Performance Characteristics of Four Automated Natriuretic Peptide Assays

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Abstract

Measurement of circulating B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) can identify patients with heart failure and guide therapy. The limit of detection, linearity, imprecision, method comparison, analytic concordance, and reference intervals of the Access 2 BNP (Biosite, San Diego, CA), ADVIA Centaur BNP (Bayer Diagnostics, Tarrytown, NY), AxSYM BNP (Abbott Diagnostics, Abbott Park, IL), and E170 NT-proBNP (Roche Diagnostics, Indianapolis, IN) methods were evaluated. The Triage meter BNP assay (Biosite) was the comparison method. Imprecision testing showed total coefficients of variation of 4.1%, 4.4%, 5.5%, and 0.8% for the Access 2, ADVIA Centaur, AxSYM, and E170, respectively. Relative to the Triage meter, method comparison revealed a slope of 0.96 and r = *0.95, a slope of 0.77 and* r = *0.92, a slope of 1.13 and* r = *0.94, and a slope of 8.8 and* r = *0.80 for the Access 2, ADVIA Centaur, AxSYM, and E170, respectively. Overall analytic concordance values with the Triage meter were 95.9%, 92.9%, 92.4%, and 84.3% for the Access 2, ADVIA Centaur, AxSYM, and E170, respectively. All automated natriuretic peptide methods showed acceptable analytic performance.*

B-type natriuretic peptide (BNP) is a neurohormone that is synthesized in the ventricles of the heart as a 134-amino-acid polypeptide known as preproBNP, which is cleaved to produce proBNP (108 amino acids) and an N-terminal signal peptide (26 amino acids). ProBNP is cleaved to the inactive N-terminal proBNP (NT-proBNP) fragment (amino acids 1-76) and the hormone, BNP (amino acids 77-108).[1] The release of BNP and NT-proBNP is dependent on ventricular myocyte stretch in response to ventricular volume expansion. Measurements of BNP and NT-proBNP have been used to identify patients with heart failure and to monitor the efficacy of their treatment.[2] At present, there are an estimated 5 million Americans with heart failure, and nearly 50,000 new cases are diagnosed every year.[3]

Human BNP (BNP 1-32) is biologically active and is composed of the last 32 amino acids of the carboxyl terminus of proBNP. It has a relatively short circulating half-life of 20 minutes.[1] Studies have shown that there may be more than one form of circulating BNP.[4] Proteolytic degradation can occur in vivo with production of small amounts of des-SerPro-BNP (3-32). In vitro, EDTA-anticoagulated plasma collected in glass tubes can produce the active protease, kallikrein, with consequent loss of amino acids from the C-terminus of BNP.[5] Prolonged incubation of EDTA-anticoagulated plasma collected in plastic tubes produces des-SerPro-BNP owing to proteolytic degradation of the N-terminus. Presumably an aminopeptidase is involved in this process.[4] NT-proBNP comprises the first 76 amino acids of proBNP and is cosecreted with BNP. It is biologically inactive and relatively stable in circulation and in serum in vitro.[6] The circulating half-life is 1 to 2 hours.[1]

A number of commercially available automated assays for BNP and NT-proBNP measurement have become available:

the Access 2 BNP (Biosite, San Diego, CA), ADVIA Centaur BNP (Bayer Diagnostics, Tarrytown, NY), AxSYM BNP (Abbott Diagnostics, Abbott Park, IL), and Elecsys NT-proBNP assays (Roche Diagnostics, Indianapolis, IN).[7-10] These 4 automated methods were evaluated for limit of detection, linearity, imprecision, and reference intervals. Method comparison studies also were performed in which the Triage point-of-care immunoassay (Biosite) was used as the comparison method and analytic concordance was assessed.[11,12]

Materials and Methods

The Access 2 BNP immunoassay, the ADVIA Centaur BNP immunoassay, a microparticle enzyme BNP immunoassay (Axis Shield Diagnostics, Dundee, Scotland) performed on the AxSYM, and the MODULAR ANALYTICS E170 NT-proBNP immunoassay (Roche Diagnostics) automated methods were evaluated. The Triage BNP immunoassay (Biosite) performed on the Triage meter (Biosite) was used as the comparison method. All analyses were performed according to the manufacturers' instructions.

