Agreement of Different Immunoassays for Urinary Albumin Measurement

To the Editor:

Diagnosis of diabetic nephropathy (DN) is based on measurements of urinary albumin (UALB) by a sensitive analytical method with sufficient analytical precision (CV <15%) to detect changes in UALB concentration (1–3). We evaluated the comparative diagnostic accuracies of 4 UALB assays for DN.

We used urine samples from 98 diabetic outpatients. UALB was measured by 4 immunoturbidimetric (IT) assays: the Urine Pack Immuno method (Bayer) as implemented on a Roche Cobas Mira Plus (method A), Mabio Apect Diagnostic (BioSys) (method B), Albumin Tina-quant (Roche) (method C), and Microalbumin (Randox) (method D). Methods B, C, and D were implemented on a Hitachi 917 analyzer (Roche). Method A was considered as the reference standard (comparison method) because it had been used in our laboratory since 1996 and validated in previous studies (2, 4, 5). Total urinary protein was measured before UALB, and the U/CSF Protein assay (Roche) on the Hitachi 917 analyzer (Roche) was used to estimate the contribution (%) of albumin to total protein for each sample (5). Based on this estimate, we prediuted samples to allow UALB measurement within the linearity interval for each assay. Urine samples were classified as normo-, micro-, or macroalbuminuric on the basis of the UALB measured using method A (1, 4). Intra- and interassay CVs were calculated for each IT method in pooled material at UALB concentrations of 30 and 100 mg/L. The agreement between methods was evaluated by Bland-Altman plots and k coefficients.

Of 98 urine samples analyzed (58 random and 40 24-h urine), 27 urine samples were classified by method A as normoalbuminuric, 49 as microalbuminuric, and 22 as macroalbuminuric. The intra- and interassay CVs were <6% for all IT methods at both tested UALB concentrations. The analytical sensitivities ranged from 3 to 5 mg/L, and the upper limits of linearity from 160 to 400 mg/L. Method C had the highest linearity. All methods had an excellent correlation (r) with the comparison method A: 0.991, 0.996, and 0.988; P < 0.05, for methods B, C, and D, respectively. UALB results among the 4 methods were not found to be different (ANOVA; P > 0.812), and no significant differences were seen in pairwise comparisons of UALB values (n = 98) obtained with each method and those obtained with the comparison method A [median (range) 54.4 (5.0–1392.0) mg/L, method B [59.3 (2.3–1151.0) mg/L], method C [56.0 (3.0–1109.0) mg/L], and method D [48.2 (4.0–950.0) mg/L], P > 0.05. The observed mean differences (range) in UALB (mg/L) were: method A vs B, 8.17 (–1.23 to 17.58); A vs C, 13.34 (3.99–22.69); and A vs D, 35.1 (19.69–50.48). These differences are shown in Bland-Altman plots (Fig. 1).

The diagnostic agreements with the comparison method across the DN stages (values within the reference interval, or values outside the reference interval indicating microalbuminuria or macroalbuminuria), were 91.8%, 94.9%, and 91.8% for methods B, C, and D, respectively, classified as excellent by Kappa statistics (κ = 0.887, 0.929, and 0.887 for methods B, C, and D, respectively). Of the 98 urine samples, disagreement with UALB results obtained by method A occurred in 8 samples (8.2%) analyzed by method B, 5 samples (5.1%) analyzed by method C, and 8 samples (8.2%) analyzed by method D. The UALB values of these 21 samples were near the diagnostic cutoff values for the different stages of DN (4).

