Influence of Sampling and Storage Conditions on B-Type Natriuretic Peptide Immunoactivity for 3 Automated Assays

To the Editor:

B-Type natriuretic peptide (BNP) is a marker for diagnosis and prognosis of congestive heart failure (CHF). In response to the analytical uncertainties of automated BNP assays, the IFCC Committee on Standardization of Markers of Cardiac Damage published recommendations for the evaluation of in vitro NBP stability (1).

We analyzed the effect of exposure to room temperature for 24 h on whole-blood BNP immunoactivity or the effect of 1 freeze–thaw (FT) cycle on plasma BNP immunoactivity in 3 different assays. Blood samples from 4 CHF patients were collected on ice with Sarstedt tubes containing potassium EDTA (1.6 mg/mL EDTA), EDTA with aprotinin (500 kallikrein-inhibitory units/mL), and EDTA with benzamidine (80 μmol/mL). Whole blood was split into 2 aliquots: one was centrifuged (2400 g for 10 min) for immediate assay (baseline), with the remaining plasma stored at −20 °C; the other aliquot was allowed to stand at room temperature for 24 h under constant gentle mixing before centrifugation and assay. The procedure generated a total of 36 aliquots. BNP recoveries were calculated as percentage of baseline.

Baseline BNP values were 1133–4854 ng/L (mean, 1789 ng/L) with the AxSYM (Abbott Diagnostics), 593–3232 ng/L (mean, 1106 ng/L) with the ADVIA Centaur (Bayer Diagnostics), and 540–3839 ng/L (mean, 1359 ng/L) with the Beckman Coulter Access 2 (BioSite reagents). These values were not affected by protease inhibitors (one-way ANOVA for effect of protease inhibitors, P = 0.93, 0.92, and 0.95 for the AxSYM, Centaur, and Access 2, respectively). Exposure of whole blood to room temperature for 24 h led to a mean decrease of 21%. Neither the sampling conditions nor the assay affected BNP loss (Table 1). These data do not support the claims in the Access 2 and Centaur package inserts (BioSite technical bulletin, December 2004; Bayer 131075 Rev D, 2003), stating that BNP is stable in whole blood for 24 h at room temperature. After 1 FT cycle, the BNP loss averaged 10% and was equivalent for all assays, regardless of the presence of aprotinin or benzamidine, in contrast to one report showing that 1 FT cycle decreased BNP by 36% (2).

We also evaluated BNP stability during shorter storage times (room temperature and 4 °C), without agitation, using conditions close to those likely to be used with samples from outpatient clinics or physicians’ offices. EDTA samples from 5 CHF patients with baseline BNP values of 404-2746 ng/L (mean, 1623 ng/L) on the AxSYM, 232-1694 ng/L (mean, 939 ng/L) on the Centaur, and 234-1917 ng/L (mean, 985 ng/L) on the Access 2 were split and allowed to stand at room temperature and 4 °C for 4 and 8 h before centrifugation and assay. At room temperature, mean (SD) measured values after 4 h were 88 (8)%, 94 (6)%, and 94 (6)% of the baseline values on the AxSYM, Centaur and Access 2, respectively, and after 8 h, the measured concentrations were 85 (14)%, 91 (7)%, and 89 (6)% of baseline values. At 4 °C, after storage for 4 h, measured values were 95 (9)%, 90 (8)%, and 100 (17)% of baseline values on the AxSYM, Centaur, and Access 2, respectively, and after 8 h, they were 90 (10)%, 88 (10)%, and 99 (15)% of baseline values. No significant time or instrument effects were evidenced by ANOVA analysis for storage at room temperature (P = 0.37 and 0.46, respectively) and 4 °C (P = 0.48 and 0.22, respectively). The overall loss of BNP reactivity was 10%, a value comparable to the effect of 1 FT cycle. At 4 °C, loss of BNP immunoreactivity was somewhat lower, averaging 6%. Our results confirm the AxSYM claim, which was the only package insert (8G82-20) reporting the 4 h BNP stability at room temperature. In contrast, using the Biosite BNP POCT Triage® meter, Yeo et al. (3) reported a significant BNP loss after 4 h of storage at room temperature.

In conclusion, our data indicate that protease inhibition does not pro-

References

2. Analytical Services International. Tacrolimus in-duct forms that may or may not affect performance (6,7). I have ob-
served this tacrolimus, but not ascomycin, adduct. However, in my laboratory methanol ascomycin sol-

This work was presented in abstract form at the 9th Congress of the International Association of Therapeutic Drug Monitoring & Clinical Toxicology, Louisville, Kentucky, April 2005.
tect BNP from degradation at room temperature and that BNP assays can be safely performed within the 8 h after sampling, with whole blood being preferably kept at 4 °C, with all 3 instruments tested.

