Analytical Performance and Diagnostic Accuracy of Immunometric Assays for the Measurement of Plasma B-Type Natriuretic Peptide (BNP) and N-Terminal proBNP, *Aldo Clerico,[1*] Concetta Prontera,[1] Michele Emdin,[2] Claudio Passino,[2] Simona Storti,[1] Roberta Poletti,[2] Luc Zyw,[2] and Gian Carlo Zucchelli[1]* ([1] Laboratory of Cardiovascular Endocrinology and [2] Cardiovascular Medicine Department, CNR Institute of Clinical Physiology, Pisa, Italy; * address correspondence to this author at: Laboratory of Cardiovascular Endocrinology, CNR Institute of Clinical Physiology, Via Trieste 41, 56126 Pisa, Italy; fax 39-0585-493601, e-mail clerico@ifc.cnr.it)

Over the last 5 years, several immunoassay methods for the measurement of B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) became commercially available. Recent studies have confirmed the clinical usefulness of measurements of these cardiac natriuretic peptides for the prognostic stratification of patients with congestive heart failure, for the detection of left ventricular systolic and/or diastolic dysfunction, and for the differential diagnosis of dyspnea *(1–4)*. However, to answer the most urgent requests of current clinical practice and to allow widespread use of BNP and NT-proBNP assays, some key issues had to be solved, such as increasing the sensitivity, precision, and experimental practicability *(5)*. The aim of the present study was to evaluate and compare the analytical and clinical performance of five commercial natriuretic peptide immunoassays.

Throughout this report, the values in healthy individuals and patients are reported as the range, median, and 25th and 75th percentiles. We enrolled 172 healthy adults (89 women and 83 men; age range, 16–73 years; median age, 49.0 years; 25th percentile, 38 years; 75th percentile, 58 years). All participants were nonobese (body mass index, 19.4–27.6 kg/m^2, 24 kg/m^2, 22.8 kg/m^2, and 25.6 kg/m^2), normotensive (diastolic blood pressure, 60–85 mmHg, 70 mmHg, 70 mmHg, and 80 mmHg; systolic blood pressure, 90–140 mmHg, 120 mmHg, 102.5 mmHg, and 124.5 mmHg), and free from acute or chronic diseases. All participants had main plasma indices (including creatinine, urea nitrogen, glucose, uric acid, albumin, enzymes, electrolytes, and hemoglobin) within the appropriate reference intervals, and normal erythrocyte and leukocyte counts and urine analyses. All were asymptomatic and underwent a complete examination by a cardiologist, including a visit, standard 12-lead electrocardiogram, Doppler echocardiographic examination, and a bicycle stress test (when older than 50 years), which excluded silent heart disease.

We also prospectively evaluated 279 consecutive patients with heart failure (211 men and 68 women; age, 20–89 years, 66.0 years, 57.3 years, and 73 years). Cardiac morphology and function were assessed by two-dimensional echocardiography, or cardiac catheterization when needed. Idiopathic dilated cardiomyopathy was diagnosed in 136 patients (48.7%), whereas the other 143 patients suffered from secondary cardiomyopathy. Of these, 126 (45.1%) had ischemic cardiomyopathy. The inclusion criterion was a depressed (<50%) left ventricular ejection fraction [mean (SD), 31.8 (9.4)%; range, 10–48%]. Heart failure severity was evaluated clinically according to the New York Heart Association (NYHA) classification: 32 patients were in functional NYHA class I, 133 were in class II, 81 were in class III, and 33 were in class IV, respectively. Patients were treated with a multidrug treatment (78% of patients treated with frusemide, 94% with angiotensin-converting enzyme inhibitor, 70% with carvedilol, and 58% with spironolactone), which was not stopped at the time of the study.

We collected blood samples from the healthy controls and cardiac patients between 0800 and 0900 in the morning after an overnight fast; all had been resting for 20 min in a supine rest. Immediately after withdrawal, blood samples (8–10 mL) were placed in ice-chilled disposable polypropylene tubes containing EDTA (1 mg/mL of plasma) and aprotinin (500 kIU/mL of plasma). Plasma samples were rapidly separated by centrifugation at 2400*g* for 15 min at 4 °C and then frozen and stored at −20 °C in 0.5-mL aliquots in polypropylene tubes until being assayed; assays were performed as soon as possible.

Four methods for BNP were tested: an IRMA (SHIONORIA BNP; Shionogi & Co) *(6)*; two fully automated immunoassay systems [microparticle enzyme immunoassay (MEIA) method for the AxSYM® System (Abbott Laboratories Diagnostics Division), and ADVIA method for the Centaur System (Bayer Diagnostics Division)] *(7, 8)*; and a point-of-care testing (POCT) method (TRIAGE® BNP Test; Biosite® Diagnostics) *(7, 9)*. We also evaluated the fully automated electrochemiluminescence immunoassay for NT-proBNP on an Elecsys® 2010 analyzer (Roche Diagnostics) *(10, 11)*. All assays were performed according to the manufacturers' instructions.

