Assessing Analytical Quality of Hb A1c Assays Using Sigma Metrics

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Abstract

Background: The use of Hb A1c assays for the diagnosis of type 2 diabetes requires that these assays be accurate, precise and robust in the clinical laboratory. The aim of the study was to evaluate the analytical performance of four Hb A1c commercial assays using accuracy based grading and Sigma metrics.

Methods: Accuracy based grading was accomplished by testing the analytical performance of Hb A1c assays using the IFCC reference method on four commercial Hb A1c assays: Abbott ARCHITECT Enzymatic; Roche Tira-quant, Tosoh G8 HPLC and the BioRad Variant II Turbo 2.0 assays. The eight reference sample panel was tested in two separate runs, five replicates per run, for a total of n=10 test results per reference sample per assay. Mean and %CV were calculated for each sample for each assay and the Sigma metrics were calculated using a TEa = 6%.

Results: The total number of samples with Six Sigma or greater performance for each assay were as follows: Abbott ARCHITECT 6/8 (range 5-6 Sigma); BioRad Variant 5/8 (range 0.4-2 Sigma); Roche Tira-quant 2/8 (range 0.7-2 Sigma); and Tosoh G8/8 (range 0.4-2 Sigma).

Conclusion: The Abbott ARCHITECT enzymatic assay was the only assay to perform based Six Sigma assay performance across the most reference samples in this study, followed by Roche Variant II Turbo and Roche Reagent Assay. Only sub Six Sigma performance was observed with all reference samples using the Tosoh G8 assay.

Introduction

Glycated Hemoglobin A1c (Hb A1c)

Hb A1c is a naturally occurring, non-enzymatic product resulting from posttranslational modification of red blood cell (RBC) hemoglobin. It reflects the average plasma glucose concentration over the normal ~120 day average life span of the red blood cell. Hb A1c is formed by attachment of glucose to N-terminal amino acids of the hemoglobin beta chain. Hb A1c is defined as β-(N-desacyl)deoxyfructosyl Hb, a hexapeptide, and is the major glycation site of the Hb A1c molecule. Hb A1c correlates with the risk of long-term complications and may be used as a marker of glycemic control. Six Sigma methods have been introduced. The National Glycohemoglobin Standardization Program (NGSS) certifies methods for Hb A1c quantification and sets standards for all Hb A1c assays for use in diagnostic laboratories. A total allowable error goal of +/- 6% was established by the College of American Pathologists (CAP) for its accuracy-graded survey. Field method results must produce results within +/- 6% of the assigned target value for the CAP PT samples. A higher order reference method for Hb A1c is recommended by the International Federation of Clinical Chemistry (IFCC) and is linked to the values set by the NGSS.

IFCC Reference System

The IFCC reference method uses Hb from washed and lysed RBCs and cleaves the terminal end of the beta chain into a hexapeptide using the proteolytic enzyme endoproteinase Glu-C. The non-glycated non-glycated peptides are separated using either mass spectrometry or capillary electrophoresis with ultraviolet detection. The two detection methods yield equivalent results. Results are reported as mmol/mol. Annually all whole blood pools are prepared and made available to manufacturers. These samples have values assigned by the reference method and can thus be used as accuracy controls to assess bias of field methods.

Hb A1c values reported in NGSP units can be converted to IFCC SI units using the master equation:

NGSP Hb A1c (% A1c) = 0.915 (IFCC Hb A1c, mmol/mol) + 2.159

Materials & Methods

Analyzers and Assays

1. Tosoh G8 HPLC (Tosoh, San Francisco, CA)
2. Bio-Rad Variant II Turbo HPLC (Bio-Rad, Hercules, CA)
3. Roche c651, Tira-Quant immunosay (Roche, Indianapolis, IN)
4. Abbott ARCHITECT c8000, Next Gen Hb A1c (enzymatic) (Abbott, Abbott Park, IL)

Controls

Bio-Rad Hb A1c lyophilized diabetes biological control, lyophilized human whole blood. Level I control target values ranged from about 5.4 – 8.0 % Hb A1c (55 – 86 mmol/mol) for the four systems and Level II control target values ranged from about 9.2% - 10 % Hb A1c (77 – 84 mmol/mol).

