This is the final manuscript of the paper “Clinically relevant lot-to-lot reagent difference in a commercial immunoturbidimetric assay for glycated hemoglobin A1c”. The article is published in Clinical Biochemistry (https://dx.doi.org/10.1016/j.clinbiochem.2015.07.018).

Clinically relevant lot-to-lot reagent difference in a commercial immunoturbidimetric assay for glycated hemoglobin A1c

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Abstract

Objectives: Hemoglobin A1c (HbA1c) is employed for diagnosis and therapy monitoring of diabetes mellitus. The effect of a change of reagent lot on the measured values of a commercial immunoturbidimetric HbA1c assay (A1C3) was investigated.

Design and Methods: Comparison measurements of A1C3 and an automated affinity chromatography method (VIIT) were performed in 15 samples for the initial and in 20 samples for the subsequent A1C3 lot. The results of 27 and 19 measurements of a normal and of 28 and 20 of a pathological control (before and after the switch of the A1C3 reagent lot, respectively) were evaluated. Finally, the results of 6463 patient samples that had been measured with the initial and 434 that had been measured with the subsequent A1C3 lot were investigated.

Results: VIIT yielded significantly higher results than the initial A1C3 lot (bias: 0.41 % HbA1c, 4.5 mmol/mol) but agreed well with the subsequent lot (bias: -0.01 % HbA1c, -0.1 mmol/mol). Changing to the subsequent reagent lot resulted in significant increases of the mean of the normal control of 0.316 % HbA1c (3.5 mmol/mol) and of the pathological control of 0.749 % HbA1c (8.2 mmol/mol). The median of patient samples measured with the subsequent lot was significantly higher by 0.40 % HbA1c (4.4 mmol/mol).

Conclusions: The subsequent A1C3 reagent lot yields significantly higher measurement results than the initial by approximately 0.5 % HbA1c (5.5 mmol/mol). This difference is considered as clinically relevant. A combined effort of manufacturers and notified bodies is necessary to minimize lot-to-lot variation.

Keywords

HbA1c, lot variability, immunoassay

Abbreviations

A1C3, Tina-quant Hemoglobin A1c generation 3 assay; CI, confidence interval; DM, Diabetes mellitus; HbA1c, hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program; PCHn, PreciControl HbA1c norm (lot 684900); PCHp, PreciControl HbA1c path (lot 684902); VIIT, Variant II Turbo hemoglobin testing system;

Introduction

Hemoglobin A1c (HbA1c) is formed by ketoamine linkage of glucose to the N-terminal amino group of the β-chain of HbA1 [1]. Measured levels reflect mean glycemia of the preceding 90 - 120 days [2]. Elevated HbA1c levels are associated with increased risk for long-term micro- and macrovascular complications in diabetes mellitus (DM) type 1 and type 2 [3, 4]. Measurements of HbA1c have therefore been commonly used to monitor glycemic control in DM. A few years ago, HbA1c ≥ 6.5 % (47.5 mmol/mol) was additionally introduced as criterion for diagnosis of DM. In this regard, HbA1c offers comparable validity, but higher convenience than measurement of plasma glucose in fasting condition or following 75 g oral glucose tolerance test [2, 5, 6].

Inquiries from experienced clinicians concerning HbA1c results being in poor agreement with the patients' glucose measurements prompted us to investigate our routine HbA1c assay. During the evaluation process, the current reagent lot coincidentally was used up and a new one had to be loaded. The switch to the new reagent lot, however, had tremendous impact on the measured values which we describe here within.

Materials and methods

HbA1c assays

Two different National Glycohemoglobin Standardization Program (NGSP) certified HbA1c measurement methods were used. Routinely, HbA1c values were determined with the immunoturbidimetric Tina-quant Hemoglobin A1c generation 3 assay (A1C3) on the cobas c 501 module of the cobas 8000 analyzer (all from Roche, Mannheim, Germany). A1C3 reagent lots 680505 and 687516 were investigated and will be referred to as "initial lot" and "subsequent lot", respectively. All measurements were performed with the same lot of hemolyzing reagent (682129-05).