The imprecision profiles and functional sensitivities, calculated at the points on the imprecision profiles corresponding to CVs of 10% and 20%, respectively, for all immunoassays tested in this study are reported in Table 1A. Each imprecision profile was calculated by repeated measurements (n ≥10) of some plasma samples in different runs (data not shown).

We found close linear relationships among the results obtained with the four BNP methods [linear regression coefficients (*R*) ranging from 0.928 to 0.974; see Fig. 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue2/]. The correlation between the NT-proBNP assay and the BNP assays was lower (r = 0.468–0.715). Different methods gave different natriuretic peptide results for both healthy individuals and patients with heart failure (Table 1). Results obtained with the MEIA system were significantly different (P <0.0001) from those obtained with the other BNP assays. Results of Bland–Altman analyses indicated significant differences approximately proportional to the size of measurement between the MEIA and the other BNP immunoassays (Fig. 1, A–C). MEIA mean values for healthy individuals and patients with mild heart failure (NYHA classes I and II) were up to twofold greater than the values in other

Table 1. Analytical and clinical characteristics of the commercial natriuretic peptide assays.

A. Reference values, 97th percentile values, and functional sensitivities of immunoassays studied

Method	Mean (SD)	Median	Range[a]	97th percentile[b]	Functional sensitivity[c] 20% CV	Functional sensitivity[c] 10% CV
BNP, ng/L						
IRMA	11 (10)	7	0.4–66	40	9	40
MEIA	22 (30)	13	<5 to 221	105	20	
ADVIA	14 (12)	9	<3 to 6	45	7	25
POCT	10 (7)	8	<8 to 63	40	8	30
NT-proBNP, μg/L (ECLIA)[d]	49 (35)	41	7–220	155	9	22

B. Mean (SD) BNP and NT-proBNP concentrations in the entire patient group and in patients divided according to the severity of disease: Mild (NYHA class I or II) or severe (NYHA class III or IV) heart failure

Method	All HF patients(n = 279)	Mild HF (n = 166)	Severe HF (n = 113)	P[e]
BNP, ng/L				
IRMA	301 (378)	170 (235)	494 (459)	<0.0001
MEIA	400 (626)	228 (331)	696 (860)	<0.0001
ADVIA	288 (464)	162 (217)	505 (658)	<0.0001
POCT	331 (714)	148 (203)	891 (1254)	<0.0001
NT-proBNP, μg/L (ECLIA)	3017 (5235)	1329 (1703)	5497 (7302)	<0.0001

[a] Minimum–maximum.
[b] 97th percentile of gaussian distribution in a healthy population.
[c] Lowest concentration measured with a CV of 10% or 20%
[d] ECLIA, electrochemiluminescence immunoassay; HF, heart failure.
[e] *P* values for differences between mild and severe heart failure patients and healthy individuals (by ANOVA after log-transformation of original data).

