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Multi-center validation of the Response Biomedical Corporation RAMP® NT-proBNP assay with comparison to the Roche Diagnostics GmbH Elecsys® proBNP assay ☆

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Abstract

Background: NT-proBNP measurements aid in the evaluation of patients with suspected heart failure (HF) and may facilitate risk stratification in patients with HF and acute coronary syndrome (ACS). Point-of-care (POC) assays may provide more timely results and potentially improve patient outcomes.
Methods: We evaluated the analytical performance of the Response Biomedical Corporation whole blood RAMP amino-terminal pro-B type natriuretic peptide (NT-proBNP) POC assay compared to the Roche Elecsys proBNP (NT-proBNP) assay.
Results: Intra-day and total imprecision (% CV) ranged from 5.5% to 10.3% at 140, 449 and 1675 ng/L. The lowest concentration that yields a 20% CV was 57 ng/L. The lower limit of detection was 18 ng/L. The upper limit of linearity was validated to 23,428 ng/L with an average recovery of 95%. Correlation by Passing and Bablok regression yielded RAMP = 1.01 Elecsys + 14.6, $r = 0.98$ ($n = 540$; range of Elecsys values <5 to >35,000). Concordance of RAMP versus Elecsys using cut-offs of 125 ng/L for subjects <75 years and 450 ng/L for subjects ≥75 was 92% (95% CI 89–94%) for a group consisting of 127 apparently healthy individuals and 208 non-healthy subjects without HF, and 99% (95% CI 97–100%) for patients with HF, using the New York Heart Association (NYHA) functional classification. Overall, 80%, 87%, 97% and 100% of the RAMP results and 77%, 85%, 96% and 100% of the Elecsys results were greater than the age appropriate cut-off for NYHA I, II, III or IV groups. For both the RAMP and Elecsys results, the median NT-proBNP value was statistically correlated (increasing) with NYHA I, II, III or IV groups, respectively ($p < 0.0001$), with no significant difference between the two methods.
Conclusions: The POC Response Biomedical RAMP NT-proBNP assay provides comparable results that measured on the FDA cleared Roche Elecsys central laboratory platform.

Keywords: NT-proBNP; Point-of-care; RAMP®; Biomedical; Heart failure; Method validation

1. Introduction

Measurement of amino-terminal brain type natriuretic peptide (NT-proBNP) has been shown to be useful for the evaluation of patients with dyspnea, to be superior to clinical judgment for the diagnosis of acute heart failure (HF), and to provide prognostic information in patients with HF and acute coronary syndromes [1–3]. NT-proBNP measurements in patients with acute HF have been shown to reduce hospital length of stay with an associated decrease in the rate of re-hospitalization and post-discharge mortality [4] presumably due to earlier diagnosis and initiation of more secure therapy.

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Table 1
Demographics of 3 study groups: apparently healthy reference group, non-healthy non-heart failure reference group and heart failure group

Group	*N*	Age (Mean)	Male %	Female %	Comorbidity %
Apparent healthy reference	127	44.8 y Range (18–83)	41	59	N/A
Non-healthy (non-CHF) reference	208	60.5 y Range (24–100)	51	49	Diabetes 23 Hypertension 35.5 Pulmonary 8.2 Other 33.2
HF	271	63.9 y Range (19–95)	63	37	Diabetes 32.1 Hypertension 36.9 Pulmonary 5.2 Other 25.8

N/A: Not applicable.
HF: Heart failure.
y: Years.

Traditionally, NT-proBNP has been measured in the central laboratory using conventional immunodiagnostic platforms such as the Roche Diagnostics Elecsys 1010 and 2010 and modular E170, Dade Systems (Dimension and Stratus CS), Ortho Vitros ECi, Siemens Immulite, and other platforms. The Dade System Stratus CS is also suitable for use at the point-of-care.