The limit of detection for each of the automated natriuretic peptide methods was determined by performing 2 separate runs and averaging the results. In each run "zero" material, as specified later in this paragraph, was analyzed in 10 replicates, nonzero material was analyzed in 3 replicates as required by EP Evaluator Release 5 software (David G. Rhoads Associates, Kennett Square, PA), and the 2 SD limit of the zero material was calculated. On the Access 2, BNP Calibrator S0 (0 ng/L) and Calibrator S2 (111 ng/L; Biosite) were used. On the ADVIA Centaur, Multi-diluent 1 (0 ng/L) and Master Curve Material Level 3 (72 ng/L; Bayer Diagnostics) were used. On the AxSYM, BNP Calibrator A (0 ng/L) and Calibrator B (100 ng/L; Axis Shield Diagnostics) were used. On the E170, Universal Diluent (0 ng/L) and PreciControl proBNP2 diluted 1:2 with Universal Diluent (final concentration, 140 ng/L; Roche Diagnostics) were used.

Linearity was assessed on the Access 2 by performing serial dilutions of BNP Calibrator S5 with Calibrator S0 (Biosite). Calibrator S5 was diluted to give final concentrations of 0.8%, 2%, 5%, 10%, 20%, 40%, 80%, and 100% of the original (range of BNP concentrations, 42-4,916 ng/L). For the ADVIA Centaur, linearity was assessed by making serial dilutions of Master Curve Material Level 7 with Multi-diluent 1 (Bayer Diagnostics). Master Curve Material Level 7 was diluted with Multi-diluent 1 to give final concentrations of 0.78%, 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50%, 84%, and 100% of the original (range of BNP concentrations, 42-4,298 ng/L). For the AxSYM, linearity was assessed by performing serial dilutions of BNP Calibrator F with BNP Calibrator A with final dilutions of 1%, 2.5%, 6.25%, 12.5%, 25%, 50%, and 90% of the original (range of BNP concentrations, 40-3,671 ng/L). For the E170, proBNP CalCheck Level 3 was diluted serially with Universal Diluent to give final dilutions of 0.105%, 0.21%, 0.52%, 1.04%, 2.08%, 4.17%, 8.33%, 16.67%, 25%, 50%, and 100% of the original (range of NT-proBNP concentrations, 42-20,974 ng/L). All samples were assayed in duplicate for each analyzer.

Imprecision studies were performed with the Access 2, ADVIA Centaur, AxSYM, and E170 using individual manufacturers' quality control material. Three concentrations of lyophilized BNP Controls for the ADVIA Centaur and 2 concentrations of NT-proBNP PreciControls for the E170 were reconstituted according to package insert instructions. Multiple bottles for each level were pooled and divided into aliquots for daily use. All aliquots were stored frozen at –70°C until use. Liquid Access 2 (Biosite) and AxSYM BNP controls were stored at 4°C. Controls were assayed by each method in replicates of 2 using fresh controls for each run. Two runs were conducted per day, on each of 5 days, with a minimum of 2 hours separating each run for a total of 20 replicates for each control level.

For method comparison studies, EDTA-anticoagulated plasma specimens submitted for BNP testing were obtained from –20°C frozen storage following completion of clinical testing. Specimens chosen contained BNP concentrations that spanned a concentration range of 6 to 4,730 ng/L by the Triage meter. The subjects were between 5 and 104 years old. On retrieval, samples were stored at –20°C for up to 2 weeks and then moved to –70°C storage until analysis. Before analysis samples were thawed, mixed thoroughly at low speed, and centrifuged at 2,000*g* for 10 minutes to remove any particulate matter. A total of 197 samples were analyzed by the Access 2, ADVIA Centaur, AxSYM, E170, and Triage meter methods. All testing was carried out according to manufacturers' specifications.