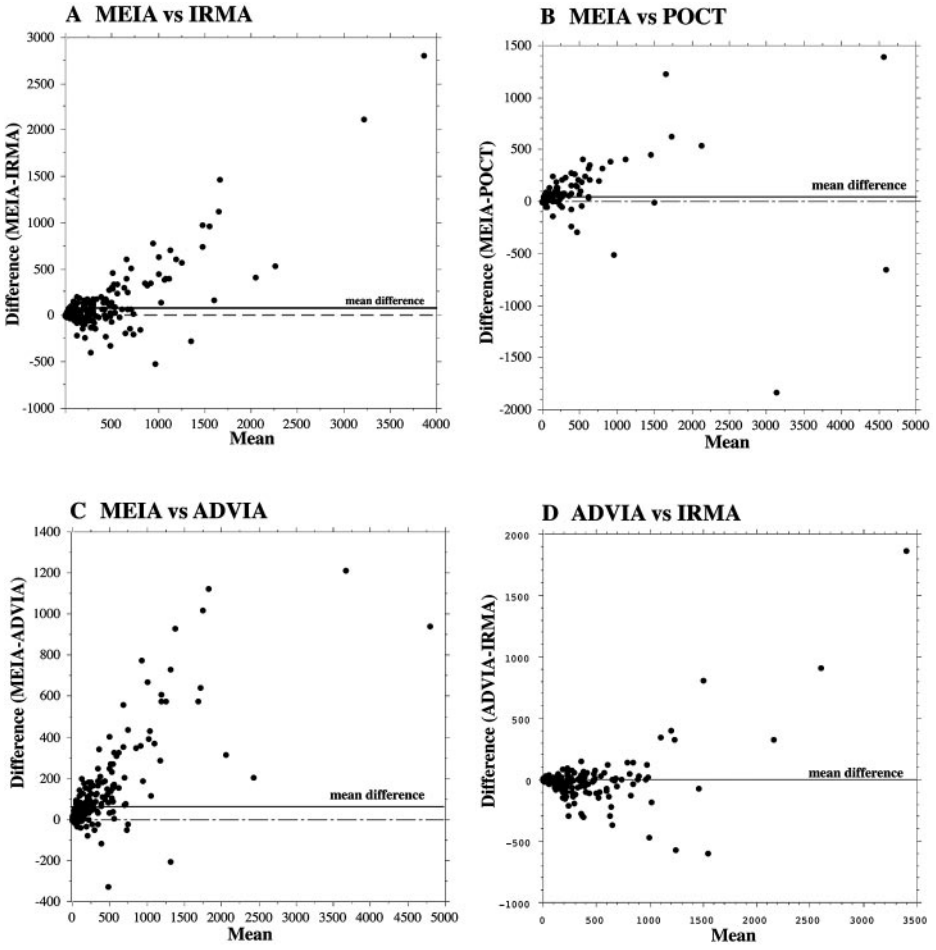

Fig. 1. Bland–Altman plots comparing the results obtained with the MEIA and IRMA methods (*A*), the MEIA and POCT methods (*B*), the MEIA and ADVIA methods (*C*), and the ADVIA and IRMA methods (*D*).

BNP immunoassays. We found better agreement between the results obtained with the IRMA and ADVIA methods (Fig. 1D; see also Fig. 1D in the online Data Supplement). We also observed a significant difference between the results obtained with these two methods ($P = 0.0032$); the mean (SD) difference was 2.1 (140.8) ng/L.

All immunoassay methods could differentiate between healthy individuals and patients with different degrees of heart failure as well as between patients with mild (NYHA classes I and II) and severe (NYHA classes III and IV) heart failure (Table 1B).

We tested the diagnostic accuracy of immunoassay methods for BNP and NT-proBNP by ROC curve analysis. All immunoassay methods clearly differentiated between the group of healthy individuals and the two groups of cardiac patients with mild (NYHA classes I and II) or severe (NYHA classes III and IV) heart failure [areas under the curves (AUC), 0.865–0.999]. The electrochemiluminescence immunoassay for NT-proBNP (AUC = 0.954; 95% confidence interval, 0.920–0.978) showed the best power, compared with the other immunoassays (AUC values ranging from 0.865 for the ADVIA to 0.902 for the IRMA; $P < 0.01$), for separating healthy individuals from patients with mild symptoms of heart failure. The MEIA method showed different diagnostic characteristics compared with other the BNP immunoassays (McNemar χ^2 test, $P = 0.0036$ vs ADVIA, 0.0083 vs IRMA, and 0.0153 vs POCT TRIAGE) in this group of patients, whereas there were no significant differences in performance among the other immunoassays. All immunoassay methods performed well (AUC values, 0.982–0.999) in differentiating between healthy individuals and patients with severe heart failure.

The main goal of this study was to evaluate the analytical performance of several BNP and NT-proBNP immunoassays in samples subjected to the same preanalytical conditions. This protocol was chosen to better focus on performance differences among the tested methods to reduce as much as possible the other confounding causes of variability typical of multicenter studies or metaanalysis of published data. However, the clinical results of this study cannot be extrapolated to other clinical settings. Comparison of our results with those of other recent studies *(7–14)* suggests that diagnostic accuracy can strongly depend on patient selection and on the cardiac natriuretic peptide assayed (BNP, N-terminal pro-A-type natriuretic peptide, or NT-proBNP), as well as on the analytical performance and diagnostic accuracy of the immunoassay chosen.

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New PCR-Based Assay for Detection of Common Mutations Associated with Rifampin and Isoniazid Resistance in *Mycobacterium tuberculosis*, *Svetlana Dubiley,[1*] Angelina Mayorova,[2] Anna Ignatova,[1] Eugene Kirillov,[2] Valentina Stepanshina,[2] Alexander Kolesnikov,[1] and Igor Shemyakin[2]* ([1] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia; [2] State Research Center for Applied Microbiology, Obolensk, Moscow Region, Russia; * address correspondence to this author at: Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, 16/10 Miklukho-Maklaya, Moscow, 1179997 Russia; fax 7-095-3357103, e-mail lana@ibch.ru)

Tuberculosis imposes a major burden of death and disease on the human population. Each year, tens of millions of people become infected with *Mycobacterium tuberculosis*, several millions develop clinical disease, and more than 2 million die of tuberculosis *(1, 2)*. The successful treatment of tuberculosis depends heavily on timely diagnostics and selection of an adequate treatment strategy. Use of genotyping tools based on molecular techniques decreases the time necessary for the detection of drug resistance in *M. tuberculosis* from several weeks to a few days or even less, and a patient's treatment regimen can