Guidelines concerning the optimal turnaround time for BNP and NT-proBNP have not been firmly established, although in an ED setting a turnaround time of less than 60 min for natriuretic peptide assays might be optimal. If true, this target would be challenging for the typical central laboratory to deliver, and some institutions would require point-of-care testing. Furthermore, the potential utility of a rapid whole blood point-of-care test for NT-proBNP in the emergency department (ED) setting or in the physician's office would be significant. Currently only the Dade Stratus CS is both FDA cleared and suitable for use at the point-of-care. In this study, we report the results of a multi-center prospective evaluation of the RAMP NT-proBNP (Response Biomedical) rapid whole blood assay with a comparison to the Roche Elecsys 2010 method in the central laboratory.

2. Materials and methods

This study was a multi-center evaluation of the RAMP (Response Biomedical, Burnaby, British Columbia, Canada) NT-proBNP assay on the RAMP reader portable platform. Study sites included; Massachusetts General Hospital, Boston, MA; Hennepin County Medical Center, Minneapolis, MN; Mayo Clinic, Rochester, MN and San Francisco General Hospital, San Francisco, CA. This study was approved by the institutional review board of each study center respectively.

2.1. Analytical methods

The RAMP reader is a fixed wavelength fluorescence spectrophotometer that performs immunoassays using a single-use disposable cartridge. The RAMP Reader currently has Food and Drug Administration 510(k) clearance for myoglobin, CK-MB and troponin I. For the RAMP assay, an EDTA whole blood specimen was collected from each subject. An aliquot of the sample was removed with the transfer device provided in the assay kit, mixed in the provided pre-measured buffer and applied to a single-use disposable cartridge. Results were displayed on the instrument screen in approximately 15 min. Testing for NT-proBNP on the RAMP platform was performed according to the manufacturer's instructions on EDTA whole blood samples. Testing for NT-proBNP was also performed on the Roche Diagnostics (Indianapolis, IN) Elecsys 2010 immunoassay platform using a simultaneously collected heparinized plasma sample according to the manufacturer's instructions. The heparinized plasma samples were immediately frozen at −20 °C at each study site and shipped to the study core laboratory (Hennepin County Medical Center) for Elecsys 2010 NT-proBNP testing.

Intra-day and day-to-day imprecision for the RAMP NT-proBNP assay was assessed by replicate analysis of three concentrations of control materials (140, 449 and 1675 ng/L) in duplicate each day for 10 days. Analytical sensitivity (lower limit of detection) was determined by measuring 20 replicates of the zero calibrator material followed by determination of the mean plus two standard deviations. A sensitivity profile to determine the concentration producing a 20% CV for the RAMP NT-proBNP assay was determined by measuring ten replicates of 25 clinical specimens and normal donors in the range of 28–299 ng/L of NT-proBNP.

Linearity and percent recovery of the RAMP NT-proBNP assay was determined by serial dilution of a high (23,428 ng/L) and a mid-range (12,236 ng/L) EDTA plasma sample. Each sample and 5 serial dilutions were measured in three replicates. The mean, standard deviation and coefficient of variation were calculated for each replicate and a linear regression analysis of the measured versus the expected NT-proBNP concentration was performed.

2.2. Subjects and clinical evaluation

Following informed consent subjects were enrolled in the study and EDTA whole blood and heparinized plasma samples were collected simultaneously. At one site (MGH) patients were not consented and samples were collected from discarded specimens in our emergency department satellite laboratory in

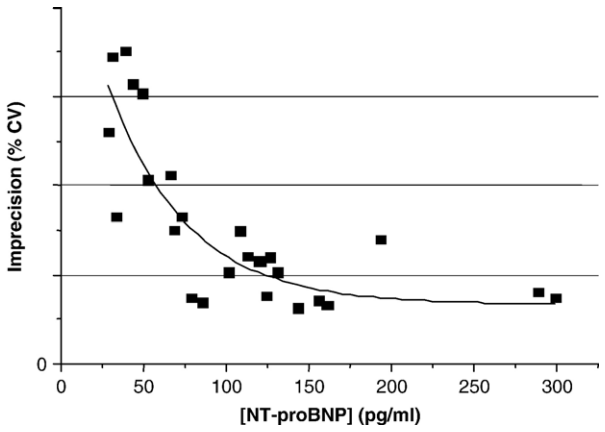


Fig. 1. Functional sensitivity of the RAMP NT-proBNP assay: functional sensitivity = 57 ng/L at 20% CV. CV: Coefficient of variation.