Use of different IT methods for UALB measurement in this study did not notably alter the classification of DN. Considering all methods, on average only 6.6% of samples were misclassified. Twelve of 21 samples misclassified were in the normoalbuminuria range by the reference method. In only 2 cases were patient samples labeled as DN by method A misclassified as normoalbuminuric by another method. These 2 samples were from microalbuminuric patients (UALB = 18.9 mg/L and 30.8 mg/24 h) and were erroneously classified as normoalbuminuric (UALB = 16.5 mg/L and 25.5 mg/24 h). The misclassification of 5 macroalbuminuric patient samples as microalbuminuric by method D was not clinically relevant, because the diagnosis of DN was maintained and the clinical management of micro- and macroalbuminuric patients is similar. The UALB values in these patients were near the lower cutoff limit for macroalbuminuria. Because UALB biological variation can be as much as 30%, it is recommended that the final diagnosis of DN be confirmed in a second urine sample (1–3). By reducing the effects of analytical variation, analysis of a second urine sample should be helpful in evaluating UALB values close to the adopted cutoffs for the diagnosis of micro- and macroalbuminuria. The immunoassay methods for UALB evaluated in this study appear to give results that can be applied interchangeably without clinically relevant misclassification of different stages of DN.

Grant/Funding Support: This work was supported by a Grant from Programa de Apoio a Núcleos de Excelência do Ministério de Ciência e Tecnologia (Pronex).
Fig. 1. Bland-Altman difference plots for urinary albumin immunoassays.

(A), Method A vs B, (B), A vs C, (C), A vs D. Relative differences (%) are shown on the y-axis. Solid line = zero line; dashed line = mean difference; dotted lines = ±2 SDs of difference. Reagent sets: method A, Urine Pack Immuno (Bayer); method B, Malb Aptec (BioSys); method C, Albumin Tina-quant (Roche); method D, Microalbumin (Randox).
Financial Disclosures: None declared.

Acknowledgment: We thank the Clinical Chemistry Unit at Clinical Pathology Division, HCPA, for providing urine samples and UALB results.

References


Influence of Thyroid Hormone Autoantibodies on 7 Thyroid Hormone Assays

To the Editor:

Spurious results of thyroid function tests (TFT) can be recognized when they do not reflect the clinical status of the patient or are not internally consistent [e.g., increased FT₄ with nonsuppressed thyroid-stimulating hormone (TSH)]. Potential causes of spurious TFT results include nonspecific binding of endogenous circulating factors, such as heterophilic antibodies, with assay reagents (1), the presence of albumin variants found in familial dysalbuminemic hyperthyroxinaemia (2), and thyroid hormone autoantibodies (THAA) (3). We describe a patient who had discordant TFTs due to circulating THAA and report differences in TFT results obtained on a variety of automated immunoassay platforms.

A 19-year-old man presented with tiredness spanning a 12-month period following a prolonged viral illness. A family history of hypothyroidism prompted a request for TFTs. These showed increased FT₄ of 60 pmol/L (reference interval 10–25 pmol/L) and FT₃ of 8.1 pmol/L (reference interval 2.5–6.5 pmol/L), but an nonsuppressed TSH of 0.9 mU/L (reference interval 0.3–5.5 mU/L). The tests were performed on an Immulite 2500 (Siemens Medical Solutions Diagnostics), Elesys E170 (Roche Diagnostics), TOSOH AIA 1800 (Tosoh Bioscience), Architect c800 (Abbott Limited Diagnostics), UniCel Dxi 800 (Beckman Coulter UK), and Wallac Delfia (PerkinElmer UK).

Measurements of TSH obtained with the different systems did not show marked assay-dependent variation (Table 1). The Immulite 2500 was the only assay that gave an increased FT₃ result of 9.1 pmol/L (reference interval 2.5–6.5 pmol/L). Although no T₃ autoantibodies were detected, endogenous antibodies to T₃ may possibly cross-react with the T₃ label used in the Immulite assay. FT₄ results varied from within the reference range with Wallac Delfia to >200% of the upper reference limit with the Advia Centaur, suggesting that observed differences in FT₄ results are likely due to differences in assay design. One-step procedures showed the greatest positive interference; high results were observed with both the Advia Centaur and Immulite 2500. The TOSOH AIA 1800, another 1-step assay, appeared less affected, a finding that may be attributable to a different tracer
used for signal detection. Both the Advia Centaur and Immulite 2500 use chemiluminescent substrates, whereas the TOSOH uses a fluorogenic tracer. The different molecular sizes of the labels may influence whether they are recognized by the THAA.