To verify reference intervals, EDTA-anticoagulated plasma specimens from apparently healthy subjects who were not taking any prescription medications were retrieved from –70°C storage and assayed by each automated method. The sample population consisted of 60 men and 60 women between 19 and 61 years of age, with a median age of 30 years. All samples were subjected to the same handling procedures before analysis by each method. All studies using samples obtained from humans were approved by the institutional review board of the University of Utah, Salt Lake City.

Epitope-mapping studies were performed using the Access 2, AxSYM, and ADVIA Centaur BNP methods. Five different human BNP fragments were synthesized at the University of Utah Health Sciences Center Core Research Facility, Salt Lake City, and contained the following amino acid sequences: 1-32, 3-32, 4-32, 10-32, and 1-31. These peptides were purified by reverse phase high-performance liquid

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chromatography and cyclized by reduction and oxidation to form a disulfide bond between the cysteines at positions 10 and 26. A second purification step was performed after cyclization by reverse phase high-performance liquid chromatography, and the purity of each peptide was confirmed by mass spectrometry. Lyophilized peptide fragments were dissolved in a 1:1 solution of dimethyl sulfoxide and water and stored at –70°C. Synthetic peptides were quantified by amino acid analysis after acid hydrolysis. For epitope-mapping studies, each of the synthetic peptides was diluted in a protein-containing diluent provided by Abbott Diagnostics to 1:500,000 and 1:1,000,000 dilutions using volumetric glassware. A sample of the diluent and aliquots of the diluted peptides were run in duplicate on the Access 2, AxSYM, and ADVIA Centaur BNP assays. BNP concentrations were calculated by subtracting the average diluent concentration from the average of the concentrations measured for each dilution of each peptide. The percentages of recovery were determined based on the molar concentrations of peptide diluted in the matrix according to quantification by amino acid analysis.

EP Evaluator Release 5 software was used for limit of detection calculations, linearity assessment, complex imprecision calculations, reference interval determinations, and diagnostic concordance. Passing-Bablok analysis was performed using Analyse-It, version 1.63, for all method comparison studies (Analyse-It Software, Leeds, England).

Results

The limit of detection for each method was assessed and compared with the manufacturers' claimed values. The Access 2 had an average limit of detection of 0.4 ng/L with a manufacturer's claim of 1 ng/L; the ADVIA Centaur an average limit of detection of 0.8 ng/L with a manufacturer's claim of 2 ng/L; the AxSYM an average limit of detection of 9 ng/L with a manufacturer's claim of 15 ng/L; and the E170 an average limit of detection of 3 ng/L with a manufacturer's claim of 5 ng/L. Linearity for all 4 automated methods was assessed. All *r* values were more than 0.99 by linear regression analysis. The target value for each linearity sample was calculated based on the samples with the lowest and highest concentrations within the analytic measurement range for each method. All methods had a maximum average deviation from the target recovery of less than 10%. Imprecision for each method was determined using individual manufacturers' quality control materials as described. All methods demonstrated total imprecision of less than 6% **Table 1**.

Reference intervals were determined for each method using EDTA-anticoagulated plasma samples as described. The range of BNP concentrations observed for samples from healthy control subjects on the Access 2 was between 3 and 81 ng/L with a 97.5% upper reference limit of 42 ng/L. The range of BNP concentrations observed on the ADVIA Centaur was between 0 and 80 ng/L, with a 97.5% upper reference limit of 37 ng/L. The range of BNP concentrations observed on the AxSYM was from 0 to 144 ng/L, with a 97.5% upper reference limit of 79 ng/L. The range of NT-proBNP concentrations observed for samples from healthy subjects on the E170 was between 5 and 147 ng/L, with a 97.5% upper reference limit of 114 ng/L.

