The observed change in calibration of the Siemens method increased GFR values. For instance, a cystatin C concentration corresponding to a GFR of 60 mL·min⁻¹·(1.73 m²)⁻¹ in 2005 increased to approximately 80 mL·min⁻¹·(1.73 m²)⁻¹ in 2010.

Owing to the nonlinear relationship between cystatin C and the estimated GFR, the bias in cystatin C concentrations in patients with a normal kidney function and those with a moderately decreased kidney function had the greatest effect on the estimated GFR. Therefore, it is essential that the calibration of the cystatin C method be consistent, because small changes in cystatin C will introduce a large bias in GFR estimates. The Siemens direct carbohydrate-deficient transferrin method was reported to have a downward shift in calibration between 2006 and 2007 (5). Both Siemens methods are based on particle-enhanced nephelometry, and the downward shifts in calibration for the 2 methods coincided in time and approximate magnitude.

The results of this study indicate that a downward shift in calibration for the Siemens cystatin C method occurred between March 2006 and December 2008. Cystatin C-based equations to estimate GFR that were derived from results obtained from older lots of reagent and calibrator cannot be used with the current Siemens method. The results emphasize the need for an international cystatin C reference material, which has recently been developed by the Institute for Reference Materials and Measurements.

**Editor’s Note:** Siemens, when contacted, declined to comment on this letter.

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**References**


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**No Effect of Anticoagulant on Hb A1c Analysis by the IFCC Reference Procedure**

**To the Editor:**

The use of glycohemoglobin (Hb A₁c)¹ as a diagnostic marker has been limited because of the lack of a standardized method. Hb A₁c estimation with the commercially available instruments is based on different analytical and immuno-

¹ Nonstandard abbreviations: Hb A₁c, glycohemoglobin; ICH, International Council for Standardization in Haematology; R_{external} calibration curve of glycated and nonglycated hexapeptide calibrants; Sx, Hb A₁c signal; Sy, Hb A₁c signal; R_{chex} ratio of Hb A₁c signal to the Hb A₁c signal.
logical principles that are method and reagent dependent. Consequently, several global initiatives have been taken toward optimizing and standardizing Hb A1c estimation. An IFCC working group carried out one such initiative, which is now considered the gold standard for Hb A1c measurement (1–4). This method is based on the proteolytic digestion of the N-terminal β chain of Hb A1c and Hb A0, followed by chromatographic separation of the hexapeptides and quantification by either liquid chromatography–mass spectrometry or capillary electrophoresis with ultraviolet detection.

Anticoagulants are used to prevent the coagulation of blood. The IFCC method uses EDTA as the anticoagulant. The effects of other anticoagulants have not been studied, however, although they are known to influence glucose analysis. We therefore analyzed the effect of the different anticoagulants (sodium heparin, lithium heparin, sodium fluoride, and sodium citrate) on Hb A1c measurement by the IFCC method.

Fresh blood samples were collected from 3 volunteers who had different Hb A1c percentages (low, medium, high) into Vacutainers (Becton Dickinson) containing different anticoagulants. Hemolysates were prepared and stored as per the IFCC method until analysis. Fresh blood samples were collected and prepared as per the IFCC method. The Hb A1c percentages obtained for samples obtained from the volunteers. The Hb A1c percentages obtained for different anticoagulants for volunteers with high, medium, and low glycemic index values were, respectively: EDTA, 9.07%, 6.13%, and 3.25%; lithium heparin, 8.57%, 6.16%, and 3.29%; sodium citrate, 8.71%, 5.98%, and 3.12%; sodium fluoride, 9.22%, 6.14%, and 3.13%; and sodium heparin, 9.32%, 6.10%, and 3.10%. CV values were 5%.