Two-step methods appear not to be susceptible to THAA interference, because the procedure ensures that there is no contact between serum components and analog tracer (3). This characteristic was evident in the Wallac Delfia, which gave results comparable to the EDFT4. Conversely, the other 2-step assays, Abbott Architect and Beckman DXI 800, gave borderline low results, which may have been related to the nature of the tracer used in these assays. Both the Abbott and Beckman assays use T₃ acridinium–labeled tracer in the second step of the reaction, when the tracer binds to unbound sites of the capture antibody. Any THAA left after the washing step will bind only the analyte and not the tracer, and hence increase the signal and decrease the FT₄ concentration detected. The Elecsys E170 is a competitive 1-step assay that differs in that it involves 2 incubations steps, but without a washing step in between. First the serum and a ruthenium–labeled capture antibody are mixed, then the biotin–labeled T₄ analog that binds the remaining free sites on the capture antibody is added. This assay design may account for the borderline positive interference observed (Table 1).

Our data demonstrate that the presence of THAA can lead to both positive and negative interference in FT₄ assays, and an increased FT₃ in only 1 assay. Serum TSH provides the most reliable assessment of thyroid function for patients found to have such antibodies.

**References**


**Table 1. TFT Measurements by 7 Different Immunoassays and Equilibrium Dialysis.**

<table>
<thead>
<tr>
<th>Immunoassay system</th>
<th>Principle</th>
<th>TSH, mU/L</th>
<th>FT₄, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immulite 2500</td>
<td>1-Step chemiluminescent assay</td>
<td>0.93 (0.3–5.5)</td>
<td>50.8 (10–25)</td>
</tr>
<tr>
<td>Advia Centaur</td>
<td>1-Step chemiluminescent assay</td>
<td>1.10 (0.4–5.5)</td>
<td>55.3 (9–20)</td>
</tr>
<tr>
<td>TOSOH AIA 1800</td>
<td>1-Step fluoroimmunoassay</td>
<td>1.08 (0.4–4.0)</td>
<td>27.3 (10.6–21)</td>
</tr>
<tr>
<td>Elecsys E170</td>
<td>1-Step (2-step incubation) Chemiluminescent assay</td>
<td>1.04 (0.3–4.5)</td>
<td>24.9 (10–22)</td>
</tr>
<tr>
<td>Architect</td>
<td>2-Step chemiluminescent assay</td>
<td>0.93 (0.2–5.0)</td>
<td>9.0 (9–19)</td>
</tr>
<tr>
<td>Beckman DXI 800</td>
<td>2-Step chemiluminescent assay</td>
<td>0.96 (0.4–4.5)</td>
<td>7.3 (7–17)</td>
</tr>
<tr>
<td>Wallac Delfia</td>
<td>2-step assay</td>
<td>0.97 (0.4–4.0)</td>
<td>14.1 (9–20)</td>
</tr>
<tr>
<td>Nichol FT₄ Equilibrium dialysis</td>
<td>Physical separation of free hormone, then immunoassay</td>
<td>NA</td>
<td>16.1 (10–36)</td>
</tr>
</tbody>
</table>

*Patient results outside reference intervals are in bold type. NA indicates not measured.
Analytical Quality of Calcitonin Determination and Its Effect on the Adequacy of Screening for Medullary Carcinoma of the Thyroid

To the Editor:

Calcitonin, a 32–amino acid calcium-lowering peptide secreted by the C cells (parafollicular cells) of the thyroid, is used as a marker for medullary carcinoma of the thyroid (MCT). However, calcitonin is not specific for MCT, because it is also secreted by other neoplasms, including breast cancer and small cell lung cancer.

Secretion of calcitonin is regulated primarily by the concentration of extracellular calcium, but can also be stimulated by gastrin. Although calcitonin concentrations are higher in men than in women and tend to decline with age, many laboratories use a cutoff value of 10 ng/L instead of a population-based reference interval. Prior studies have shown that basal calcitonin concentrations are below this threshold in a normal population (1,2) and in 90% of patients suffering from other nodular thyroid diseases (3). In clinical practice, a patient with a calcitonin concentration higher than 10 ng/L should undergo a pentagastrin test to exclude MCT.

