any immunoassay, with or without an extraction and a purification step, requires validation by a definitive or reference method before being considered a gold standard (2).

We have not made an arbitrary statement that extraction/chromatography RIAs for testosterone do not work. Our point is that there are no convincing data showing that they do work. The only existing reports, from two independent sources (1, 5), that compared extraction/chromatography RIA with gas chromatography–mass spectrometry show that there are considerable problems with these analyses. Until an extraction/chromatography RIA has been properly validated the results generated from such tests are subject to question.

We appreciate the opportunity to further emphasize the need for proper validation of steroid immunoassays.

References

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Effect of Different Sample Types and Stability after Blood Collection of N-Terminal Pro-B-Type Natriuretic Peptide as Measured with Roche Elecsys System

To the Editor:

N-Terminal pro-B-type natriuretic peptide (NT-proBNP; 76-amino acid peptide) is cosecreted with BNP and can be measured by automated immunoassay (Elecsys 2010; Roche UK). This is a fully automated electrochemiluminescence “sandwich” immunoassay based on capture (biotinylated) and detection (ruthenium) polyclonal antibodies against NT-proBNP amino acids 1–21 and 39–50 (1), respectively. Reports on in vitro stability are conflicting (2–4). We assessed the effect of different sample types on measurement of NT-proBNP and evaluated the effect of delayed separation of serum from cells on stability. We compared plasma in lithium heparinate (14 IU/mL of blood; Greiner Labotechnik; cat no. 9045-22-1) and serum in serum separator (SST) tubes from patients attending a heart failure clinic. For sample type comparison, blood from 60 patients was collected into SST and lithium heparin tubes. Blood was also collected in plain glass and SST tubes (n = 10) to investigate whether there was significant adsorption of NT-proBNP by the gel separator (plastic tubes; Greiner Labotechnik Bio-One GmbH).

To assess stability, blood from 12 individuals was collected in four SST tubes. The first tube was centrifuged, aliquoted, and stored at −20 °C for baseline measurement. The remaining serum was left on the gel at 22 °C; aliquots were taken and stored at −20 °C after 24, 48, and 72 h. This simulates the situation in the laboratory after the sample has been received. The second, third, and fourth samples were centrifuged after 24, 48, and 72 h at 22 °C, aliquoted, and frozen until assay. Samples from the same patient were thawed and analyzed within the same run. The be-
between-day CV were 8.4% (34 ng/L), 3.3% (421 ng/L), and 3.6% (15 938 ng/L; n = 20) for pooled serum and 2.7% (216 ng/L) and 1.5% (4087 ng/L; n = 20) for the Elescys PreciControl proBNP calibrator. All data followed a gaussian distribution after log transformation. NT-proBNP was 4% lower in lithium heparin plasma than in serum by the paired t-test (P < 0.001; n = 60), with a regression equation of log hep (ng/L; n = 780 Letters) = 0.99 log SST (95% confidence interval (CI) r = 0.03 ng/L (12.2 to 7.1%) at 72 h (n = 12; all P < 0.001 relative to baseline). Dasgupta et al. (5), found that NT-proBNP is stable for 48 h in SST tubes (Becton Dickinson) after centrifugation. We have demonstrated stability up to 72 h. However, we observed a decrease when blood was left unseparated for 24 h or longer. The manufacturers and Sokoll et al. (6) state that there is no difference in results obtained with serum and heparin plasma, whereas we have demonstrated a difference of 4%. Sokoll et al. (6) used lithium and ammonium heparin compared with the lithium heparinate tubes used here. We conclude that NT-proBNP can be precisely measured and that it is stable if serum has been separated from cells within 24 h. There is a small but statistically significant difference in results between heparin plasma and serum. This is unlikely to be of clinical importance because of the large intra-individual biological variation of NT-proBNP (33.3%) (7).

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References

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Urinary Iodine Analysis: An Alternative Method for Digestion of Urine Samples

To the Editor:

Iodide plays a central role in thyroid physiology, and iodine compounds are essential for normal vertebrate growth and development. Useful information about the iodine nutritional status of a population can be obtained by measuring urinary iodine to estimate the prevalence of iodine deficiency disorders. Several methods for measuring urinary iodine are currently available [for a review, see Ref. (1)]: most of these involve the Sandell–Kolthoff reaction (2), which is based on a colorimetric ceric–arsenic assay. However, these methods are susceptible to several problems: e.g., various contaminants can cause the reduction of cerium(IV), or pigments or drug metabolites in the urine can increase the natural yellow color of the urine, leading to false-negative results. The digestion of urine samples, which is the first step in all of the methods based on the Sandell–Kolthoff reaction, is therefore crucial.

In this study, we tested three digestion methods on 200 urine samples from patients hospitalized at the La Timone University Hospital: the conventional chloric acid method (1); an acid ammonium persulfate method (3, 4); and a method involving a HNO3–HCl mixture. For the latter method, the digestion was performed by mixing 3 mL of 14.3 mol/L HNO3 with 2 mL of 12.1 mol/L HCl just before use. Urine samples (250 μL) were digested with 125 μL of the mixture for 60 min at 110 °C, then brought to 2 mL with phosphate-buffered saline (PBS), pH 9.0.

The microassay system is based on the catalytic Sandell–Kolthoff reaction, as described previously (3, 5). In this assay, the catalytic activity of iodide on the oxidation of arsenic(III) by cerium(IV) is measured by monitoring the reduction of the yellow ceric ions.

In the case of chloric acid digestion, we noted that ~75% of the urine...