during the same time frame is beyond the scope of this observational study.

Our study highlights the fact that isoprostane concentrations have the same limitations as other measures of obstetric outcome, such as pH and Apgar score: i.e., they are increased in neonates with HIE, but may also be increased in apparently healthy infants and may be within reference values in infants who die from acute causes or in whom a pathology manifests after delivery.

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Comparison of a Fully Automated Immunoassay with a Point-of-Care Testing Method for B-Type Natriuretic Peptide,

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The clinical relevance of B-type natriuretic peptide (BNP) as a diagnostic tool and prognostic marker in patients with cardiovascular diseases has been confirmed recently (1–3). Over the last 5 years, several immunoassay methods for the measurement of BNP, which share some analytical characteristics, such as full automation, turn-around time <60 min, lower imprecision, and/or better analytical and functional sensitivity, have become commercially available (1, 4). The analytical performance and diagnostic accuracy of immunoassays for BNP were compared recently (5, 6). In the present study, we evaluated the analytical performance and diagnostic accuracy of an automated immunochemiluminescent assay for BNP (ACCESS System; Beckman Coulter). We also compared its performance and accuracy with the TRIAGE BNP test (Biosite). The 2 immunoassays use as antigen the intact BNP 1–32 peptide and the same mouse anti-human BNP antibodies. The capture antibody (Scios) recognizes the peptide ring, whereas the detection antibody (Biosite) recognizes an epitope between amino acids 5 and 10 at the NH_2 terminus (6, 7). All statistical analyses were performed by use of parametric tests after log transformation of the original data. Nonparametric tests were also performed and showed identical statistical trends.

We collected blood samples from 91 healthy individuals [51 women and 40 men; mean (SD) age, 43.2 (13.4) years; range, 16–71 years] and 214 patients with idiopathic or secondary cardiomyopathy (only 21 with a left ventricular ejection fraction >50%). Blood was collected between 0800 and 0900 after the individuals had fasted overnight and had rested for 20 min in a supine position. Immediately after withdrawal, blood samples (8–10 mL) were placed in ice-chilled disposable polypropylene tubes containing EDTA (1 mg/mL of plasma). Plasma samples were obtained shortly after venipuncture by centrifugation for 15 min at 4 °C and then were frozen and stored at −20 °C in 0.5-mL aliquots until being assayed.

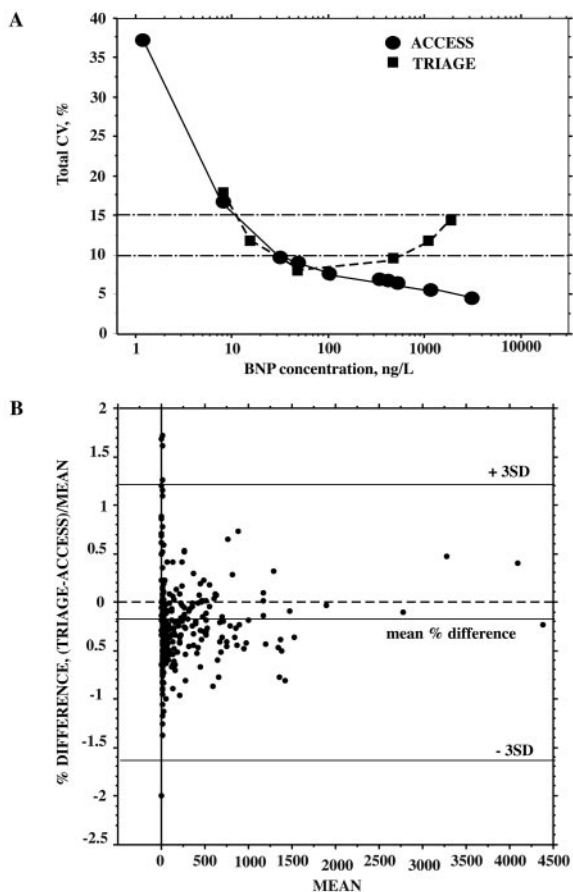We found that plasma samples can be kept at 37 °C for

Fig. 1. Total imprecision profiles (*A*) and Bland–Altman difference plot (*B*) for the tested immunoassays.