It is important to note, however, that these interpretive guidelines have been developed using the Cisbio international reagent set for calcitonin measurement. This immunoradiometric assay uses 2 antibodies, one directed against the 11–17 and the other against the 24–32 sequence of the peptide. IRMA reagent sets, and particularly the Cisbio international kit, have been recommended for calcitonin measurement in the past and are still recommended by some scientific societies. However, in recent years, most medical laboratories have moved from radioisotopic methods to fully automated methods, and such a change may pose problems, because some chemiluminescent methods lack adequate sensitivity and specificity (4).

In our laboratory, we planned to move from an immunoradiometric method (Cisbio) to an automated method (Liaison, Diasorin). When both reagent sets were calibrated against the 2nd International Standard 89/620, the Cisbio reagent set clearly indicated that 1 μIU of the international standard corresponded to 3.6 pg of Cisbio calcitonin. Because this information was not provided by Diasorin, we created our own standard curve for Liaison. For that purpose, we used a sample with a very low calcitonin concentration higher than 10 ng/L should undergo a pentagastrin test to exclude MCT.

Basing the lowest reportable limit for a test on the determined LOQ (5), we reported any results obtained with the Liaison reagent set below 12 ng/L as “<12 ng/L.” However, with the application of a 10 ng/L threshold for pentagastrin testing as described above, the LOQ of 12 ng/L meant that patients with calcitonin concentrations between 9 and 12 ng/L (2% of women and 10% of men, in our experience) would potentially be missed with assays performed using the Liaison reagent set.

Such an outcome is problematic for a screening test. We suggest that the “old” 10 ng/L cutoff obtained with the Cisbio reagent set should be confirmed by a multicenter study involving several different calcitonin assays.
Stabilization of Glucose in Blood Specimens: Mechanism of Delay in Fluoride Inhibition of Glycolysis

To the Editor:

The recent report by Gambino (1) brought attention to the often overlooked fact that fluoride does not prevent loss of plasma glucose during the first 30–90 min (or longer) after blood collection (2). Although fluoride is effective in preventing later loss of glucose (1, 2), the mechanism of its delay in its action is a matter of some interest.

Fluoride acts primarily by inhibiting enolase in the glycolytic pathway. Fluoride strongly inhibits the enzyme in the presence of inorganic phosphate. The inhibitory species is the fluorophosphate ion, which when bound to magnesium forms a complex with enolase and inactivates the enzyme. The delay in fluoride’s prevention of glucose loss in blood samples is sometimes attributed to a postulated delay in the entry of fluoride ion into the blood cells in which the glycolytic enzymes reside. Several observations cast doubt on this explanation, however.

As part of a project for quality improvement of sample-handling requirements, we collected blood from a volunteer into four 5-mL Vacutainer (BD) tubes, 2 containing sodium fluoride/potassium oxalate, 10 mg/8 mg, and 2 with lithium heparin, 51 U, and plasma separating tube gel. The tubes were not centrifuged. After sample collection, 1 tube of each type was kept at room temperature, and another was put immediately into an ice water bath. The tubes were kept upright and mixed immediately before removing aliquots at 0, 15, 30, 45, 60, and 90 min. Each aliquot was centrifuged immediately for 1 min at 5585 g in a microcentrifuge. The plasma was removed and lactate and glucose were measured on an Architect Analyzer (Abbott). The study was repeated on blood samples obtained from a second volunteer on a different day. The results for tubes at room temperature are summarized in Fig. 1. In the tubes of blood stored in ice water, with or without fluoride, decreases in glucose and increases in lactate were ≤0.1 mmol/L even at 90 min (data omitted for clarity).

