Assessing Analytical Quality of HbA1c Assays Using Sigma Metrics

Dave Armbruster[1], Gregory Maine[1], Joseph Litten[2], Saeed Jortani[3], Sarah Pickering[3]

[1]Abbott Diagnostics, Abbott Park IL, USA; [2]Winchester Medical Center, Winchester, VA, USA; [3]Kentucky Clinical Trials Laboratory, Louisville, KY, USA

IFCC-EFLM EuroMedLab Paris 2015 Congress • 21 – 25 June, 2015

ABSTRACT

Background: The use of HbA1c assays for the diagnosis of type 2 diabetes requires that these assays be accurate, precise and robust in the clinical laboratory. The aim of this study was to evaluate the analytical performance of four HbA1c commercial assays using accuracy based grading and Sigma metrics.

Methods: Accuracy based grading was accomplished by testing eight frozen whole blood samples from the European Reference Laboratory for Glycohemoglobin (ERL) with HbA1c values determined by the IFCC reference method on four commercial HbA1c assays: Abbott ARCHITECT Enzymatic; Roche Tina-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 3.5-30 Sigma); Bio-Rad Variant 5/8 (range 0.4–21 Sigma); Roche Tina-quant 2/8 (range 0–7.2 Sigma); and TOSOH G8 0/8 (range 0–4.2 Sigma).

Conclusion: The Abbott ARCHITECT enzymatic assay demonstrated accuracy based Six Sigma assay performance across the most reference samples in this study, followed by the Bio-Rad and Roche assays. Only sub Six Sigma performance was observed with all reference samples using the TOSOH G8 assay.

INTRODUCTION

GLYCATED HEMOGLOBIN A1C (HbA1c)

HbA1c is a naturally occurring, non-enzymatic product resulting from exposure of hemoglobin to glucose. It reflects the average plasma glucose concentration over the normal ~120 day average life span of the red blood cell. HbA1c is formed by attachment of glucose to N-terminal amino acids of the hemoglobin beta chain. HbA1c is defined as β-N-valine glycated HB (β-N(1-deoxy) fructosyl Hb, a hexapeptide, and is the major glycation site of the HbA1c molecule. HbA1c correlates with the risk of long-term diabetes complications and its measurement has been widely used for monitoring of long-term blood glucose control and compliance in individuals with diabetes mellitus. Several studies, including the Diabetes Control and Complications Trial (DCCT), have shown long-term control of diabetes can prevent complications such as cardiovascular disease, retinopathy, nephropathy, and neuropathy. More recently, HbA1c is used to diagnose diabetes.

OBJECTIVE

The objective of this study was to compare the performance of four common HbA1c methods. A whole blood reference panel was obtained from the IFCC reference system laboratory (Streetziekenhuis Koningin Beatrix, Winterswijk, The Netherlands) with target values assigned by the IFCC reference method. The accuracy of each assay was assessed by the observed bias (difference between target value and observed value) and the Six Sigma metric for each assay. Comparison of the results of each field method to the reference panel assigned target values represents an "absolute bias" (difference between routine clinical laboratory methods and the accepted reference method) as opposed to a "relative bias" (difference between two routine methods or a routine method and a mean value as estimated by an EQA/PT program). This approach was purposely chosen to come as close as feasible to knowing "scientific truth."

INTRODUCTION (CONT'D)

DIABETES CLINICAL GUIDELINES

American Diabetes Association (ADA) Recommendations for diagnosis and monitoring of diabetes.

Diabetes diagnostic threshold: ≥ 6.5 %HbA1c (≥ 48 mmol/mol)

Pre-diabetes diagnostic range: 5.7–6.4 %HbA1c (39–46 mmol/mol)

Glycemic control target: ≤ 7.0 %HbA1c (≤ 53 mmol/mol)

The major advantages and disadvantages of using HbA1c for diagnosis over traditional glucose measurements are shown below:

ADVANTAGES

- Convenient (no fasting required)
- Low day-to-day variability
- Reflects long-term glucose exposure

DISADVANTAGES

- Result not accurate in some medical conditions (e.g. Sickle cell anemia, hemoglobinopathies)
- Not for diagnostic use in children, pregnancy, or other conditions in which blood glucose may change rapidly
- Influenced by ethnicity and age

METHODOLOGIES

HbA1c is measured by a variety of methods. Some are based on charge differences, some on structural differences, and some on chemical reactivity. The most common methods are immunoassay and high performance liquid chromatography (HPLC). Recently, enzymatic methods have been introduced. The National Glycohemoglobin Standardization Program (NGSP) certifies methods used for HbA1c quantification and sets standards for all HbA1c assays for use in diagnostic laboratories. Beginning in 2013, a total allowable error goal of +/- 6% was implemented 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 has been established by the International Federation of Clinical Chemistry (IFCC) and is linked to the values set by the NGSP.