Method comparison studies using EDTA-anticoagulated plasma samples revealed varying differences in agreement between the automated methods and the Triage comparison method with slopes ranging from 0.77 to 8.9 and correlation

Table 1
Summary of Imprecision Data for Four Automated Natriuretic Peptide Assays*

Method/Sample	Mean Concentration (ng/L)	Coefficient of Variation (%)			
		Within-Run	Between-Run	Between-Day	Total
Access 2					
L1	879	2.9	1.7	2.3	4.1
L2	408.0	1.6	2.2	1.3	3.0
L3	2,079.7	1.9	0.0	0.8	2.1
ADVIA Centaur					
L1	44.5	2.3	3.8	0.0	4.4
L2	430.6	2.2	0.0	1.4	2.6
L3	1,571.8	1.8	0.4	1.7	2.5
AxSYM					
L1	101.4	5.1	2.1	0.8	5.5
L2	423.2	4.4	1.4	2.2	5.2
L3	1,423.4	3.8	3.0	2.3	5.4
MODULAR ANALYTICS E170					
L1	259.6	0.7	0.4	0.2	0.8
L2	6,039.2	0.4	0.7	0.0	0.8

* For proprietary information, see the text.

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coefficients of 0.80 to 0.95 **Figure 1**. Passing-Bablok analysis showed an average negative bias of 10 ng/L at the 100-ng/L cutoff between the Access 2 and the Triage comparison method. Analytic concordance data showed the Access 2 as having 95.9% agreement with the Triage with 3 false-positive and 5 false-negative results **Table 2**.

Passing-Bablok analysis showed an average negative bias of 26 ng/L at the 100-ng/L cutoff between the ADVIA Centaur and the Triage comparison method. Concordance data showed the ADVIA Centaur as having 92.9% agreement with the Triage with 1 false-positive and 13 false-negative results.

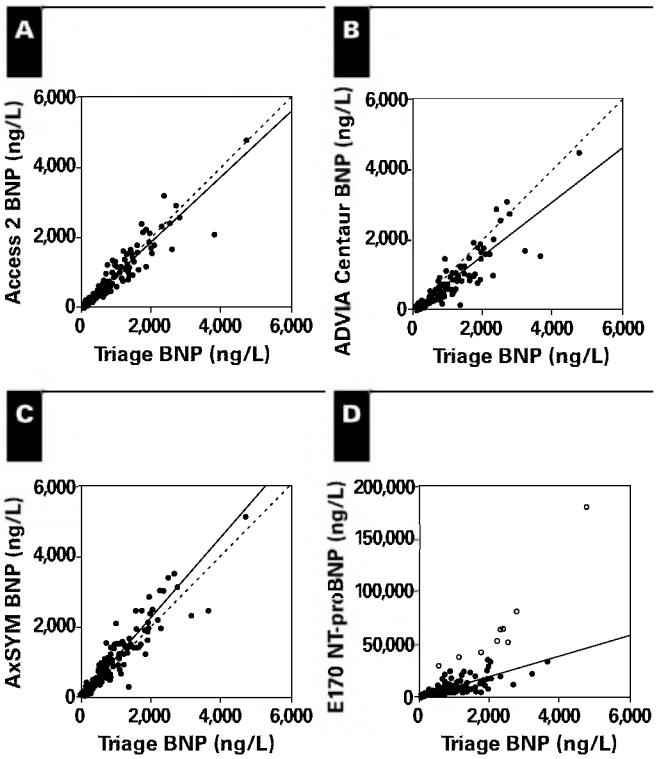

Figure 1 Comparison of 4 automated natriuretic peptide assays with the Triage meter B-type natriuretic peptide (BNP) comparison method. A method comparison study was performed using 197 samples. The dotted line is the line of identity (x = y). Passing-Bablok regression analysis was performed and is indicated by a solid line. **A**, The Access 2 BNP method gave a slope of 0.96 ± 0.02 and an intercept of –6 ± 4 ng/L; $r = 0.95$. **B**, The ADVIA Centaur BNP method gave a slope of 0.77 ± 0.02 and an intercept of –3 ± 3 ng/L; $r = 0.92$. **C**, The AxSYM BNP method gave a slope of 1.13 ± 0.03 and an intercept of –6 ± 4 ng/L; $r = 0.94$. **D**, The E170 NT-proBNP method gave a slope of 8.9 ± 0.5 and an intercept of –225 ± 56 ng/L; $r = 0.80$. Nine samples that were noted to give higher NT-proBNP values are indicated by open circles and were excluded from final statistical analysis. For proprietary information, see the text.