If fluoride does not enter blood cells rapidly, then it cannot rapidly inhibit the production of lactate, which is produced from pyruvate, the final product of glycolysis. By contrast, as shown in Fig. 1, fluoride completely blocked the production of lactate for >30 min during a time when consumption of glucose was brisk in the presence of fluoride. In portions of the blood that were incubated without fluoride (Fig. 1), the quantity of lactate produced was as expected, that is, almost 2 mol of lactate were produced for each 1 mol of glucose consumed (e.g., the concentration of lactate increased at 90 min by 1.1 mmol/L in samples from each volunteer, whereas the concentration of glucose decreased by 0.6 mmol/L, Fig. 1). These data are similar to those reported by Feig et al. (3) for washed human erythrocytes, which showed consumption of glucose at 10 min after addition of fluoride, but negligible production of lactate at that time point. Moreover, Astles et al. (4), although focusing on somewhat later time points after blood collection, also reported that fluoride rapidly inhibited production of lactate in whole blood.

A parsimonious explanation of these findings is that after fluoride is mixed with blood it rapidly blocks enolase (within <5 min), and that enzymes upstream of enolase in the glycolytic pathway remain active. Thus glucose continues to be metabolized to glucose 6-phosphate, which is further me-

References

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DOI: 10.1373/clinchem.2007.100636
tabolized to other phosphorylated metabolites of glucose, all of which accumulate in the cells. Thus the glucose concentration continues to decrease in the plasma. By contrast, lactate is stable because, with enolase inhibited, no phosphoenolpyruvate is formed and thus there is no substrate for pyruvate kinase and there is no production of pyruvate or lactic acid.

With the glycolytic pathway blocked, other pathways may also metabolize phosphorylated sugars. Such metabolism will continue until equilibrium states are reached for the several reactions involved. In particular, the rate of phosphorylation of glucose to glucose 6-phosphate will decrease because this rate depends on the supply of ATP, the concentration of which decreases in erythrocytes by almost 90% at 60 min after addition of fluoride (3).

The proposed explanation for delay in the action of fluoride does not require postulating the existence of a barrier to the movement of fluoride into erythrocytes and leukocytes. In fact, studies of lactate transport into human erythrocytes indicate that fluoride exchange across the erythrocyte membrane is rapid (5). We conclude that the delay in fluoride’s ability to stop the use of glucose reflects continuing metabolism of glucose despite inhibition of the downstream target enzymes inhibited by fluoride.

**Grant/Funding Support:** LMM’s postdoctoral training in clinical chemistry and laboratory medicine is supported by a Past Presidents’ Scholarship from the Van Slyke Foundation of the American Association for Clinical Chemistry.

**Financial Disclosures:** None declared.

**Acknowledgments:** The authors thank Judy Hundley, Bob Miller, Victoria Reynolds, and the staff of the clinical chemistry laboratory at the University of Virginia for excellent technical support.

**References**


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**Fig. 1.** Changes in lactate and glucose concentrations over time.

Specimens of whole blood were collected and stored at room temperature in Vacutainer tubes (BD) containing either lithium heparin (filled squares) or fluoride/oxalate (open diamonds). Over a 90-min period, samples were removed from each tube and centrifuged to obtain plasma for measurements of lactate and glucose. Panels A and B, volunteer 1; panels C and D, volunteer 2.
Variability of Lipoprotein-Associated Phospholipase A2 Measurements

To the Editor:

We read with interest the recent report in this journal by Khuseyinova and colleagues regarding the variability of serial lipoprotein-associated phospholipase A2 (Lp-PLA2) measurements in postmyocardial infarction patients (1). Because Lp-PLA2 plays a role in inflammation but, unlike other inflammatory proteins, is not an acute-phase reactant (2), nonspecificity issues associated with acute-phase reactants may be averted. Indeed, recent reviews verify that several epidemiologic studies have demonstrated a strong association between Lp-PLA2 and ischemic events (2–4). Thus, as indicated by Khuseyinova and colleagues, Lp-PLA2 appears to be a promising emerging biomarker for coronary heart disease and stroke. We applaud the effort of this group to evaluate the performance of Lp-PLA2 measurements.