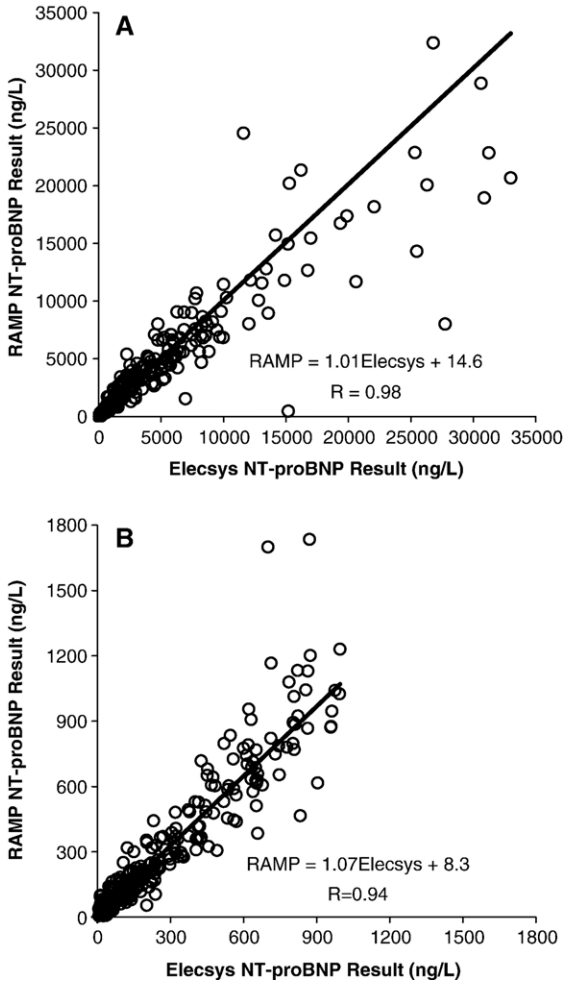


Fig. 2. Comparison of the RAMP NT-proBNP versus the Elecsys NT-proBNP.

conformance with an IRB waiver for discarded samples with medical record review. The protocol was reviewed and approved by the institutional review board at each institution.

Patients were treated according to standard medical care at each respective institution. Diagnoses that were recorded were use to classify patients into New York Heart Association (NYHA) stages I, II, III or IV by investigators at each site who were blinded to the results of the RAMP assay but not to other test results obtained for clinical purposes.

2.3. Patient groups

The reference group included two subgroups. The first consisted of 127 apparently healthy individuals and the second subgroup 208 non-healthy subjects with dyspnea, coronary artery disease, acute coronary syndrome, hypertension, diabetes, pulmonary disease (including pulmonary embolus), or other diagnoses but without acute or chronic heart failure. The heart failure group consisted of 271 patients classified into NYHA stages I, II, III or IV by retrospective chart review by study investigators.

2.4. Statistical analysis

Statistical analysis was performed using the Analyze-It (Analyze-It Software, Leeds, England) for Microsoft Excel software (Microsoft Corporation, Redmond, WA).

3. Results

The demographics and clinical characteristics of the three subject groups are shown in Table 1. The healthy reference group was on average younger than the other two groups ($p<0.001$) with a greater preponderance of women ($p<0.001$). The non-healthy, non-CHF group was similar to the heart failure group in age, gender composition, and comorbidities.

Imprecision ranged from 5.5–9.4% (intra-day CV) to 8.9–10.3% (day-to-day CV).