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 glycated and non-glycated peptides are separated using HPLC. The glycated and non-glycated peptides are quantitated using either mass spectrometry or capillary electrophoresis with ultraviolet detection. The two detection methods yield equivalent results. Results are reported as mmol/mol. Annually eight whole blood pools are prepared and made available to manufacturers. These samples have values assigned by the reference methods and thus can be used as accuracy controls to assess bias of field methods.

HbA1c values reported in NGSP units can be converted to IFCC SI units using the master equation:

NGSP HbA1c (% A1c) = 0.915 (IFCC HbA1c, mmol/mol) + 2.15%

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 c501, Tina-Quant immunoassay (immunoturbidimetry) (Roche, Indianapolis, IN)
4. Abbott ARCHITECT *c*8000, Next Gen HbA1c (enzymatic) (Abbott, Abbott Park, IL)

CONTROLS

Bio-Rad HbA1c lyphochek diabetes bilevel control, lyophilized human whole blood. Level I control target values ranged from about 5.4–6.0% HbA1c (35–36 mmol/mol) for the four systems and Level II control target values ranged from about 9.2–10 % HbA1c (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 mean, 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 HbA1c for each assay
- %CV = Precision as measured for each assay with each IFCC reference panel sample

RESULTS

Table 1. Target values off IFCC reference panel samples, observed HbA1c values, bias, and precision (%CV) for the field methods, and Six Sigma metrics for each assay.

		ARCHITECT 1			Roche			TOSOH			Bio-Rad Variant Turbo 2.0		
Ref Value %A1c	TEa	Bias	Precision	Sigma Metric	Bias	Precision	Sigma Metric	Bias	Precision	Sigma Metric	Bias	Precision	Sigma Metric
4.99	6	1.4	1.0	3.5	5.0	4.6	0.0	1.8	3.4	0.9	3.8	3.3	0.4
5.70	6	0.9	0.9	5.3	6.8	1.8	0.0	1.6	2.9	1.4	1.6	0.5	7.5
6.72	6	0.3	0.8	8.4	3.7	2.9	1.0	4.0	3.4	0.8	2.4	0.9	4.7
7.55	6	1.1	0.6	10.3	0.8	1.9	3.5	6.5	1.2	0.9	2.0	0.0	> 18
8.44	6	1.7	0.5	13.8	0.8	1.1	6.7	5.6	1.3	2.1	2.4	0.6	10.2
9.33	6	2.9	0.0	> 20	0.9	1.5	5.8	6.5	1.2	2.4	2.9	0.0	> 21
10.36	6	1.4	0.3	29.6	2.0	1.2	7.2	3.9	4.9	1.3	0.5	0.5	18.1
11.26	6	1.9	0.4	22.3	2.5	1.5	5.9	4.4	1.6	4.2	1.4	2.4	4.0

	ARCHITECT 1	Roche	TOSOH	Bio-Rad Variant Turbo 2.0
≥ 6 Sigma	6	2	0	5
3–5.9 Sigma	2	3	1	2
< 3 Sigma	0	3	7	1

CONCLUSIONS

HbA1c is a critical assay because of the worldwide diabetes epidemic. Analytical performance of HbA1c 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 HbA1c is internationally accepted as the "gold standard" for this analyte. 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 range (AMR) for any field assay 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 NGSP accepted measure of accuracy. Six Sigma metrics also allows assay quality to be objectively assessed on the basis of TEa, bias, and precision.

Objective comparison of analytical quality of common HbA1c field methods on the basis of bias and 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 analyzed using the TOSOH G8 assay.

REFERENCES

1. Standards of medical care in diabetes – 2013. *Diabetes Care.* 2013;36:S11-66.
2. Jeppsson J-O, Koblold U, Barr J, et al. Approved IFCC reference method for the HbA1c in human blood. *Clin Chem Lab Med.* 2002;40:78-89.
3. Hoelzel W, Weykamp C, Jeppsson J-O, et al. IFCC referencesystem for measurement of hemoglobin A1c in human bloodand the national standardization schemes in the United States, Japan, and Sweden: A comparison study. *Clin Chem.* 2004;50:166-174.
4. Weykamp C, John WG, Mosca A, et al. The IFCC reference measurement system for HbA1c: A 6-year progress report. *Clin Chem.* 2008;54:240-248.
5. Weykamp C, John WG, Mosca A. A review of the challenge in measuring hemoglobin A1c. *J Diabetes Sci Technol.* 2009;3:439-445.
6. Weykamp C, Mosca A, Gillery P, Panteghini M. The analytical goals for hemoglobin A1c measurement in IFCC units and National Glycohemoglobin Standardization Program units are different. *Clin Chem.* 2011;57:1204-12-5.
7. Goodall I. HbA1c standardisation destination – Global IFCC standardisation how, why, where and when. *Clin BiochemRev.* 2005;26:5-19.

ADD-120023-GBL-EN (v3.1)