The Hb A1c percentages obtained in the study showed no variation with respect to the use of different anticoagulants. One of the reasons for this uniformity could be due to the lysis of erythrocytes before Hb A1c estimation. Moreover, the chromatographic separation of the digested products followed by their resolution as the m/z ratios of their protonated adducts [(M+H)+] is also independent of anticoagulants.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References

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Necessity of Fractionated Urine Collection for Monitoring Patients with Cystinuria

To the Editor:

Cystinuria is an inherited form of nephrolithiasis caused by mutations in the SLC7A9 [solute carrier family 7 (cationic amino acid transporter, y+ system), member 9] and SLC3A1 [solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, activator of cystine, dibasic and neutral amino acid transport), member 1] genes, which encode the luminal transporter b(0,+ ) AT subunit and rBAT subunit, respectively. The disease is characterized by impaired proximal tubular and intestinal reabsorption of cystine and the dibasic amino acids ornithine, lysine, and arginine (1). Treatment of cystinuria aims to prevent new stone formation and consists of increased fluid intake, urine alkalization, and cystine-binding drugs for avoiding urinary cystine supersaturation, which occurs when the urinary cystine concentration is >250 mg/L at pH 4.5–7.5 or >500 mg/L at pHs >7.5 (2). The effectiveness of this treatment is often disappointing. Patients undergo frequent surgeries, which are often followed by an early relapse. Monitoring urinary cystine is the cornerstone of cystinuria management; however, most textbooks do not provide clear recommendations as to how such monitoring should be performed. The traditional way to monitor the cystine concentration has been analysis of a 24-h urine sample; however, achieving a 24-h cystine concentration below concentrations indicating risk does not prevent formation of renal stones (2–5). Stone formation in such cases can be due to diurnal variation in urinary cystine excretion (2–4), but it also can be due to diurnal variation in urinary pH and fluid intake.

We evaluated the benefit of fractionated 24-h urine collection for 9 patients with homozygous cystinuria (ages 8–65 years, 4 males) (Table 1). We collected 4 urine fractions from each of the participating patients and detected 15 fractions with cystine supersaturation (42% of all investigated collection fractions). The night period (0200–0700) was the most risky, because the concentration of cystine solubility was exceeded at this time in 6 of the 9 cases. On the other hand, the daytime period also required careful attention (9 of the 15 urine fractions with supersaturation were collected during the day), possibly because of a dietary load with methionine (2), insufficient fluid intake, or decreased urinary pH.

Twelve (80%) of the 15 fractions with supersaturation would have been missed if only standard 24-h samples had been analyzed. Only 1 of the patients would have been considered “at risk” for deviation of cystine stones according to the 24-h collection results.

For optimal monitoring of patients with cystinuria, we stress the necessity of 6-h urine collections, not only to distinguish day and night portions but also for detecting day periods at risk for cystine supersaturation and thus nephrolithiasis.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article.

Table 1. Diurnal variation of cystine concentration.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cystine, mg/L pH</th>
<th>Cystine, mg/L pH</th>
<th>Cystine, mg/L pH</th>
<th>Cystine, mg/L pH</th>
<th>Cystine, mg/L pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0700–1300</td>
<td>1200–1900</td>
<td>1900–0200</td>
<td>0200–0700</td>
<td>24-h Collection</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>398</td>
<td>124</td>
<td>8.5</td>
<td>305</td>
<td>683</td>
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<tr>
<td>2</td>
<td>576</td>
<td>7.5</td>
<td>325</td>
<td>8.0</td>
<td>365</td>
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<tr>
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<td>5.5</td>
<td>210</td>
</tr>
<tr>
<td>4</td>
<td>138</td>
<td>7.0</td>
<td>94</td>
<td>5.0</td>
<td>183</td>
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<tr>
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<td>6</td>
<td>446</td>
<td>8</td>
<td>204</td>
</tr>
</tbody>
</table>

*Cystine supersaturation: a cystine concentration >250 mg/L at pH <7.5 or >500 mg/L at pH >7.5.

1 Human genes: SLC7A9, solute carrier family 7 (cationic amino acid transporter, y+ system), member 9; SLC3A1, solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, activator of cystine, dibasic and neutral amino acid transport), member 1.