quantities expressed per mass unit of excreted creatinine preferably be replaced by the analyte quantity expressed per unit volume.

References

Joris Delanghe
Jean-Paul De Sleijpe
Marc De Buyzere
Johan Robbrecht
Roger Wieme
Alex Vermeulen

Central Lab. and Dept. of Endocrinol.
University Hospital (IB2)
De Pinteelaan 185
B-9000 Gent, Belgium

Detergent Enhances Interference of Urate in Determinations of Fructosamine

To the Editor:

Fructosamine in serum (S-FA) is usually determined by a two-point kinetic assay based on the reduction of nitroblue tetrazolium (NBT), essentially as originally described by Johnson et al. (1). Particular attention has been paid to calibration of the method and various calibrators ("etalons"") (2)) have been proposed and marketed. Recently, Phillipou et al. (3) published data indicating some advantage of adding a detergent, Triton X-100, to the usual reagent. This addition diminishes the influence of lipemic sera and gives a visible spectrum for the calibrator (DMF, 1-deoxy-1-morpholinofructose), comparable with that for glycated albumin.

We analyzed 50 patients' specimens (with S-FA ranging between 1.95 and 5.20 mmol/L) and compared the results obtained by our previous routinely used method (Fructosamine, Roche application for Cobas-Bio; incubation of 50 μL of sample and 200 μL of reagent, for 10 min at 37 °C with a first reading at 550 nm and a second reading 15 min later and calibrated with glycated serum proteins of human origin) with those obtained with the same reagent with added Triton. We saw no correlation between the concentrations (r = 0.09) measured by the two methods. The general trend for the results in a scatter-plot indicated that the method involving Triton gave higher values than those without detergent. Urate is known to interfere with the NBT reaction (Roche application note) but to what extent is not well described. Here we report a marked enhanced influence of urate in physiological concentrations on the nonspecific reduction of NBT in the presence of Triton X-100.

We increased the urate concentrations of four sera with respective S-FA concentrations of 1.35, 1.82, 2.06, and 3.64 mmol/L by adding urate to obtain four series of samples with urate concentrations 50, 100, 150, 250, and 500 μmol/L greater than the initial concentrations, which were 270, 290, 300, and 360 μmol/L, respectively. We then repeated the S-FA determination, using the Roche reagent fortified with Triton X-100 (analytical grade) to give a final concentration of 20 g/L. As shown in Figure 1, urate increases the S-FA somewhat in the original method but by almost 10-fold as much when Triton X-100 is also present.

We modified the reagent further by adding uricase (EC 1.7.3.3, Boehringer Mannheim) to the NBT-Triton reagent to give final amounts of 0.01 to 0.1 U per sample. As shown in Figure 1, even 0.01 U has a marked effect on the measurement of S-FA, but at least 0.05 U per sample seems necessary to reduce the analytical interference from urate to give the same result as that obtained by the original reagent.

We then also analyzed these same specimens by the original method (x) and with additions of Triton as above and uricase, 0.05 U per sample (y). The correlation coefficient (r) was 0.58, indicating a significant correlation between

![Fig. 1. Fructosamine as determined with the Roche Fructosamine reagent and with additions](image)

**Fig. 1.** Fructosamine as determined with the Roche Fructosamine reagent and with additions

Top to bottom: added Triton (20 g/L), added uricase 0.01 U per sample, original reagent, and added uricase, 0.05 and 0.1 U per sample. The scale on the right refers to the Triton graph. Means ± SD are shown. n = 4 each
between the two sets of data. The slope (analytical sensitivity) was 0.5 and the intercept (background) 1.01 mmol/L, indicating that the NBT-Triton X-100–uricase reagent (FAU) is less sensitive than the original method and produces a considerable background. There was no correlation between the results for S-FA, S-FAU, or the difference between the two (S-FA – S-FAU) and the concentrations of triglyceride or cholesterol in the specimens.

If the same reference intervals are used for both methods, 14 of the 50 results would have been judged differently by the FAU results. Of these, three were false positives. The patients with discrepant results were identified and their glucose hemoestasis was further investigated by determining Hb A1c and glucose. In both the false-positive and false-negative groups, results for these latter two analytes agreed better with S-FA values than with S-FAU.