Precision

Precision was determined for each of the IFCC reference panel samples. Each of the reference samples was analyzed on two separate analytical trials in replicates of 5 (n = 10). The % SD, and % CV were calculated for each assay/analyzer for each of the IFCC reference samples.

Sigma Metric Calculation

Sigma metrics were calculated as follows:

• Sigma metric = (TEa – bias) /% CV (all values expressed as percent (%))
• TEa = Total Error Allowable (+/ -6% of IFCC concentration)
• Bias = Target value of IFCC sample – observed mean Hb A1c for each assay
• % CV = Precision as measured for each assay with each IFCC reference panel sample

References


Conclusion

Hb A1c is a critical assay because of the worldwide diabetes epidemic. Analytical performance of Hb A1c assays has improved dramatically and may now be used to diagnose diabetes in addition to monitoring glycemic control. However, analytical quality is imperative and must be initially proven and then monitored for the early detection of patients at risk of developing diabetes. The IFCC reference method for Hb A1c is internationally accepted as the “gold standard” for this assay. The IFCC reference system provides commutable whole blood samples with reference method target values. Thus “true bias” of assays, instead of “relative bias,” can be measured throughout the analytical measurement system by testing a panel of IFCC reference samples. A TEa of +/- 6% has been established based on clinical needs for diagnosis. Comparison of observed bias to this TEa target is the NGSS accepted measure of accuracy. Sigma metrics also allow assay quality to be objectively assessed on the basis of TEa, bias, and precision.

Objective comparison of analytical quality of common Hb A1c field methods on the basis of Six Sigma metrics demonstrated some marked differences. The Abbott ARCHITECT enzymatic assay demonstrated the best accuracy across the IFCC reference samples based on bias and Sigma metrics, followed by the Bio-Rad and Roche assays, respectively. Surprisingly, in this study the greatest bias and the lowest Sigma metrics performance were observed with the reference samples when analyzing using the Tosoh G8 assay.

Table 1. Target values off IFCC reference panel samples, observed Hb A1c values, bias, and precision (%CV) for the field methods, and Six Sigma metrics for each assay.

<table>
<thead>
<tr>
<th>Reference Value %HbA1c</th>
<th>Abbott</th>
<th>Roche</th>
<th>Tosoh</th>
<th>BioRad</th>
<th>Sigma Metric</th>
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<tbody>
<tr>
<td>4.99</td>
<td>5.9</td>
<td>5.3</td>
<td>5.0</td>
<td>5.0</td>
<td>1.4</td>
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<td>5.70</td>
<td>0.9</td>
<td>6.8</td>
<td>1.8</td>
<td>1.6</td>
<td>7.5</td>
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<tr>
<td>6.72</td>
<td>1.3</td>
<td>8.4</td>
<td>2.9</td>
<td>5.0</td>
<td>2.4</td>
</tr>
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<td>7.55</td>
<td>1.6</td>
<td>13.8</td>
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<td>8.44</td>
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<td>28.3</td>
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<td>4.0</td>
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<td>10.36</td>
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<td>8.4</td>
<td>2.0</td>
<td>2.0</td>
<td>1.5</td>
</tr>
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<td>11.26</td>
<td>2.0</td>
<td>23.3</td>
<td>4.9</td>
<td>2.0</td>
<td>4.0</td>
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<table>
<thead>
<tr>
<th>Hb A1c (%)</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tbody>
<tr>
<td>Sigma Metric</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>3.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Average precision calculation was as follows: Bias: 0.9 (mean); %CV: 4.0 (mean). TEa was set as +/- 6% and Sigma calculations were done using Eq 1 for each assay.

TEa = Total Error Allowable (+/- 6% of IFCC concentration)