
To the Editor:

We have developed an improved reference measurement method for hemoglobin A1c (Hb A1c) based on liquid chromatography–isotope dilution–tandem mass spectrometry (LC-ID-MS/MS) with traceability to the SI unit. The method has a small measurement uncertainty (MU) and gives results in good agreement with the accuracy-based IFCC reference method.

Kaiser et al. (1) demonstrated the possibility of using ID-MS for Hb A1c measurement, which involved the proteolysis of hemoglobins using endoproteinase Glu-C and hydrolysis of hexapeptide calibration standards using HCl. However, large MU and significant difference in results compared with the IFCC reference method were observed. Using the same approach, we developed an improved procedure based on LC-ID-MS/MS and used the method for participation in an IFCC ring trial for reference laboratories (RELA 2013) for Hb A1c, in which our results for 2 lyophilized samples were compared against those from the IFCC reference method, including 6 approved IFCC network laboratories for Hb A1c.

Key steps in our ID-MS procedure to preserve the unbroken traceability to the SI unit were the hydrolysis of the hexapeptide calibration standards [β-chain N-terminal Val-His-Leu-Thr-Pro-Glu (VE) and 1-deoxyfructoxyl-Val-His-Leu-Thr-Pro-Glu (GE)] and the proteolysis of Hb A0 and Hb A1c. The use of hexapeptide calibration standards allows the traceability to amino acid certified reference materials, which is more reliable than the traceability to reference protein standards in the IFCC reference procedure (1). To ensure accurate measurement, complete hydrolysis of the hexapeptides must be achieved. We found that the use of 1% phenol in 6 mol/L HCl markedly reduced the hydrolysis time from the previously reported 65 h (1) to 24 h. In addition, amino acids that are stable in acidic environment, such as leucine and proline, were suitable for the quantification of the hexapeptides. This was demonstrated by the consistency between the results of leucine and proline (deviation <0.6%) for both VE and GE. Valine was found to be unsuitable, possibly owing to its linkage to a deoxyfructoyxy group in GE. Because amino acid analysis is affected by both peptide and amino acid impurities, these impurities would need to be quantified (by HPLC and LC-MS/MS, respectively) to ensure the accuracy of the analysis and subsequently the Hb A1c measurement. In our case, VE had satisfactory purity, but GE contained VE as an impurity. The concentration of GE calibration solution was therefore corrected by the quantification of VE in GE to remove the positive bias in the Hb A1c measurement.

In proteolysis, incomplete reaction will result in significant deviations between the amounts of hexapeptides obtained and the true amounts of Hb A1c and Hb A0. We found that adding additional endoproteinase Glu-C was crucial to ensure complete proteolysis [125 g/mg hemoglobin vs the reported amount of 10 μg (2)]. We believe that the complete proteolysis of hemoglobins markedly improves the imprecision and accuracy and further enhances the robustness of our ID-MS method.

Table 1. LC-ID-MS/MS measurements of the samples in RELA 2013.

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>VE concentration, μmol/g (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.500</td>
<td>3.147</td>
</tr>
<tr>
<td>SD, μmol/g</td>
<td>0.024</td>
<td>0.034</td>
</tr>
<tr>
<td>CV, %</td>
<td>0.69</td>
<td>1.08</td>
</tr>
<tr>
<td>GE concentration, μmol/g (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.12854</td>
<td>0.2909</td>
</tr>
<tr>
<td>SD, μmol/g</td>
<td>0.00075</td>
<td>0.0025</td>
</tr>
<tr>
<td>CV, %</td>
<td>0.58</td>
<td>0.86</td>
</tr>
<tr>
<td>Hb A1c value (obtained), mmol/mol</td>
<td>35.42</td>
<td>84.6</td>
</tr>
<tr>
<td>U, mmol/mol</td>
<td>0.98</td>
<td>2.4</td>
</tr>
<tr>
<td>% Ua</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Hb A1c value (target), mmol/mol</td>
<td>35.02</td>
<td>83.9</td>
</tr>
<tr>
<td>Deviation from target value, mmol/mol</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Relative deviation from target value, %</td>
<td>1.14</td>
<td>0.83</td>
</tr>
</tbody>
</table>

* Expanded uncertainties were obtained by multiplying standard uncertainty by a coverage factor of 2. U, the expanded measurement uncertainty of the obtained Hb A1c value; %U, relative expanded measurement uncertainty.

b The means of the participating laboratories’ results in RELA 2013 were used as the target values. One outlier for sample A was excluded.

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1 Hb A0, hemoglobin A0; LC-ID-MS/MS, liquid chromatography–isotope dilution–tandem mass spectrometry; MU, measurement uncertainty; VE, β-chain N-terminal Val-His-Leu-Thr-Pro-Glu, GE, 1-deoxyfructoxyl-Val-His-Leu-Thr-Pro-Glu, NGSP, National Glycohemoglobin Standardization Program.

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Letters to the Editor

Cardiac Troponin Testing Is Overused after the Rule-In or Rule-Out of Myocardial Infarction

To the Editor:

No good studies have systematically evaluated appropriate clinical utilization of cardiac troponin testing in the clinical setting of the rule-in and rule-out of myocardial infarction (MI)\(^1\) (1, 2). Our collective 100-plus years of clinical and laboratory experience suggested that provider test ordering and use of cardiac troponin has been excessive after a diagnosis of MI or no MI has been determined. There is no evidence that supports continuation of cardiac troponin testing after a diagnosis is made (2–5). The goal of this study was to determine whether clinicians appropriately use cardiac troponin I (cTnI) in the assessment of patients at moderate to high risk of acute coronary syndrome (ACS) and MI.

After receiving institutional review board approval, we retrospectively reviewed electronic health/medical records in 100 consecutive patients who had serial cTnI orders in the cardiac renal unit, where patients at moderate to high risk for MI are evaluated. Diagnoses were adjudicated by either an emergency medicine physician or a cardiologist as MI or no MI according to the 2012 Third Universal Definition of MI guidelines (2). A cTnI order set consisted of measurements obtained at 0, 3, 6, and 9 h (Ortho-Clinical Diagnostics Vitros ES, 99th percentile 0.034 μg/L) (5). Clinicians were not limited to any number of order sets. Excessive cTnI measurements were defined as any cTnI ordered and measured after the provider made the diagnosis of MI or no MI. All cTnI results during hospitalization were tabulated.

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References


Lingkai Wong\(^2\)
Hong Liu\(^2\)
Sharon Yong\(^2\)
Qinde Liu\(^2\)*
Tong Kooi Lee\(^2\)

\(^2\) Chemical Metrology Division
Applied Sciences Group
Health Sciences Authority
Singapore

* Address correspondence to this author at:
1 Science Park Rd #01-05/06, The Capricorn Singapore Science Park II Singapore 117528
E-mail liu_qinde@hsa.gov.sg.

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Nonstandard abbreviations: MI, myocardial infarction; cTnI, cardiac troponin I; ACS, acute coronary syndrome; PCI, percutaneous coronary intervention.