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Have you stress tested your assay?

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ABSTRACT

Objectives: When a clinical assay is stressed with extraordinarily high volume of specimens over a short period of time, extra caution may be needed to avoid systematic errors and biases. Here we report our experience with a HgbA1c assay used for high volume wellness screening purpose, to illustrate the importance of stress testing during assay validation.

Design and Methods: Over 15,000 whole blood specimens were tested for HgbA1c in a period of 2 months. HgbA1c was tested by an immunoturbidimetric method on a high through-put automation line. The HgbA1c population distribution in our study was compared to that from the NHANES database. Daily distributions of HgbA1c values $\geq 6\%$, means and medians were plotted. Correlation studies were performed between the high through-put immunoturbidimetric assay and a medium through-put HPLC method.

Results: We observed a shift of HgbA1c distribution to the higher values compared to the NHANES. A bias of 15–20% was noted from further stress testing where large number of samples were batched and tested using the immunoturbidimetric assay. A 5–7% higher bias remained after implementing a cuvette washing program after each HgbA1c sample. We hypothesized this bias was caused by build-up of blood cell fragments in the cuvettes when continuous whole blood samples are run through the system. Our experience suggests stress testing needs to be incorporated early in the test validation process for high volume batched screening applications. This seemingly extra validation step may save significant troubleshooting and retesting efforts down the road.

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1. Introduction

Stress testing is a term usually used in engineering to describe the process of putting a system through exertion or stress. It involves testing beyond normal operational capacity, often to a breaking point, in order to determine the stability of the given system. Similarly, when it comes to clinical diagnostics, stress testing may be necessary to reveal the performance of your assays in today's high through-put, fast-paced clinical testing environment. Here we report our experience using stress testing to troubleshoot a Hemoglobin A1c (HgbA1c) assay used in a high volume wellness screening application.

HgbA1c is recommended as a marker for diabetes diagnosis and monitoring [1]. Compared to fasting glucose and glucose tolerance test, HgbA1c has the advantage of convenience and free from acute perturbations [2]. With the current focus on

Abbreviations: ; HgbA1c, hemoglobin A1c; NHANES, National health and nutritional examination survey; NGSP, National glycohemoglobin standardization program; IFCC, International federation of clinical chemistry; DCCT, Diabetes control and complications trial; HPLC, high performance liquid chromatography; QC, quality control; HMH, Houston Methodist Hospital

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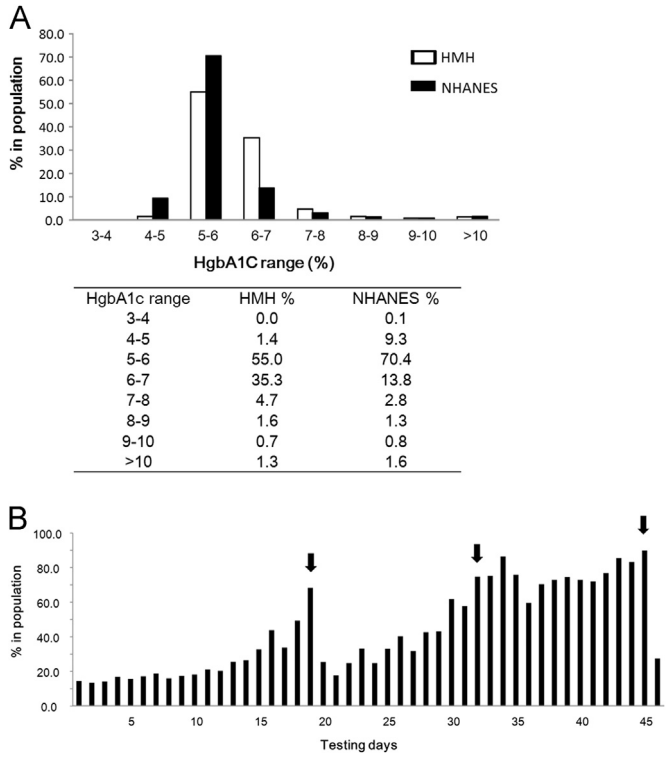
wellness and disease prevention, HgbA1c level is frequently a clinical performance measure and is incorporated in the physician quality reporting system. Therefore, it is a frequently ordered test in both primary and specialty care settings.

