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 Eclesys Preci-Control 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 = 1.06 log SST − 0.085 (r > 0.99). The 95% confidence interval (CI) for the slope was 0.99–1.01, and for the intercept was −0.13 to −0.03 ng/L (r > 0.99). A Bland–Altman plot is shown in Fig. 1 to aid interpretation. We found no significant difference between serum from plain glass and SST tubes (P = 0.15; n = 10). NT-proBNP was stable in serum left on top of the SST gel at room temperature for 72 h with no significant mean decrease from baseline at 24 h (0.2%; 95% CI, −1.8 to 1.5%), 48 h (0.8%; −2.6 to 0.9%), or 72 h (1.2%; 95% CI, −3.0 to 0.6%; n = 12). For serum that was left unseparated in the SST tube up to 72 h before separation, NT-proBNP decreased by a mean of 4.4% (−6.2 to −2.5%) at 24 h, 6.9% (−9.3 to −4.6%) at 48 h, and 9.6% (−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 intraindividual 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...
samples had a slightly brown color. This color was probably attributable to the oxidation of iodide into iodine, and to confirm this hypothesis, we subjected 10 samples of a KI solution (10.8 μmol/L) to chloric acid digestion. All of the digested samples showed a brown color. Iodine analysis performed by titration showed that a mean (SD) of 42 (8)% of the initial iodide content was transformed into free iodine, which has no catalytic activity in the Sandell–Kolthoff reaction. Similar results were also obtained with perchloric acid digestion. In the case of ammonium persulfate digestion, 48% of the urine samples showed a yellow color. To assess the effects of this natural yellow color of the urine samples on the urinary iodine measurements, we placed 5 μL of digested urine plus 95 μL of PBS (pH 9.0) or 20 μL of digested urine plus 80 μL of PBS (pH 9.0) in wells of a 96-well microtiter plate. After we added 60 μL of arsenious acid and incubated the mixture at room temperature for 10 min, we monitored the absorbance at 405 nm (A_{405 nm}) in the absence of cerium(IV) sulfate. With the 20-μL samples, the absorbance ranged from 0.024 to 0.209 [mean (SD), 0.126 (0.093)]. However, with the 20-μL samples, only 8% of the urine samples were slightly colored after the HNO_3–HCl digestion, and none of them gave absorbance values >0.05 [mean (SD), 0.018 (0.011)]. To assess the resulting percentage error attributable to the natural yellow color of the digested urine, we measured the urinary iodide content of two aliquots (5 and 20 μL) of samples with A_{405 nm} values >0.024. As can be seen from Table 1, the urinary iodide content measured in 20-μL urine samples digested with ammonium persulfate was significantly lower than the values obtained with either 5-μL samples digested with ammonium persulfate or 5- and 20-μL urine samples digested with the HNO_3–HCl mixture. The extent of the error resulting from the natural yellow color of the samples depended on both the iodide concentration in the microplate well and the intensity of the natural yellow color. Accordingly, for the 5-μL sample, the natural yellow color of the urine samples digested with ammonium persulfate gave a relatively low absorbance value [mean (SD), 0.021 (0.016)], which did not affect the loss of the yellow color of cerium(IV) attributable to the catalytic activity of the iodide. A similar conclusion can be reached about the digestion method involving the use of the HNO_3–HCl mixture because the absorbance of the natural yellow color of the digested urine samples was suitably low, regardless of the volume used.

This is the first time to our knowledge that the problem of the natural yellow color of urine samples has been investigated in connection with the determination of urinary iodine. The presence of the yellow color leads to underestimation of the urinary iodide concentration. Therefore, when testing a population with a normal iodine diet, the results obtained with the two most commonly used digestion methods will be consistent if the volume of sample in the well is not >5 μL.

In addition to urinary iodide concentration assessments, there are other fields in which iodine measurements are required: the methods used to measure the iodine present in human tissues and food products, for example, must ensure complete digestion without the production of a yellow color in the digested products. For these applications, only the HNO_3–HCl digestion method, which yields a moderate yellow color, if any, is compatible with larger sample volumes of up to 40 μL (5, 6); it also shows greater sensitivity, which makes it possible to analyze trace amounts of iodine in these samples.