Fig. 1 shows the results for analysis of the sensitivity profile for the RAMP NT-proBNP assay. The 10% CV was 123 ng/L and the functional sensitivity (defined as the concentration producing a 20% coefficient of variation) was determined to be 57 ng/L.

The analytical sensitivity was determined to be 18 ng/L.

For determination of the linearity and percent recovery for the RAMP NT-proBNP assay, analysis by Passing and Bablok regression of the actual versus expected NT-proBNP concentration yielded $y=1.00x-68$ (percent recovery range 86–100%) for the high concentration sample in the range of 23,428 to 631 ng/L and $y=1.00x-86$ (percent recovery range

		Elecsys		
	Ref	+	-	Total
RAMP	+	104	21	125
	-	6	204	210
	Total	110	225	335

		Elecsys		
	HF	+	-	Total
RAMP	+	247	3	250
	-	0	21	21
		247	24	271

		n	%				n	%	
	Sensitivity	110	94.55			Sensitivity	247	100.00	
	Specificity	225	90.67			Specificity	24	87.50	
	PV+	125	83.20			PV+	250	98.80	
	PV-	210	97.14			PV-	21	100.00	
	C	**335**	**91.94**			**C**	**271**	**98.89**	

Ref: Reference
HF: Heart Failure
PV: Predictive Value
C: Concordance

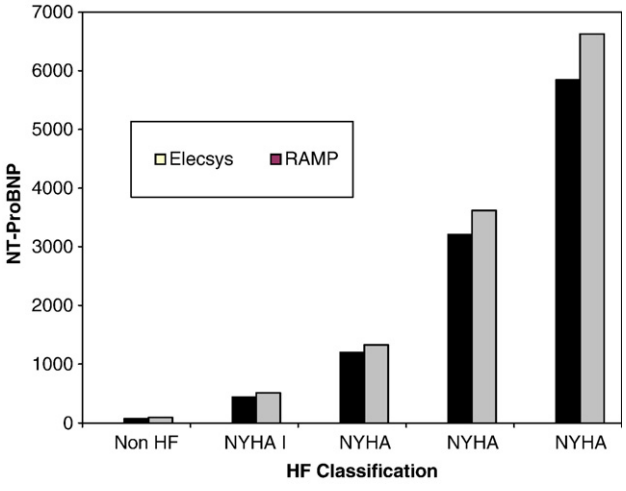
Fig. 3. Concordance between the RAMP NT-proBNP assay and the Elecsys NT-proBNP assay for a reference population (apparently healthy and non-healthy non-heart failure) and for patients with heart failure. Ref: Reference. HF: Heart failure. PV: Predictive value. C: Concordance.

86–100%) for the mid-range sample in the range 12,236 to 382 ng/L respectively.

Fig. 2 shows a comparison of the Roche Elecsys 2010 NT-proBNP to the Response RAMP NT-proBNP assay using data from all three patient groups (healthy, non-healthy without HF and HF). Analysis by Passing and Bablok regression analysis over the full range of sample results yielded RAMP = 1.01 Elecsys + 14.6, $r = 0.98$, $n = 540$ (Fig. 2A). Elimination regression using all data where the Elecsys values were less than 1000 ng/L yielded RAMP = 1.07 Elecsys + 8.3, $r = 0.94$ (Fig. 2B).

Concordance of RAMP versus Elecsys results was assessed using age specific cut-offs of 125 ng/L for subjects <75 years and 450 ng/L for subjects ≥75 years (per Roche package insert). For the reference group the concordance was 92% (95% CI 89–94%, $p = 0.225$, kappa = 0.823). For the heart failure group the concordance was 99% (95% CI 97–100%, $p = 0.641$, kappa = 0.928) (Fig. 3). The reference group was comprised of 127 apparently healthy individuals and 208 non-healthy subjects without heart failure. In the 208 non-healthy reference subset, 50% of RAMP and 54% of Elecsys results were <125 ng/L for subjects <75 years ($p = 0.501$), and 52% of RAMP and Elecsys results were <450 ng/L for subjects ≥75 years. In the 127 apparently healthy reference subset, 82% of RAMP and 89% of Elecsys results were <125 ng/L for subjects <75 years ($p = 0.101$), and 100% of RAMP and Elecsys results were <450 ng/L for subjects ≥75 years. In the heart failure group, 90% of RAMP and 89% of Elecsys results were >125 ng/L for subjects <75 years ($p = 0.745$), and 99% of RAMP and 97% of Elecsys results were >450 ng/L for subjects ≥75 years ($p = 0.563$).