The distribution of Surate concentrations in our hospital clientele was studied in a random group of 350 men and 350 women. Both distributions were skew, with medians of 377 and 318 μmol/L. The upper quartile was 432 and 379 μmol/L for men and women, respectively, and the maximum concentrations were 803 and 675 μmol/L. Although these figures do not give any indication of the prevalence of increased Surate in a population, it illustrates the skew distribution and indicates that more than a quarter of the individuals studied have Surate concentrations 50 μmol/L or more above “normal” values. Thus, to take advantage of the improved compatibility between samples and synthetic reagents, (stalon) and to reduce the influence of lipemia by using a detergent, one must diminish the analytical interference of urate by use of uricase.

These results reveal an analytical interference of urate with the NBT reaction that is enhanced by added detergent. Uricase inhibits this interference but the remaining apparent concentration of fructosamine is lower than that estimated with the conventional technique. In about a third of the specimens we analyzed, the two methods gave different results; in most cases, the results obtained by the unmodified method agreed better with independently determined glucose hemostasis. The modifications tested do not seem to improve the performance of the original method.

References

Anders Knallner
Dept. of Clin. Chem.
Karolinska Hospital
Stockholm, Sweden

Underestimation of Urinary Glucose by an Automated Urine Dipstick Analyzer Based on Glucose Oxidase with Iodine as Indicator Chromogen

To the Editor:
While evaluating a new quantitative urinary glucose method for our laboratory, we discovered unexpectedly that our Clinitek® 200 instrument, in which the Multistix® 10 SG dipstick is used (both from Miles, Elkhart, IN 46515), yielded results significantly lower than predicted by quantitative methods. The Astra® (Beckman, Brea, CA 92621) and Ektachem® 700 (Kodak, Rochester, NY 14650) gave quantitative results for glucose that agreed well, suggesting underestimation by the dipstick method. Therefore, we examined ~7000 urine specimens received in our inpatient and outpatient laboratories during 10 weeks. We then quantified urinary glucose by the Astra method and also used a semiquantitative dipstick method (Chemstrip® uG; Bio-Dynamics, Indianapolis, IN 46250) in the more strongly glucose-positive urines. The evaluation included 180 urines reading ≥1.0 g/L by the automated Clinitek 200 method. Results by the two dipstick methods are compared with those by the quantitative method in Table 1.

As in most general clinical laboratories, only a small proportion of urines submitted to our laboratory are glucose-positive. Less than 1% of all urines were found to be discordant. However, with the Clinitek 200, 36% (64 of 180) of the more strongly glucose-positive (≥1.0 g/L) gave dipstick results more than fourfold below those predicted by a quantitative glucose method. None of the same urines gave “unacceptable” results with the Bio-Dynamics Chemstrip® uG method.

The manufacturer’s technical representative, who visited our laboratory, first speculated that the discrepancy was due to excessive urine remaining on the glucose pad, causing spuriously increased optical reflectance, and thus a falsely low reading for glucose. However, in our hands, and theirs, the wiping technique required to obtain “correct results” was not reproducible.

Table 1. Discordant and Concordant Quantitative and Semiquantitative Urinary Glucose Results

<table>
<thead>
<tr>
<th>Astra glucose (quant.)</th>
<th>Clinitek 200 (sem.)</th>
<th>Number of urine specimens</th>
<th>Chemstrip uG (sem.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/L</td>
<td>1.0</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>0.51–1.75</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.76–3.75</td>
<td>0</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>3.76–7.50</td>
<td>1(1)</td>
<td>3(0)</td>
<td>19</td>
</tr>
<tr>
<td>7.51–15.00</td>
<td>1(1)</td>
<td>4(2)</td>
<td>30(0)</td>
</tr>
<tr>
<td>15.1–25.00</td>
<td>2(2)</td>
<td>12(6)</td>
<td>6</td>
</tr>
<tr>
<td>25.1–35.00</td>
<td>1(1)</td>
<td>13(13)</td>
<td>5</td>
</tr>
<tr>
<td>&gt;35.0</td>
<td>1(1)</td>
<td>37(37)</td>
<td>23</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of urine specimens with quantitative (“quant.”) glucose concentrations more that fourfold greater than that predicted by the semiquantitative dipstick result. Bold numbers indicate the number of urine samples with semiquantitative (“sem.”) dipstick results falling in the color block predicted by the quantitative glucose concentration.