The AxSYM demonstrated an average positive bias of 7 ng/L at the 100-ng/L cutoff compared with the Triage method. The data showed the AxSYM as having 92.4% concordance agreement with 10 false-positive and 5 false-negative results compared with the Triage.

With the E170 NT-proBNP method, there were 9 statistical outliers, confirmed by repeated testing, that were excluded from Passing-Bablok analysis. Before exclusion of the outliers, Passing-Bablok regression analysis gave a slope of 9.7, an intercept of –298.6, and a correlation coefficient of 0.77. Passing-Bablok analysis also showed a high positive proportional bias between the E170 and Triage methods. Analytic concordance results were calculated to show how the 2 methods compare. The E170 had 84.3% agreement with 26 false-positive and 5 false-negative results compared with the Triage method. Creatinine concentrations were measured, and glomerular filtration rates were calculated for the 9 outliers to determine whether the elevated NT-proBNP concentrations were associated with renal insufficiency. Creatinine concentrations were elevated for all 9 outliers, ranging in concentration from 1.5 to 6.4 mg/dL (133-566 µmol/L). The glomerular filtration rate was calculated for each of the 9 outliers using the Abbreviated Modification of Diet in Renal Disease study equation without correction for race because race was unknown.[13] Values ranged from 10 to 39 mL/min per 1.73 m^2.

In **Table 3**, the results of epitope-mapping performed with BNPs and each of the 3 BNP methods are summarized.

Table 2
Analytic Concordance of Four Automated Natriuretic Peptide Methods With the Triage Meter BNP Method*

	Triage BNP		
	≥100 ng/L	<100 ng/L	Total
Access 2 BNP†			
≥100 ng/L	142	3	145
<100 ng/L	5	47	52
Total	147	50	
ADVIA Centaur BNP‡			
≥100 ng/L	134	1	135
<100 ng/L	13	49	62
Total	147	50	
AxSYM BNP§			
≥100 ng/L	142	10	152
<100 ng/L	5	40	45
Total	147	50	
E170 NT-proBNP‖			
≥ Cutoff	142	26	168
< Cutoff	5	24	29
Total	147	50	

BNP, B-type natriuretic peptide.
* Data are given as number of samples in each category. For proprietary information, see the text.
† Overall concordance with Triage, 189/197 (95.9%).
‡ Overall concordance with Triage, 183/197 (92.9%).
§ Overall concordance with Triage, 182/197 (92.4%).
‖ Overall concordance with Triage, 166/197 (84.3%). Age-appropriate cutoffs of <125 ng/L for age younger than 75 years and <450 ng/L for 75 years or older were used.

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The Access 2 method recognized peptides 1-32, 3-32, 4-32, and 1-31. The ADVIA Centaur recognized peptides 1-32, 3-32, 4-32, and 10-32. The AxSYM method recognized 1-32, 3-32, and 4-32.

One additional experiment was conducted with the Triage meter and the Access 2 BNP methods. College of American Pathologists proficiency testing survey material was analyzed singly using 3 lots of Triage meter reagents and 1 lot of Access 2 reagent. Sample BNP-03 from 2003 gave Triage meter results of 241, 343, and 341 ng/L with each of the 3 lots of reagent, and the Access 2 method gave a result of 758 ng/L. Sample BNP-04 from 2003 gave Triage meter results of 908, 1,200, and 973 ng/L with each of the 3 lots of reagent, and the Access 2 method gave a result of 2,679 ng/L.

Table 3
Summary of Molar Immunoreactivity of B-Type Natriuretic Peptides*

	Method		
Peptide	Access 2	ADVIA Centaur	AxSYM
1-32	82	126	106
3-32	69	126	118
4-32	159	175	164
10-32	<1	163	<1
1-31	87	<1	<1

* Data are given as percentages. For proprietary information, see the text.