(*A*), the CV values corresponding to desirable imprecision (15% CV) and optimum imprecision (10% CV), respectively, are indicated with *dashed lines*. (*B*), the difference plot shows the difference between methods as a percentage (*y axis*) plotted vs the mean value (*x axis*). *Solid horizontal lines* indicate the mean difference and the ± 3 SD values. The *dashed line* indicates no difference.

20 different days of several plasma samples (7 on the TRIAGE and 12 on the ACCESS) with BNP concentrations covering the analytical working range, are shown in Fig. 1A. According to the IFCC goals for desirable (15% total CV) and optimum (10% total CV) imprecision (*7*), the 2 methods had similar functional sensitivities (~10 ng/L and 30 ng/L, respectively). The imprecision for samples with BNP values <10 ng/L was not satisfactory in either method. We assessed the within-run and total imprecision of the ACCESS according to the NCCLS EP5-A guideline (including experimental protocol, number of measurements, and data calculation) (*9*) by repeatedly assaying 2 plasma samples with BNP concentrations of 52.6 and 1095 ng/L in duplicate on 20 different working days (both in the morning and in the afternoon). For the 2 samples, within-run imprecision was 3.4% and 1.6%, respectively, and total imprecision was 8.4% and 5.9%.

After exclusion by statistical analysis of the 17 samples with BNP values <5 ng/L in the TRIAGE assay, we found a close linear relationship ($R = 0.919$; n = 287) between the 2 methods: ACCESS = 62.5 (14.1) + 0.932 (0.024) TRIAGE (P <0.0001 for both). The Bland–Altman plot of the same data confirmed that there was a difference between the values measured with the 2 immunoassays; this difference increased proportionally as the measured concentration increased (Fig. 1B) (*10*). There was a slight but significant mean difference between the values [mean (SD) difference for TRIAGE − ACCESS, −45.2 (217.8) ng/L (minimum–maximum, −1152 to 1667 ng/L; P <0.0001); mean % difference, −0.2% (minimum–maximum, −2.0% to 1.7%)].

All 91 healthy persons were nonobese, normotensive, and free from acute or chronic diseases. Moreover, all had values within the reference intervals for creatinine, urea, glucose, uric acid, albumin, electrolytes, cardiac markers, and hemoglobin as well as for erythrocyte and leukocyte

4 h without a significant decrease in measured (on an ACCESS system) BNP concentration [mean (SD) measured value, 97.8 (5.3)% of the original concentration; mean difference from the original concentration, 10.3 ng/L; 95% confidence interval (CI), −20.4 to 41.1 ng/L (P = 0.1147); median measured value, 790 ng/L (range, 37–2904 ng/L); n = 15]. We observed a slight but significant difference when plasma samples were kept at 4 °C for 24 h [mean measured value 89.0 (5.0)% of the original concentration; mean difference from the original concentration, 27.5 ng/L; 95% CI, 13.7–41.4 ng/L (P <0.0001); median measured value, 160.5 ng/L (range, 12–824 ng/L); n = 24].

We assessed the detection limit of the ACCESS by repeatedly measuring (n = 20) the calibrator with a BNP concentration of 0 ng/L in 3 different runs. The mean (SD) measured concentration was 0.3 (0.1) ng/L (range, 0.3–0.4 ng/L). We confirmed the results of a previous study from our laboratory indicating that the detection limit of the TRIAGE is <8 ng/L (manufacturer-reported detection limit, 5 ng/L) (*8*). The imprecision profiles of the TRIAGE and ACCESS, obtained by repeated measurements over

Table 1. Characteristics of the heart failure patients enrolled for the evaluation of diagnostic accuracy.

		NYHA class	
	All	**I-II**	**III-IV**
No. of patients	193	122	71
Mean (SD) age, years	64 (13)	62 (13)	67 (12)
F/M, n	45/148	28/94	16/55
Mean (SD) BMI,[a] kg/m²	26 (4)	27 (2)	25 (3)
Idiopathic/postischemic/ secondary cardiomyopathy, %	49/36/15	57/26/17	37/51/12
Mean (SD) LVEF, %	34 (10)	37 (11)	27 (7)
Diabetes, %	14	11	18
Treatment, %			
Frusemide	84	36	75
Beta-blockers	71	64	79
ACE inhibitor	78	79	77
ARB	7	4	12
Spironolactone	32	16	59

[a] BMI, body mass index; LVEF, left ventricular ejection fraction; ACE, angiotensinogen-converting enzyme; ARB, angiotensin receptor blocker.

counts and basic urine analysis. 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. The median ACCESS and TRIAGE BNP concentrations were 10.5 ng/L (range, 1–53 ng/L; 97.5th percentile, 45 ng/L) and 8.9 ng/L (range <5 to 52 ng/L; 97.5th percentile, 35 ng/L), respectively. The distribution of BNP values measured by the 2 methods in the healthy group is shown in Fig. 1 of the Data Supplement that accompanies this Technical Brief at http://www.clinchem.org/content/vol51/issue7/.

