### Table 1. Odds ratios for a poor outcome from CK-MB and cTnI values in non-AMI patients with chest pain.

<table>
<thead>
<tr>
<th>Cutoff conc, µg/L</th>
<th>Odds ratio (95% CI)</th>
<th>( x^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>3.1 (0.88-15.9)</td>
<td>3.20</td>
<td>0.735</td>
</tr>
<tr>
<td>3.5</td>
<td>3.1 (0.88-15.9)</td>
<td>3.20</td>
<td>0.735</td>
</tr>
<tr>
<td>4.0</td>
<td>6.5 (1.46-29.1)</td>
<td>6.04</td>
<td>0.140</td>
</tr>
<tr>
<td>4.5</td>
<td>4.2 (0.94-18.6)</td>
<td>3.51</td>
<td>0.061</td>
</tr>
<tr>
<td>5.0</td>
<td>5.6 (1.21-26.0)</td>
<td>4.84</td>
<td>0.278</td>
</tr>
<tr>
<td>cTnI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>4.1 (0.97-17.7)</td>
<td>3.66</td>
<td>0.0557</td>
</tr>
<tr>
<td>1.25</td>
<td>4.6 (1.06-19.8)</td>
<td>4.17</td>
<td>0.0412</td>
</tr>
<tr>
<td>1.50</td>
<td>7.5 (1.65-34.0)</td>
<td>6.81</td>
<td>0.009</td>
</tr>
<tr>
<td>1.60</td>
<td>8.8 (1.90-40.6)</td>
<td>7.67</td>
<td>0.0056</td>
</tr>
<tr>
<td>2.00</td>
<td>4.2 (0.82-21.1)</td>
<td>2.97</td>
<td>0.085</td>
</tr>
<tr>
<td>2.25</td>
<td>5.1 (0.97-26.9)</td>
<td>3.69</td>
<td>0.055</td>
</tr>
<tr>
<td>2.50</td>
<td>2.9 (0.47-17.8)</td>
<td>1.33</td>
<td>2.91</td>
</tr>
</tbody>
</table>

The CK-MB cutoff concentration for AMI diagnosis was 5.0 µg/L. For the premarket cTnI assay, we previously determined a normal range of 0–0.5 µg/L from 150 healthy individuals, and a cutoff value of 2.5 µg/L for AMI diagnosis [8]. Logistic regression analysis was performed with a PC-based statistical package (Version 4.0; Crunch Software, Oakland, CA).

During the study period, 9 patients had adverse outcomes. There were 3 AMIs during hospitalization and 3 after discharge, and there were 4 cardiac deaths overall. Different cutoff concentrations for CK-MB and cTnI were considered in determining what values produced the best discrimination between good and poor outcomes. Table 1 lists the odds ratios and 95% confidence intervals (CI) for the prediction of short-term risk. Cutoff values of 4.0 µg/L for CK-MB and 1.6 µg/L for cTnI produced results with the highest odds ratios: 6.5 (95% CI, 1.5-29) and 8.8 (1.9-41), respectively. The results obtained with these lower cutoff concentrations were more predictive than those based on the preestablished AMI cutoff values of 5.0 and 2.5 µg/L, respectively.

These odds ratios show that poor outcome was significantly more frequent in the high-cTnI group than in the low-cTnI group. Although also prognostic in this study, the utility of CK-MB for risk stratification remains questionable, given the conflicting results of previous studies [4]. cTnI may have more promise because it has higher specificity for heart damage than does CK-MB [9]. Odds ratios for cTnI in this study were similar to those in our previous study of cTnT [10]. Whether or not cTnI is equal to or better than cTnT remains to be determined. The specificity of cTnT has been debated, particularly in patients with chronic renal failure [11-13]; use of cTnT for risk stratification would be less useful if results are indeed falsely increased in these patients.

We thank Behring Diagnostics, Westwood, MA, for loan of equipment, gifts of reagents, and financial assistance to conduct this study.

References


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Choice of Materials for Long-Term Quality Control of Blood Folate Assays

To the Editor:

As a national reference laboratory in the nutritional anemias, we report >12 000 diagnostic blood folate estimations annually. The accuracy and long-term reproducibility of our folate microbiological assays are thus important, and care is taken to ensure both the quality and stability of our in-house control materials for the assay. We recently compared the long-term performances of these in-house human blood controls and of those from a commercial source.

Commercially available lyophilized human blood controls (Lyphocheck Whole Blood Controls, lot 28200; ECS Division, Bio-Rad Labs., Anaheim, CA) reportedly performed well during a 2-year evaluation [1]. These two controls, which were reconstituted, diluted...
with ascorbic acid, and stored at −70 °C, gave CVs of 7.64% and 3.95% over 102 separate runs, when assayed with the QuantaPhase 121-radioassay kit (no. 190-6002; Bio-Rad).