Discussion

The limit of detection for each method was lower than the manufacturers' claimed limit of detection. Linearity was acceptable for all methods, with an r value of more than 0.99 by linear regression and recoveries for each point falling within ± 10% of the target value. The imprecision of all of the automated methods was adequate, with total coefficients of variation of less than 6%. It is important to note that imprecision at or near the clinical decision threshold of 100 ng/L for the Access 2, ADVIA Centaur, and AxSYM was acceptable. The Access 2 demonstrated a total coefficient of variation of 4.1% at a mean concentration of 87.9 ng/L. The ADVIA Centaur demonstrated a total coefficient of variation of 4.4% at a concentration of 44.5 ng/L. The AxSYM demonstrated a total coefficient of variation of 5.5% at a concentration of 101.4 ng/L. The lowest concentration assessed for imprecision on the E170 was 259.6 ng/L, which gave a total CV of 0.8%. This concentration was higher than the manufacturer's 125-ng/L cutoff for patients 74 years and younger but lower than the 450-ng/L cutoff for patients 75 years and older.

The upper 97.5% reference limit for all methods fell below the manufacturers' clinical decision thresholds. The AxSYM and E170 had upper limits that were only slightly lower than the manufacturers' diagnostic cutoff values. The AxSYM had an upper reference limit of 79 ng/L with a diagnostic cutoff of 100 ng/L, and the E170 had an upper reference limit of 114 ng/L with a diagnostic cutoff of 125 ng/L. The Access 2 and ADVIA Centaur, however, had upper reference limits of 42 ng/L and 37 ng/L, respectively, that were much lower than the diagnostic cutoff of 100 ng/L for each assay.

These results suggest that there will be intermethod differences in sensitivity and specificity. Of the BNP methods evaluated, the ADVIA Centaur would be expected to demonstrate the highest clinical specificity with the lowest sensitivity, whereas the AxSYM would be expected to have the highest clinical sensitivity and the lowest clinical specificity. The E170 would be expected to have the highest clinical sensitivity with the lowest specificity of all methods, assuming approximately equal areas under the receiver operating characteristic curves for BNP and NT-proBNP.[14] A limitation of our reference interval study was that the age range of the 120 reference subjects tested was 19 to 61 years with only 10% older than 45 years. The manufacturers' clinical decision cutoff values were determined using samples from patients younger than 45 years to older than 75 years with heart failure and from age-matched healthy volunteers. No clinical information was available for the samples used in our study. Additional studies that compare the clinical sensitivity and specificity of these assays in the same group of patients with heart failure are needed to better understand the clinical implications of the assay differences we noted.

Method comparison results revealed differences between the automated methods and the Triage comparison method. The E170 showed the poorest agreement with the Triage method. The differences between the E170 and the Triage comparison method can be attributed to the fact that the 2 methods measure different analytes: the Triage measures BNP, whereas the E170 measures NT-proBNP. Our slope of 8.9 was comparable to that in a previous report in which slopes ranged from 6 to 20 in a multicenter evaluation.[9] There were 9 outliers by the E170 method, even after repeated testing, compared with the Triage comparison method. Although the cause for these outliers is uncertain, creatinine concentrations for these samples were all higher than 1.4 mg/dL (124 µmol/L). The glomerular filtration rate was less than 40 mL/min per 1.73 m^2 for each subject from whom these samples were collected. This suggests that renal insufficiency might have contributed to elevations in the NT-proBNP results that were out of proportion to BNP elevations in these subjects.[2]

The differences between the comparison method and the ADVIA Centaur might be due to calibration differences between the methods. The ADVIA Centaur yielded lower results with a substantial negative bias at the clinical decision

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threshold of 100 ng/L compared with the Triage method. We observed a slope of 0.77 that was comparable to a previous report showing a slope of 0.78 compared with the Triage meter.[7] The ADVIA Centaur also demonstrated a higher number of false-negative results than the other methods compared with the Triage.

The AxSYM demonstrated good overall agreement with the Triage comparison method with a slope of 1.13 and correlation coefficient of 0.94. It demonstrated concordance agreement of 92.4% with fewer false-negative than false-positive results compared with the Triage method. We were unable to find published studies comparing the AxSYM and Triage methods. However, a comparison of the ADVIA Centaur and AxSYM methods found a slope of 1.55.[8] Passing-Bablok analysis of the AxSYM vs ADVIA Centaur gave a slope of 1.49, consistent with the findings of the previous study.

