample buffer decreases potential interference from human anti-mouse antibodies (HAMA) and Rheumatoid Factor (RhF). In addition, a warning that heterophilic antibodies may interfere with the assay is included in the limitations section of the package insert.

f. Assay cut-off:

> 125 pg/mL in people < 75 years old and > 450 pg/mL ≥ 75 years old suggests, in conjunction with other clinical information, that congestive heart failure (CHF) is present. The assay cutoffs were established based on the Roche Elecsys proBNP assay: the RAMP NT-proBNP assay is traceable to the Roche method.

2. Comparison studies:

   a. *Method comparison with predicate device:*

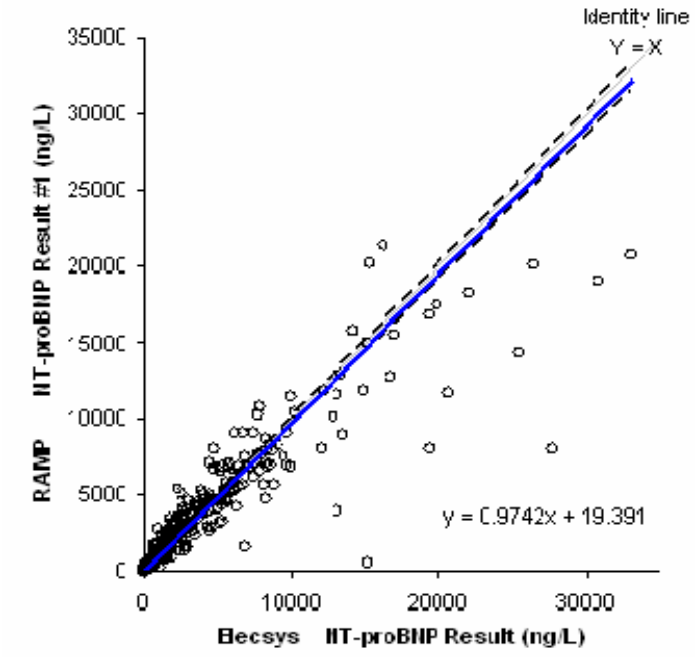
   Six hundred and ninety-nine (699) subjects were enrolled in the method comparison study at four clinical trial sites; subjects were drawn from inpatients, out-patients from heart failure clinics, and patients from an emergency room setting.

   The presenting population included 46% (323) with hypertension, 30% (208) who presented with shortness of breath, 22% (152) with diabetes, 14% (99) with pulmonary disorders, 12% (84) with coronary disease, 8% (56) with atrial fibrillation, 4% (31) with renal failure, 19% (133) were healthy, and the remainder had diagnoses not believed to be cardiac related (hepatitis, HIV, cancer, etc.).

   EDTA and heparin whole blood samples were obtained for each of these subjects. An aliquot of EDTA whole blood was used for the RAMP NT-proBNP Assay while heparinized plasma was prepared for the Roche Elecsys proBNP Assay. Passing-Bablock regression analysis of the 580 samples that contained between 34 pg/mL and 22,000 pg/mL of NT-proBNP (based on the RAMP NT-proBNP Assay results) yielded:

Comparative Method	Slope	Intercept (pg/mL)	Correlation coefficient (R)
Roche Elecsys	0.97	19.39	0.98
95% CI	0.95 to 1.00	14.20 to 24.67	0.97 to 0.98

RAMP NT-proBNP versus Elecsys NT-proBNP



b. Matrix comparison:

Not applicable; the assay only uses EDTA whole blood samples. The package insert cautions the operator not to use heparinized whole blood, plasma, or serum.

3. Clinical studies:

a. Clinical Sensitivity:

Clinical sensitivity and specificity were calculated using data collected from 858 subjects. Of these, 299 were diagnosed with CHF using local hospital criteria, 189 individuals without CHF but with potentially confounding co-morbidity (diabetes, renal insufficiency, hypertension or chronic obstructive pulmonary disease) and 370 reference individuals. This reference group includes an additional 159 subjects added from an additional clinical site without concomitant testing in the Elecsys system. Of these, 55% (87/159) were male and 8% (12/159) were more than 75 years old. None of these patients had reported co-morbidities. Sensitivity and specificity by age are shown in the following table:

Sensitivity and Specificity of RAMP NT-proBNP Assay – (cut-points 125/450 pg/mL for Ages <75 / ≥75)