To evaluate the diagnostic accuracy of the 2 methods, we measured only samples from the 193 patients with a clinically ascertained diagnosis of heart failure. The clinical characteristics of these patients are reported in Table 1. Cardiac morphology and function were assessed by 2-dimensional echocardiography, or cardiac catheterization when needed. The median (range) ACCESS and TRIAGE BNP concentrations measured were 171 (2–4833) ng/L and 118 (<5 to 4930) ng/L, respectively. The distributions of BNP values measured by the 2 methods in these patients grouped according to the severity of heart failure [New York Heart Association (NYHA) functional classes I to IV] are shown in Figs. 1 and 2 of the online Data Supplement. However, the results obtained with both the TRIAGE and ACCESS indicated a significant difference between the mean BNP values in healthy persons vs heart failure patients (P <0.0001 by Scheffè test after ANOVA).

We used ROC curve analysis to evaluate the diagnostic accuracy of the 2 methods in differentiating between healthy persons and patients with heart failure. We found no difference in diagnostic accuracy between the 2 methods for differentiating healthy persons from patients with mild (NYHA class I and II; n = 122; P = 0.196) or severe (NYHA class III and IV; n = 71; P = 0.697) heart failure. For the TRIAGE, the areas under the curves were 0.840 (SE, 0.027; 95% CI, 0.788–0.893) for patients with mild disease and 0.998 (SE, 0.002; 95% CI, 0.995–1.000) for patients with severe heart failure, whereas for the ACCESS system, the areas under the curves were 0.870 (SE, 0.023; 95% CI, 0.825–0.916) for patients with mild disease and 0.997 (SE, 0.002; 95% CI, 0.993–1.000) for patients with severe heart failure. The BNP values corresponding to a sensitivity of 95% in differentiating healthy persons from patients with mild heart failure were 7.5 ng/L (corresponding specificity, 40%) for the ACCESS and 5.1 ng/L for the TRIAGE (corresponding specificity, 29%). The BNP values corresponding to a specificity of 95% were 41 ng/L (corresponding sensitivity, 65%) for the ACCESS and 29 ng/L (corresponding sensitivity, 63%) for the TRIAGE (Fig. 3 of the online Data Supplement). However, it is important to emphasize that the 95% sensitivity with the TRIAGE was obtained at a cutoff near the assay detection limit. The decision cutoff values from the current study are strictly related to our specific clinical setting, comparing healthy persons and patients with clinically ascertained heart failure. In routine clinical practice, several groups of individuals/patients suspected of having a specific disease are usually compared.

The present data confirm and extend previous results suggesting that BNP results are method dependent and that a single predefined common cutoff value cannot be used (5, 6). Furthermore, we demonstrated that immunoassays that use the same antibodies and calibration materials do not automatically give the same results. However, the cutoff and reference values of these 2 methods are similar.

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Invasive Trophoblast Antigen (Hyperglycosylated Human Chorionic Gonadotropin) as a First-Trimester Serum Marker for Down Syndrome, *Martin J.N. Weinans,[1*] Ulrich Sancken,[2] Raj Pandian,[3] Jody M.W. van de Ouweland,[4] Henk W.A. de Bruijn,[4] Jozien P. Holm,[1] and Albert Mantingh[1]* ([1] Antenatal Diagnosis Unit, Department of Obstetrics and Gynaecology, and [4] Department of Pathology and Laboratory Medicine, University Hospital, Groningen, The Netherlands; [2] Institut für Humangenetik der Universität Göttingen, Göttingen, Germany; [3] Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; * address correspondence to this author at: Department of Obstetrics and Gynaecology, University Hospital, PO Box 30.001, 9700 RB Groningen, The Netherlands; fax 31503611806, e-mail martinweinans@planet.nl)

Hyperglycosylated human chorionic gonadotropin (hCG) is a variant of hCG with more asparagine (N)-linked