Our current in-house quality-control materials are made from fresh whole blood and are stored frozen at −20 °C. To evaluate the reconstituted commercial controls as a potential alternative to our in-house materials, we compared the long-term quality-control performances of both sets of materials when stored together at −20 °C, rather than at the −70 °C used in the earlier study [1].

We prepared the Lyphochek controls exactly as recommended [1]. Briefly, vials of lyophilized human blood (Lyphochek Levels 2 and 3) were reconstituted with deionized water, mixed, and allowed to stand for 30 min. They were then diluted 1:11 in fresh 10 g/L ascorbic acid to give an ascorbated hemolysate [1]. We also prepared a more-dilute hemolysate suitable for direct addition to microtiter plates for microbiological assay. This was prepared by a further 1:40 dilution of the above hemolysate in 5 g/L sodium ascorbate.

The in-house controls (n = 4), having a range of folate values, were prepared by diluting fresh anticoagulated (EDTA) whole blood 1:11 into fresh 10 g/L ascorbic acid, mixing, and incubating at 37 °C for 30 min to allow complete deconjugation of folate polyglutamates. Cell debris was removed by centrifugation, and the supernatant liquid was filtered through a 0.2-μm syringe filter (Nalge, Rochester, NY). Again, a more-dilute hemolysate was prepared by 1:40 dilution in 5 g/L sodium ascorbate.

Both commercial and in-house undiluted and diluted hemolysates were dispensed in 500-μL aliquots into cryovials (Sarstedt, Wexford, Ireland) and stored at −20 °C. They were thawed and assayed weekly by microbiological assay [2] over a period of 16 months.

The long-term performance of undiluted in-house controls (n = 4) was good, with individual reproducibility (CV) over the 16 months of 5.2%, 5.6%, 2.3%, and 5.4%. Equivalent reproducibility data for diluted in-house materials assayed in parallel were 3.5%, 3.4%, 4.3%, and 4.0%, respectively. We continued to assay these diluted hemolysates weekly for a further 10 months and obtained good reproducibility over the full 26-month period (CVs of 3.9%, 7.1%, 3.6%, and 5.5%, respectively). On the other hand, analysis of both the undiluted and diluted commercial control hemolysates was discontinued after the initial 16 months because of poor reproducibility (CV >50%) due to folate instability (Fig. 1).

The whole-blood folate concentrations of the in-house (n = 3) and commercial controls stored and assayed weekly in parallel are shown in Fig. 1 as monthly means. Diluted commercial hemolysates (Fig. 1b) were more stable than undiluted commercial hemolysates (Fig. 1a) over 16 months, but still showed substantial degradation of folate activity.

We then compared stabilities of the commercial hemolysates stored at −20 °C and −70 °C, using materials from the same freeze-dried batch, which were still within the expiration date. Lyphochek whole-blood control levels 2 and 3 were again reconstituted as described. Hemolysates were prepared as before (diluted 1:11 in 10 g/L ascorbic acid) and also further diluted (1:40 with 5 g/L sodium ascorbate). These preparations were stored at both −20 °C and −70 °C for 10 months and assayed weekly in parallel.

Analysis of the monthly means (n = 10) confirmed the poor reproducibility of both undiluted and diluted ascorbated hemolysates at −20 °C, whereas at −70 °C the identical aliquots remained quite stable, whether stored undiluted (CVs of 3.6% and 3.8%) or diluted (1.8% and 5.5%).

The relative instability of folates at −20 °C in reconstituted commercial hemolysates as compared with fresh in-house whole-blood hemolysates in ascorbate is disconcerting and may relate to analytical supplementation by the manufacturers so as to achieve target values. Lyphochek controls are designed to be stored lyophilized at 4 °C, and the manufacturer makes no claims for the stability of ascorbated hemolysates. They do state, however, that the reconstituted frozen material (at −10 °C to −20 °C) will be stable for 30 days; we did not evaluate that average condition.

The controls gave excellent reproducibility previously when stored at −70 °C [1], and our study confirms that. The results presented here also emphasize the importance of assessing the performance of all such controls before using them.

References
Letters

Screening for Sulfite Oxidase Deficiency with Urinary Thiosulfate/ Sulfate Ratios Determined by Anion Chromatography

To the Editor:

Detection of sulfite oxidase deficiency by urine screening tests is hampered by the rarity of the disorder and the complex chemical character of the excreted compounds [1]. False negatives are well recognized, both with use of the commercial urine test strip and the various “screening” assays for sulfite and thiosulfate [2–5]. False positives are also not uncommon [6]. However, several novel analytical methods have recently been reported, including a robust assay for urinary S-sulfocysteine [7] and a reliable method for quantifying total sulfite in serum [8]. In their original description of the commercial assay, Wadman et al. [9] referred to the potential utility of quantitative ion chromatography. However, urine is a complex matrix precluding simple separation and conductimetric detection of trace anions, as we discovered in attempting to establish a normal reference range for thiosulfate. We found anion-chromatographic separation of urinary thiosulfate satisfactory if a “heart-cut” method [10] was used, directing the void volume fraction (with abundant anions such as chloride) and the tailing fraction to waste.