CHF Patients		
Age (years)	**≤ 75**	**> 75**
N	217	82
Sensitivity	0.89	0.99
95% CI	(0.84-0.93)	(0.92-1.0)
Non-CHF no comorbidity		
Age (years)	**≤ 75**	**>75**
N	340	30
Specificity	0.85	0.72
95% CI	(0.80-0.88)	(0.53-0.87)
Non-CHF with comorbidity		
Age (years)	**≤ 75**	**>75**
N	124	65
Specificity	0.43	0.48
95% CI	(0. 43-0.52)	(0.35-0.60)

* age-stratified cut-points are applied as appropriate

CHF Population by NYHA Classification

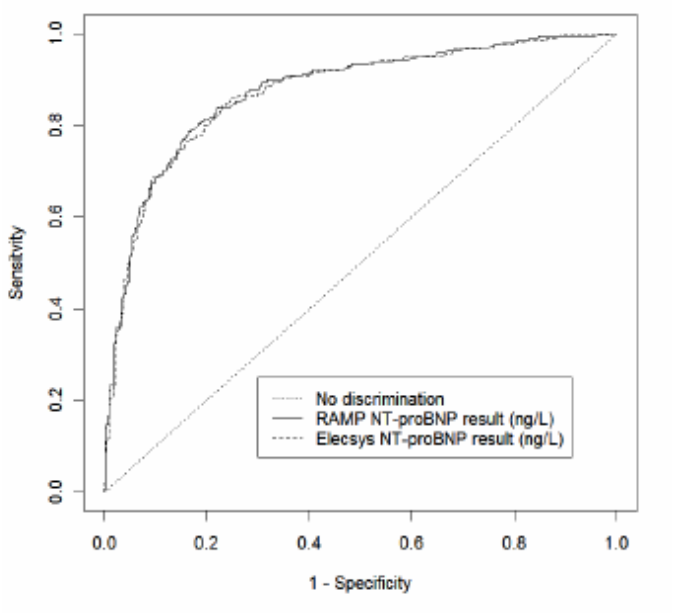
The 299 subjects diagnosed with heart failure were evaluated using the RAMP NT-proBNP Assay. The descriptive statistics for NT-proBNP concentrations are presented according to NYHA Functional Classification in the table below:

All				
NYHA Class	I	II	III	IV
n	58	91	84	66
Mean	1686	2831	5737	8308
SD	3161	4356	5939	7090
Median	832	1479	3608	6628.
95th percentile	5560	8104	20177	> 22000
Male				
n	32	56	55	40
Mean	1737	2870	5799	8855
SD	3924	4641	6182	7612
Median	724	1318	3623	5772
95th percentile	4722	10742	21068	> 22000
Female				
n	26	35	29	26
Mean	1624	2771	5618	7466
SD	1918	3921	5551	6251
Median	907	1622	3598	6937
95th percentile	5438	7306	16727	21839

*b. Clinical specificity**:***
See clinical sensitivity above.

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

4. Clinical cut-off**:**
The Receiver Operator Characteristics (ROC) – All patients
The ROC analyses for both the RAMP NT-proBNP and Roche Elecsys proBNP assays for the 699 subjects in the method comparison study, both inpatients and outpatients, are shown below. The area under the curve (AUC) for both the RAMP NT-proBNP Assay and Elecsys proBNP assays is 0.87.



5. Expected values/Reference range:
   The non-CHF population included apparently healthy subjects (n=133) as well as subjects with hypertension, shortness of breath, diabetes, pulmonary disorders, coronary disease, atrial fibrillation, renal failure, and other non-cardiac related conditions. The CHF population included both stable and acutely decompensated subjects.

RAMP proBNP levels (pg/mL) in non-CHF Subjects, with and without co-morbidity:

	No co-morbidity				With co-morbidity		
Age (years)	**>75**	**≤75**	**ALL**	**Age (years)**	**>75**	**≤75**	**ALL**
n	30	340	370	**n**	65	124	558
Mean	449.7	132.8	158.5	**Mean**	1013.0	870.5	349.4
SD	810.9	671.2	687.8	**SD**	1524.6	3445.1	996.0
Median	88.0	24.5	28.0	**Median**	512.0	185.95	66.0
95th percentile	2447.4	216.4	451.0	**95th percentile**	3986.0	2463.2	1543.9
% <125 pg/mL		84		**% <125 pg/mL**		44	
% < 450 pg/mL	74			**% < 450 pg/mL**	48		

RAMP proBNP levels (pg/mL) in CHF Subjects

Age (years)	**>75**	**≤75**	**ALL**
N	80	203	283
Mean	4970.1	3133.1	3652.4
SD	5185.8	3755.0	4280.2
Median	3300.5	1735.0	2040.0
95th percentile	19005.0	11373.3	12800.0
% >125 pg/mL		89	
% >450 pg/mL	100		

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.