References
2. Mueller T, Gegenhuber A, Dieplinger B, Poelz W, Damien Gruson1*

Damien Gruson1*
Michel F. Rousseau2
Virginie de Coninck1
Sylvie A. Ahn2
Jean-Marie Ketelslegers3
1 Unit of Diabetes and Nutrition
2 Division of Cardiology
3 Catholic University of Louvain
1200 Brussels, Belgium

* Address correspondence to this author: Unit of Diabetes and Nutrition, 54 Avenue Hippocrate, B-1200 Brussels, Belgium. Fax 32-2-7645418; e-mail gruson_damien@yahoo.fr.

DOI: 10.1373/clinchem.2005.062455

Use of B-Type Natriuretic Peptide Testing in a Community Teaching Hospital 4 Years After Implementation and Agreement of Results with Discharge Diagnoses

To the Editor:
We previously reported on the use of B-type natriuretic peptide (BNP) testing in the 400-bed Hennepin County Medical Center at the inception of testing in August 2001 (1). We now describe its use and report the agreement of the results with diagnoses in the patient records in this urban teaching hospital 4 years later.

We queried all BNP orders for June through August 2005 and found 975 test orders on 608 patients. To determine a diagnosis of heart failure, physician discharge dictations and ICD-9 codings were reviewed after Institutional Review Board approval. Final diagnoses were likely influenced by the BNP concentrations in some cases, potentially leading to overestimation of apparent diagnostic accuracy.
The Biosite Triage and Beckman Coulter Access BNP assays were performed according to the manufacturers’ guidelines and were highly correlated (r = 0.99; n = 50), with Bland–Altman analysis demonstrating a mean (SD) difference of 16 (23) ng/L. Total imprecision (CV) near the 100 ng/L cutoff was <12% for both assays.

In 2001, most BNP tests were requested by the emergency department (44%) and cardiology units (41%), noncardiac intensive care unit (ICU; 3.6%), general medicine (3.5%), and miscellaneous clinics (4.1%) (1). In 2005, the emergency department ordered 41% and the cardiology units only 10%, diluted by the increase in test utilization by noncardiac ICUs (16%), general medicine units (15%), miscellaneous clinics (13%), cardiology clinics (3.5%), and miscellaneous inpatient services (1.2%; Table 1). Thirty percent of patients had a diagnosis of heart failure, and 70% did not. One half (50%) of the group were female. The mean (SD) ages of the heart failure and non-heart failure groups were 63 (15) and 58 (16) years, respectively (P < 0.01). The median (interquartile range) BNP concentration was significantly greater for heart failure patients than for non-heart failure patients [482 (13–1024) ng/L vs 61 (5–161) ng/L, respectively; P < 0.01]. BNP was increased (≥100 ng/L) in 92.8% of patients with a diagnosis of heart failure and was within reference values in 53.6% of those without heart failure. In patients without a diagnosis of heart failure, 272 BNP tests had values ≥100 ng/L, representing 28% of ordered tests in these patients (without heart failure). Many of these patients had renal, other cardiovascular, pulmonary, or liver disease and/or multisystem pathology, which can also increase BNP. In patients with a diagnosis of heart failure, 28 BNP results were <100 ng/L. Possible explanations include confounding variables such as

Table 1. Mean (SD) measured BNP immunoreactivities, as percentages of baseline value, for samples subjected to different sampling and storage conditions and tested with various automated assays.

<table>
<thead>
<tr>
<th>Assay method</th>
<th>Storage conditions</th>
<th>EDTA</th>
<th>EDTA + aprotinin</th>
<th>EDTA + benzamidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>AxSYM BNP</td>
<td>RT</td>
<td>73 (12)b,d</td>
<td>79 (21)b,d</td>
<td>68 (15)b,d</td>
</tr>
<tr>
<td></td>
<td>FT</td>
<td>90 (11)f,a</td>
<td>100 (5)f,a</td>
<td>88 (25)f,a</td>
</tr>
<tr>
<td>ADVIA Centaur BNP</td>
<td>RT</td>
<td>80 (5)b,d</td>
<td>85 (6)b,d</td>
<td>80 (7)b,d</td>
</tr>
<tr>
<td></td>
<td>FT</td>
<td>87 (7)f,a</td>
<td>89 (7)f,a</td>
<td>85 (6)c,e</td>
</tr>
<tr>
<td>Biosite Access 2 BNP</td>
<td>RT</td>
<td>80 (8)b,d</td>
<td>85 (8)b,d</td>
<td>78 (5)b,d</td>
</tr>
<tr>
<td></td>
<td>FT</td>
<td>90 (8)c,e</td>
<td>91 (6)c,e</td>
<td>89 (3)c,e</td>
</tr>
</tbody>
</table>

a RT, 24 h at room temperature; FT, 1 freeze-thaw cycle.
b,c BNP values vs baseline (paired t test after log transformation of the variable); d P < 0.05; e P > 0.1.
f Assay and protease inhibitor effects (two-way ANOVA); g P = 0.13 and 0.25, respectively (interaction, P = 0.98); h P = 0.53 and 0.44, respectively (interaction, P = 0.90).