In conclusion, the ammonium persulfate method for urine digestion is advantageous from the point of view of its safety and ease of operation, but close attention must be paid to the volume of the urine sample used, which may limit the sensitivity of the analysis. On the other hand, although the HNO_3–HCl digestion method has most of the drawbacks associated with the use of concentrated acids (e.g., it is hazardous and requires the use of an expensive fume hood), its accuracy is only slightly affected by the natural yellow color of the digested urine samples, regardless of the sample volume tested. The method based on the HNO_3–HCl mixture could provide a particularly useful means of assessing the iodine content in various media, and there exist many potential applications for this efficient digestion process.

Table 1. Iodide content of urine samples (n = 50) showing a natural yellow color (A_{405 nm} > 0.024) after digestion with ammonium persulfate or HNO_3–HCl.

<table>
<thead>
<tr>
<th>Volume sample, μL</th>
<th>HNO_3–HCl</th>
<th>Ammonium persulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.08 (1.83)</td>
<td>1.95 (1.89)</td>
</tr>
<tr>
<td>20</td>
<td>1.98 (1.41)</td>
<td>1.05 (0.98)*</td>
</tr>
</tbody>
</table>

*p < 0.01.*

References


Which Types of Alcohol-Use Disorder Will Asialotransferrin Detect?

To the Editor:

Approximately 20% of patients seen in clinical practice have an underlying alcohol-use disorder (1). In the last 20 years, specialist work on alcohol misuse has focused mainly on diagnosis at the dependence stage. However, there is also a need to direct attention to alcohol abuse, the long, little-studied, and insufficiently treated disease during which processes damaging to health and social functioning are initiated. Recently, Legros et al. (2) concluded that as a biomarker, asialotransferrin offered the best differentiation between moderate and abusive alcohol consumption.

From a methodologic point of view, clinical investigations concerning biomarkers of alcohol abuse or alcohol dependence have to define their study populations according to internationally accepted clinical categories of alcohol misuse (DSM IV and CIM 10) or to patient populations well defined by alcohol intake (e.g., 0–10, 10–20 g/day, and so forth). This is the basic requirement to make these investigations reproducible, comparable, and interpretable by doctors working in care.

Legros et al. (2), in their excellent work on the efficacy of asialotransferrin and disialotransferrin, recommended the "analysis of the asialo-Tf [asialotransferrin] isofom, which will be present in 92% of alcohol abusers and absent in 95% of moderate alcohol consumers", and Arndt (3) cited numerous advantages, including high specificity, simple standardization of the analytical definition, and the possibility of producing specific antibodies for direct assay, as strong arguments in favor of asialotransferrin. In this sense, the results of the study by Legros et al. (2) are very promising.

Nevertheless, we found in the study by Legros et al. the methodologic problem of definition of the study population. The “alcohol abusers” in that study were probably poorly identified. No inclusion criteria according to alcohol abuse defined by DSM IV (F305) and checked by the corresponding MINI questions were used (4). One criterion for inclusion in the study was an AUDIT score >11. Patients with AUDIT scores between 7 and 11 were excluded. The mean AUDIT score for the included patients was, in fact, 27. However, the internationally validated cutoff for the AUDIT questionnaire is 8; higher AUDIT scores seem associated with greater severity of alcohol misuse, and scores >12 are characteristic of alcohol dependence (5).

Another inclusion criterion of this study was a daily ethanol intake >50 g. In fact, the alcohol abusers included drank, on average, 166 g/day, with a range from 70 to 310 g/day. In a recent study determining the nutritional intake of alcohol-dependent patients, Manari et al. (6) reported that the dependent patients included had mean ethanol consumption of 162 g/day. Nicolas et al. (7) reported a mean ethanol intake in alcohol-dependent patients of 177 g/day. Legros et al. (2) may therefore not have fully differentiated between alcohol abuse and alcohol dependence according to the DSM IV criteria. Alcohol-dependent patients may thus have been unwittingly included in that study. Testing patients with dependence but using the term “abuse” to classify them will tend to overestimate test sensitivity, producing serious consequences in screening practice.

References


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Unexpected Relationship between Plasma Homocysteine and Intrauterine Growth Restriction

To the Editor:

In their report, Infante-Rivard et al. (1) presented evidence that homo-