With all assay development, multiple iterations of assays are often developed before a given analyte is ready to be measured on a widespread basis, and Lp-PLA2 is a good example. It is important to keep these multiple iterations separate because they tend to differ. We too have had consistent results with the second-generation assay, the one reported by Khuseyinova and colleagues. This second-generation assay is no longer commercially available, however, and a third-generation assay has replaced it. We have completed extensive evaluations of both the second- and third-generation assay methods for Lp-PLA2. In our hands, the third-generation assay behaved quite differently from the second, with much greater variability and lot-to-lot variation, and values that were very different from those obtained with the second-generation assay for some patient samples. A comparison of the 2 iterations in 476 patient samples yielded the following correlation data \[3^{\text{rdGen}} = (0.557 \times 2^{\text{ndGen}}) + 67.0; R^2 = 0.712\]. As a result of analytic problems and our inability to get the third generation assay to perform to reasonable specifications, we delayed and actually stopped reporting values obtained with the third-generation assay. In particular, results obtained while performing the assay in a manual format did not match values obtained using a Triturus® automated immunoassay analyzer. Thus, both the method and instrumentation used to perform the method should be considered during method validation. Although these issues were ultimately resolved and we did institute the third-generation assay in a manual format, we still monitor this assay very carefully and go through several steps to verify the performance of each new lot of reagents.

A fourth-generation assay has recently been cleared by the FDA for use and we have evaluated it in our laboratory. This automated method is more user friendly and has performance characteristics more similar to and perhaps even better than those of the second-generation assay. We found the automated method to be linear down to 30 \(\mu\)g/L \((y = 1.052x + 9.6, R^2 = 0.998)\). Interassay CVs ranged from 6.6% to 10.4% for 6 samples over the concentration range of 94–441 \(\mu\)g/L \((n = 11\) replicates per sample). Discrepancies were observed when we compared the automated and third-generation assays \(\text{[automated} = (1.20 \times 3^{\text{rdGen}}) + 3.4; R^2 = 0.480; n = 65]\), further demonstrating differences that may be observed from one iteration to the next. We are optimistic about the long-term success of the automated method, but areas of fine tuning are needed before it is ready for wider dissemination. The important conclusion to draw from this situation is that from the research and the clinical perspectives, care must be exercised in documenting what iteration of a given assay is being used, especially when comparing values over time. We do include a comment about this concern in all of our reports.

Grant/Funding Support: JPM has received research grant funds from diaDexus in the past.

Financial Disclosures: ASJ is or has been a consultant for most of the major diagnostic companies.

Acknowledgments: We acknowledge Shannon D. Hodel-Hanson, Stacy J. Hartman, and Jennie N. Ward for their work in validation of the diaDexus Lp-PLA2 methods.

References


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Effect of Plasma Protein Depletion on BNP-32 Recovery

To the Editor:

Depletion of abundant proteins from plasma and serum is an important initial step in many biomarker discovery platforms (1). Decreasing the concentrations of highly abundant proteins (e.g., albumin, IgG, and antitrypsin) facilitates the use of contemporary proteomics technologies, such as gel electrophoresis and mass spectrometry, for detection and identification of low-abundant proteins. Furthermore, decreasing abundant protein concentrations may also improve immunoprecipitation recovery efficiencies for targeted low-abundant species by decreasing nonspecific binding (i.e., shielding the antigen-binding domain) to the antibody and/or solid supports. A notable pitfall to depletion strategies is the potential for unintentionally removing low-abundant plasma or serum proteins. These low-abundant species may be bound specifically or nonspecifically to the depletion ligand, depletion target protein (e.g., carrier proteins), or the solid support(s). Thus, it is important to critically evaluate the effectiveness of abundant plasma protein depletion for enhancing the study of low-abundant protein biomarker(s).

We have been actively developing a targeted biomarker discovery platform for characterizing the circulating forms of B-type natriuretic peptide (BNP) that includes protein depletion strategies, immunoprecipitation, gel electrophoresis, isotope dilution (absolute quantification), and nanoflow liquid chromatography coupled to high-performance hybrid Fourier transform mass spectrometry (2). BNP has emerged as a very important biomarker for the clinical diagnosis and management of heart failure (3). Despite the increasing clinical use of BNP, very little is known about the secretion, post-translational processing, and receptor binding of BNP at the molecular level (4). This gap in knowledge between the diagnostic and prognostic utility of BNP and the basic understanding of the molecular form(s) and function(s) of BNP is presently hindering the advancement of heart failure research. Increasing evidence in our laboratory (2) and others (reviewed by Goetze (4) and Liang et al. (5)) has shown that BNP circulating in heterogeneous forms that interfere with the commercially available BNP tests and potentially affect the pathophysiology of heart failure. These heterogeneous forms could have significant diagnostic, prognostic, and therapeutic value for the management of heart failure. The present obstacle to identifying circulating forms of BNP is the detection-limit gap that exists between the circulating concentrations of BNP, recovery efficiencies from plasma, and the high-performance hybrid Fourier transform mass spectrometry method. We sought to establish whether BNP-32 bound specifically or nonspecifically to the 6 most abundant proteins in plasma.