Through the efforts of NGSP and IFCC that started in early 1990s, analytical methods for HgbA1c are generally well standardized and characterized. Vendors certify their methods through NGSP to document traceability to the DCCT reference method which is based on a BioRex 70 HPLC system [3]. This is usually achieved by running result comparison of a low number (usually 10–20) of fresh blood samples. No significant bias against DCCT method supports traceability. Downstream in a clinical laboratory, before assay go-live, typically 20–30 samples are used to perform comparison studies as part of assay verification. Does this approach give us enough confidence in the real clinical performance of the assay, in the often varied and dynamic practice settings?

Recently, we had the opportunity to conduct wellness testing, including plasma lipid panel and whole blood HgbA1c for an employer. In a period of 2 months, 200–700 samples/day were analyzed for the above tests in batches during the low clinical volume hours (typically early 2nd shift). For routine clinical testing, our lab uses a Bio-Rad D-10™ HgbA1c platform which is a medium through-put HPLC instrument. The D-10™ HgbA1c program utilizes principles of cation exchange HPLC, where whole blood samples are automatically diluted and injected into the analytical cartridge. Working upon a programmed buffer gradient, the hemoglobins are separated based on their ionic interactions with the cartridge material and measured subsequently by a filter photometer at 415 nm [4]. This platform is not amenable to the high volume needs for the wellness testing application. We therefore turned to the Tina-quant® HbA1c Gen. 2 assay on the Roche Cobas c502 module, as part of a high through-put automation line. The test is described as a competitive turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood [5]. It involves three major steps: hemolysate preparation from whole blood, HgbA1c and anti-HgbA1c antibody complex formation, and polyhapten precipitation. The resulting insoluble complex can then be measured turbidimetrically: the higher the HgbA1c concentration, the higher the turbidity.

Before going live with the HgbA1c immunoturbidimetric assay for wellness testing, as part of validation we conducted a method comparison study by running 25 patient samples using the immunoassay and the HPLC method. Good correlation and no apparent bias was seen in this comparison study (data not shown). Our result is consistent with a prior method comparison study where Bio-Rad D-10™ and Roche immunoturbidimetric assay were used to measure HgbA1c for 110 patients with Type 2 Diabetes Mellitus. According to the authors, the two methods showed no essential performance difference in quantifying HgbA1c level [6].



HgbA1c range	HMH %	NHANES %
3-4	0.0	0.1
4-5	1.4	9.3
5-6	55.0	70.4
6-7	35.3	13.8
7-8	4.7	2.8
8-9	1.6	1.3
9-10	0.7	0.8
>10	1.3	1.6

Fig. 1. Distribution of HgbA1c values in the wellness testing population at HMH compared to the NHANES database. (A) Distribution of HgbA1c in study population at HMH ($n=15690$, open column) and in the population surveyed in 2011–2012 NHANES for Glycohemoglobin ($n=6145$, filled column) (data available through the open access: http://wwwn.cdc.gov/nchs/nhanes/2011-2012/GHB_G.htm). Distribution numbers in percentage are presented in the table below the bar graph. (B) Daily distribution of HgbA1c that is $\geq 6\%$ in HMH study population. Black arrows point toward the time when maintenance change of cuvettes took place on the automation line.