We have recently reported our method [11], with which we determined a reference range modestly lower than previously published [12]. We believe this difference may reflect a positive bias introduced by background urinary thiocyanate in the commonly used cyanalysis procedure. In our utilization of anion chromatography to help detect sulfite oxidase deficiency, we normalized the thiosulfate excretion data with respect to urinary sulfate rather than to creatinine [13] for two reasons: (a) excretion of the sulfite oxidase product, inorganic sulfate, is limited in affected patients [14], so the ratio is likely to be more efficient in detecting the inborn error; and (b) sulfate and thiosulfate can both be quantified by using the same chromatographic conditions.

Table 1 shows the results for clean, early-morning urines from controls and two patients (courtesy of A. Feigenbaum, Hospital for Sick Children, Toronto, ON): one with molybdenum cofactor deficiency and the other with isolated sulfite oxidase deficiency (confirmed by J. Johnson, Duke University, Durham, NC). Preliminary studies of the obligate heterozygote parents revealed no overlap of values with those in the affected children (data not shown). Thus, ion chromatography continues to be an important alternative method for assay of inorganic sulfur ions. The method may be a useful adjunct for studies of sulfite sensitivity and for clinical referral laboratories charged with screening for sulfite oxidase deficiency.

Supported in part by a grant from PSI Foundation of Ontario.

References


Table 1. Urinary thiosulfate and sulfate in sulfite oxidase-deficient patients and controls.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, years</th>
<th>n</th>
<th>Thiosulfate (S2O3-), μmol/L</th>
<th>Sulfate(SO4-), mmol/L</th>
<th>S2O3-/SO4- ratio, μmol/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated sulfite oxidase deficiency</td>
<td>2.4</td>
<td>1</td>
<td>2197</td>
<td>3.80</td>
<td>578</td>
</tr>
<tr>
<td>Molybdenum cofactor deficiency</td>
<td>4.3</td>
<td>1</td>
<td>1220</td>
<td>1.92</td>
<td>635</td>
</tr>
<tr>
<td>Controls: Infants</td>
<td>&lt;2</td>
<td>8</td>
<td>7.14 ± 1.63 (0.3–18.3)²</td>
<td>5.75 ± 0.81 (2.7–8.9)</td>
<td>1.66 ± 0.59 (0.27–2.94)</td>
</tr>
<tr>
<td>Children</td>
<td>2–10</td>
<td>21</td>
<td>8.46 ± 2.04 (0.3–39.2)</td>
<td>10.8 ± 0.6 (4.7–24.4)</td>
<td>0.83 ± 0.08 (0.04–2.98)</td>
</tr>
<tr>
<td>Children</td>
<td>10–18</td>
<td>14</td>
<td>10.78 ± 3.81 (0.8–26.2)</td>
<td>14.8 ± 1.6 (1.5–27.2)</td>
<td>0.54 ± 0.11 (0.01–1.27)</td>
</tr>
<tr>
<td>Adults</td>
<td>&gt;18</td>
<td>20</td>
<td>9.41 ± 1.16 (0.4–27.3)</td>
<td>17.1 ± 1.8 (5.0–36.1)</td>
<td>0.49 ± 0.09 (0.03–1.94)</td>
</tr>
</tbody>
</table>

² Assayed in duplicate by ion chromatography, with intraassay CV = 4.5% and interassay CV = 11.7% [11].
³ Analyzed in duplicate with intraassay CV = 3.5% and interassay CV = 5.9%, under conditions identical to those used for thiosulfate assay.
⁴ Mean ± SE (and range).
⁵ Taken from ref. 11. In that study, the difference between 24-h urines and early-morning specimens was much smaller than the interindividual variation.

* Corresponding author.

Spokespersons for Bio-Rad Labs. comment:

To the Editor:
The commercially available Lymphocheck Whole-Blood Control from Bio-Rad Labs. is supplied in lyophilized form and may conveniently be stored at refrigerated temperatures over its shelf life. The product is neither designed nor recommended to be stored frozen for longer than 30 days; further, no claims are made for storage of the control material under ascibulated conditions. By contrast, in the study above, the control material was both ascibulated and stored frozen in excess of 30 days. Those specimens stored at −70°C performed identically to their previously reported behavior (above ref. 1).

Freezer space being at a premium in the clinical laboratory, one cannot expect that clinical chemists will want to spend additional time in ascibuting, aliquotting, and freezing control material. The format of Lymphocheck Whole-Blood Control provides clinicians with a material for quality control of folate test procedures along with the convenience of long-term refrigerated storage and simplified reconstitution.