The general experimental setup in this study involved tracking radiolabeled BNP-32 ([125I]-[Tyr]-BNP-32; specific activity = 1322.66 Ci/mmol: Phoenix Pharmaceuticals) in human plasma (single individual samples collected from donors who gave informed consent and were deidentified in accordance with the Mayo Clinic’s Institutional Review Board) through the Multiple Affinity Removal System (MARS) (4.6 × 100 mm MARS 6, Agilent Technologies). Unbound (%B = 0) and bound (%B = 100) fractions were collected from the MARS eluent and the [125I]-[Tyr]-BNP-32 in each fraction was measured (1480 Wizard 3 Gamma Counter, Spectrofuge) as shown in Fig. 1. In the first experiment, 123 μL of plasma was combined with 123 μL of an 8 μg/L stock solution of [125I]-[Tyr]-BNP-32 in 110 mmol/L PBS (0.22 g NaH2PO4 + 1.28 g Na2HPO4 + 0.40 g NaCl + 0.01 Na3 in 100 mL deionized H2O; pH adjusted to 7.4 with NaOH) and 154 mmol/L NaCl at 37 °C, then 25 μL aliquots of the [125I]-[Tyr]-BNP-32/plasma sample were taken at 0, 1, and 2 h, diluted with 75 μL buffer A (Agilent Technologies; more information on buffer A can be found at http://www.chem.agilent.com/en-US/Support/FAQs/Proteomics/MARS/Pages/KB000818.aspx), filtered, and injected. The purpose of this experiment was to determine the effects of [125I]-[Tyr]-BNP-32 incubation time in plasma on its binding to high-abundant proteins. Results for the 3 time intervals sampled showed that 98% of the [125I]-[Tyr]-BNP-32 was eluted in each of the 3 unbound fractions (Fig. 1). The data clearly indicate that [125I]-[Tyr]-BNP-32 does not bind to any of the top 6 most abundant proteins in plasma (albumin, IgG, antitrypsin, IgA, transferrin, and haptoglobin). A second set of experiments was performed to determine the reproducibility of the [125I]-[Tyr]-BNP-32 recoveries shown in Fig. 1. The results show that [125I]-[Tyr]-BNP-32 does not...
bind to these proteins with mean (SD) 98% (2%) (n = 3) eluting in the unbound fraction.

High-abundant plasma protein depletion platforms have become increasingly popular for preparing plasma before mass spectral analysis (1). By measuring the radioactivity of the bound and unbound fractions from a MARS 6 column, we determined that 125I-[Tyr]-BNP-32 does not bind to the 6 most abundant proteins in plasma. Although we are cautious in assuming that the binding properties of BNP-32 and 125I-[Tyr]-BNP-32 are the same with regard to the 6 most abundant proteins, we can make reasonable inferences that the MARS 6 platform will not remove BNP-32. Another important issue that must also be determined is whether plasma protein depletion removes alternative molecular forms of BNP, such as glycosylated proBNP (5).

Disclosures: These data were presented at the 55th American Society for Mass Spectrometry Conference in Indianapolis, IN, June 3–7, 2007. Abstracts are published online (ASMS website) but are not refereed and cannot therefore be referenced in primary literature.

Acknowledgments: We thank Professor Jon Horowitz and Margaret Goralska (NCSU Veterinary Medicine) for access and assistance with the γ counter used in this study.

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DOI: 10.1373/clinchem.2007.098038