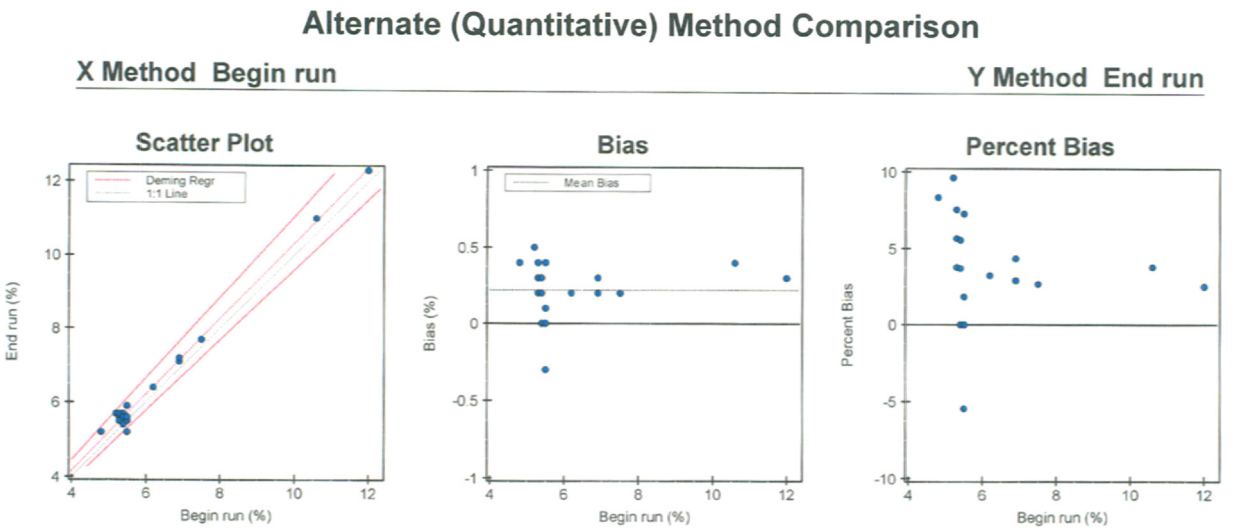


Fig. 2. Comparison of the first 20 (begin run) and the last 20 (end run) HgbA1c results in stress testing using 50 batched samples, with a cuvette washing program implemented after each sample. Fifty whole blood patient samples were run in a batch, followed by repeating the first 20 samples.

Once the wellness testing commenced, however, we started to get spotted feedback that the HgbA1c level was running higher than what it should be. The daily quality control of the immunoturbidimetric assay also showed a trend of upward drifting, and needed frequent troubleshooting. We carried out another correlation study between the immunoassay and the HPLC during this period, and still did not observe statistically significant bias.

To troubleshoot, we pulled all the wellness HgbA1c data and plotted the overall population distribution as well as daily means and medians. We observed a shift of HgbA1c to the higher values compared to the 2011–2012 NHANES population distribution (Fig. 1A), especially in the range $\geq 6\%$ where the diabetes diagnosis decision point resides (6.5%). This erroneously elevated HgbA1c level can obviously provide incorrect information regarding the employee's heath status and even cause unnecessary socioeconomic burdens. When examining the daily HgbA1c distribution in the range $\geq 6.0\%$, we were able to identify “peaks and valleys” that corresponded to the maintenance change of cuvettes on the automation line (Fig. 1B). Similar trends were noticed with daily means and medians (data not shown) over the entire course of wellness HgbA1c testing.

The above distribution analyses suggested that analytical biases might have occurred. To further confirm this, we carried out a stress testing of the immunoassay by running 200 batched HgbA1c samples, and repeating the first 20 samples after the 200 batch. A higher bias of 15–20% was observed when comparing the last 20 with the first 20 results. A 5–7% higher systematic bias was still observed when stress testing as few as 50 batched samples after a cuvette washing program was implemented after each HgbA1c sample (Fig. 2). We hypothesized this bias are caused by build-up of blood cell fragments in the cuvettes when continuous whole blood samples are run through the system in a batched mode. Cell fragment deposition and coating of the cuvettes cause the turbidity measurement to go up artificially. Feedback was given to the vendor, who subsequently released a newer generation of the assay that eliminated most bias upon stress testing ($< 0.5\%$ bias). According to the vendor, a key change in the newer generation of the assay was incorporation of stronger detergent in the wash solution, which further corroborated our hypothesis of the deposition theory.

The lesson learned is that you should always try to simulate your real clinical testing scenario and stress test your assay for high volume applications, especially if the testing is batched. After going live, monitor your QC and patient moving average or daily mean closely in order to catch unexpected result shift early on.

References

[1] International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care, vol. 32, 2009, pp. 1327–1334.
[2] E. Bonora, J. Tuomilehto, The pros and cons of diagnosing diabetes with A1c, Diabetes Care 34 (2) (2011) S184–S190.
[3] HbA1c Standardisation Destination – Global IFCC standardisation how, why, where and when a tortuous pathway from kit manufacturers, via inter-laboratory lyophilized and whole blood comparisons to designated national comparison schemes. Ian Goodall.
[4] D-10™ Hemoglobin A1c Program Instruction Manual. Package insert.
[5] Tina-quant® HbA1c Gen. 3 Turbidimetric inhibition immunoassay (TINIA) for the in vitro determination of hemoglobin A1c in whole blood or hemolysate. Package insert
[6] Farzana Yasmeen, Asim Mumtaz, Saleem-Uz-Zaman Adhami, Sajid A. Qureshi, Comparison of cation exchange hplc and immunoturbidimetric method for determination of HbA1c, Biomedica 27 (2011). \Bio-1.Doc P. 161–165 (KC) IV.