The Access 2 demonstrated the highest overall agreement with the Triage comparison method with a slope of 0.96 and a correlation coefficient of 0.95. It demonstrated the highest concordance agreement in comparison with the Triage with slightly fewer false-positive than false-negative results.

The differences between the ADVIA Centaur BNP method compared with the Triage, Access 2, and AxSYM BNP methods suggest that the ADVIA Centaur method is calibrated differently from the other 3 methods.

Our peptide immunoreactivity studies demonstrated similarities and differences in the BNP epitopes recognized by each of the automated BNP immunoassays. It is noteworthy that the recovery of intact BNP (peptide 1-32) shown in Table 3 was not as one might have expected based on our other studies with EDTA-anticoagulated plasma samples. The ADVIA Centaur gave the highest recovery, whereas we would have predicted it would have had the lowest recovery. One explanation is a matrix effect due to the artificial matrix that we used to prepare dilutions of the peptides. This matrix was chosen to minimize proteolytic degradation of the synthetic peptides in volumetric glassware. In future recovery and epitope-mapping studies, it may be more appropriate to use EDTA-anticoagulated plasma, with protease inhibitors added, to minimize any matrix effects. Matrix issues also seem to be substantial with the 2003 College of American Pathologists BNP proficiency testing survey material. The Triage meter and the Access 2 BNP methods gave results that differed by more than 100% for both survey samples. We presume that these 2 assays use the same antibodies because they have a common manufacturer, and with patient samples, they seem to be calibrated equivalently. Even with proficiency testing survey materials prepared in EDTA-anticoagulated plasma with protease inhibitors, as was the case for the 2 samples we studied, the matrix effects still can be substantial.

Matrix issues aside, our cross-reactivity studies suggest that each BNP immunoassay can recognize different circulating forms of BNP. The Access 2 and AxSYM methods recognize an epitope between amino acids 5 and 10 at the N-terminus of BNP. The ADVIA Centaur and AxSYM methods recognize an epitope at the C-terminus of BNP. The AxSYM method reportedly uses a capture antibody that recognizes amino acids 5 to 13 and a detection antibody that recognizes amino acids 26 to 32 (written communication, J. Shih, PhD, Abbott Diagnostics, August 2004). The ADVIA Centaur assay seems to recognize the same epitopes as the Shionogi assay.[4] As previously stated, des-SerPro-BNP (3-32) seems to be the major degradation product of BNP. All BNP assays that we evaluated recognize this product. Differences between BNP methods for specific samples could be due in part to degradation of BNP in these samples.

Further characterization of endogenous BNP fragments and BNP degradation in EDTA-anticoagulated plasma and the exact epitopes recognized by all immunoassays would be useful to better understand the large intermethod differences that are seen for a few samples. The clinical significance of these rare samples that show intermethod differences, if any, requires additional study as well.

All automated methods that we evaluated demonstrated acceptable analytic performance. Additional standardization efforts seem necessary to better harmonize the BNP methods. Also, because of differences in the epitopes recognized by each of the automated BNP methods, rare individual patient samples can show relatively large differences between methods, suggesting that these methods should not be interchanged for serial monitoring of individual patient conditions. The ADVIA Centaur method yields lower results on average than the other methods and likely has lower clinical sensitivity but higher specificity. The E170 NT-proBNP yields substantially higher results and cannot be used interchangeably with BNP methods. Further clinical studies need to be performed to compare the clinical sensitivity and specificity of the different automated methods using the same group of subjects.

Conclusions

All automated natriuretic peptide assays showed acceptable analytic performance. The ADVIA Centaur method gave lower average results than the Access 2, AxSYM, and Triage meter methods. The E170 NT-proBNP method showed poorer overall analytic concordance than the other methods and gave a higher percentage of false-positive results compared with the Triage meter. BNP results are method-dependent, and a single predefined common medical decision point might not be appropriate. Each of the BNP immunoassays uses antibody pairs that recognize different epitopes, which might contribute to assay discordance for individual samples. Matrix effects are known to be problematic with proficiency

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testing survey materials. They clearly are an issue for the Triage meter and Access 2 BNP methods, which use the same antibodies and similar calibrator materials.

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