Fig. 4 shows a comparison of the RAMP NT-proBNP assay to the Elecsys NT-proBNP using the New York Heart Association (NYHA) functional classification for heart failure. Overall, 80%, 87%, 97% and 100% of the RAMP results and 77%, 85%, 96% and 100% of the Elecsys results were greater than the age appropriate cut-off for NYHA I, II, III or IV groups respectively. For both the RAMP and Elecsys results, the median NT-proBNP level statistically correlated (increasing) with the NYHA I, II, III or IV groups ($p < .0001$), with no significant difference between the two methods as shown in Fig. 4.



Category	#Cases	RAMP	Elecsys	Mann-Whitney U-Test p Value
Non-CHF Reference	335	99	77	0.07
NYHA I	35	514	442	0.86
NYHA II	87	1325	1197	0.56
NYHA III	83	3618	3207	0.82
NYHA IV	66	6628	5844	0.65

Fig. 4. Median NT-proBNP for the RAMP Elecsys assay according to severity of heart failure.

4. Discussion

In this study we evaluated the POC RAMP NT-proBNP assay in comparison to the central laboratory Elecsys 2010 method. Our data demonstrates that the RAMP NT-proBNP assay compares well to the Roche Elecsys 2010 in terms of imprecision, linearity, lower detection limit, functional sensitivity, crossover by Passing and Bablok regression, and concordance for the classification of patients with heart failure. Although some components of the assay validation such as imprecision were somewhat less robust than the Roche Elecsys package insert claims (total imprecision on Elecsys 2010 of 2.2–3.2% C.V. over range of 20 to 800 ng/L) and our own MGH validation data (1.1–1.6% C.V. over range 8–5179 ng/L) this is not unexpected for a whole blood single-use disposable cartridge platform. However, all of the key performance features of the RAMP assay were within acceptable limits of performance.

The RAMP NT-proBNP assay demonstrated equal clinical performance to the Roche system in differentiating normal and non-healthy non-heart failure patients from those with acute heart failure. Furthermore, when patients were classified according to the New York Heart Association classification of heart failure, the RAMP assay also showed equivalent performance to the Roche system. These observations suggest that extrapolation of cut-points generated for the Roche NT-proBNP method for the evaluation of acute dyspnea to the RAMP NT-proBNP method would be possible.

The RAMP assay can be used in a central laboratory particularly in low volume settings and is also configured for POC use either in a satellite laboratory or at the bedside. In addition, given the growing recognition of natriuretic peptide guided HF management, the use of POC testing in physicians' offices for NT-proBNP measurement would be another obvious application for the RAMP assay. Thus the RAMP device has a number of potential uses across the spectrum of hospital applications including those in the emergency department, central laboratory, inpatient observation units and outpatient offices. Furthermore, unlike most BNP assays where methods from different manufacturers are calibrated differently, the Roche Diagnostics license for NT-proBNP mandates correlation to the parent Roche assay. For this reason NT-proBNP values from different systems should be roughly comparable. This

feature has great advantage for a POC device because diagnostic cut-off concentrations will not be different when the assay is performed on two instruments (point-of-care and central laboratory) in two locations.

In conclusion, we have validated the RAMP NT-proBNP assay and performed a comparison to the Elecsys 2010 central laboratory analyzer. The RAMP test is easy to perform using a whole blood sample and produces rapid results that may facilitate patient care in the hospital or physician's office settings where turnaround time is important for diagnosis or for improving the workflow of clinical operations.

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