For comparison measurements, the Variant II Turbo HbA_{1c} Kit - 2.0 on the Variant II Turbo hemoglobin testing system (VIIT), both from Bio-Rad Laboratories (München, Germany), was employed. VIIT is an automated HPLC analyzer quantifying HbA1c from an affinity chromatogram. VIIT analyses were performed in the local laboratory of Bio-Rad blinded for the A1C3 results.

Comparison measurements

15 EDTA whole blood samples for the initial and 20 for the subsequent lot were measured in parallel with the A1C3 and the VIIT. Samples were selected to evenly cover the complete clinically relevant range of HbA1c results from approximately 4.5 % HbA1c (25.7 mmol/mol) to 10.5 % HbA1c (91.3

mmol/mol). Methods were compared by calculating Passing & Bablok regression and agreement was evaluated via a Bland-Altman plot.

Behavior of HbA1c results over time

The laboratory information system was searched for quality control and patient samples whose HbA1c values were determined with the initial and the subsequent A1C3 lot, but with the same lot of hemolyzing reagent. We identified the normal control PreciControl HbA1c norm, lot 684900 (PCHn, Roche) and the pathologic control PreciControl HbA1c path, lot 684902 (PCHp, Roche) to meet the criteria. The former had been measured 27 and 19 and the latter 28 and 20 times with the initial and the subsequent lot, respectively. Additionally, results of 6463 unselected patient samples measured with the initial and of 434 measured with the subsequent lot were retrieved. Differences were compared in the independent samples t-Test for the control and in the Mann-Whitney U-Test for the patient samples.

Statistics software

Statistical analyses were performed with the Excel-Add in Analyse-it (Analyse-it Software, Leeds, UK). P-values < 0.05 were considered significant.

Results

Comparison of A1C3 and VIIT assays

Results of the comparison measurements between the A1C3 and the VIIT assays are depicted in Fig. 1. For the initial lot, results were only moderately comparable as illustrated by a Passing & Bablok regression fit (Fig. 1 A) of Y = 1.14 × X – 0.64 (95 % confidence interval (CI) for slope 1.06 to 1.24 and for intercept -1.30 to -0.05). The Bland-Altman plot (Fig. 1 B) revealed VIIT to yield higher results with discrepancies between both assays being more pronounced as HbA1c levels increase. A bias of 0.41 % HbA1c (4.5 mmol/mol; 95 % CI: 0.20 – 0.61 % HbA1c, 2.2 – 6.7 mmol/mol) and 95 % limits of agreement of -0.32 to 1.13 % HbA1c (-3.5 – 12.4 mmol/mol; 95 % CI: -0.68 – 0.04 and 0.77 – 1.49 % HbA1c, -7.4 – 0.4 and 8.4 – 16.4 mmol/mol, respectively) were calculated.

After changing to the subsequent A1C3 lot, a significantly improved agreement between both assays was observed. Relation of VIIT and A1CE results was then described by a Passing & Bablok regression (Fig. 1 C) of Y = 1.05 × X – 0.04 (95 % CI 1.03 to 1.08 and -0.56 to -0.24 for slope and intercept, respectively). A bias of -0.01 % HbA1c (-0.1 mmol/mol) not significantly different from zero (95 % CI: -0.08 – 0.05 % HbA1c, -0.9 – 0.5 mmol/mol) was deduced from Bland-Altman analysis (Fig. 1 D). 95 % limits of agreement were determined to be in between -0.29 % HbA1c (-3.2 mmol/mol; 95 % CI: -0.41 to -0.18 %HbA1c, -4.5 to -2.0 mmol/mol) and 0.27 % HbA1c (3.0 mmol/mol; 95 % CI: 0.15 to

0.38 % HbA1c, 1.6 to 4.2 mmol/mol). The trend to larger differences with increasing HbA1c was no longer observed.

Quality control and patient samples over time

Both control lots exhibited significant increases of values when measured with the subsequent A1C3 lot (Table 1). The mean value of PCHn increased by a mean difference of 0.316 % HbA1c (3.5 mmol/mol; 95 % CI: 0.250 - 0.382 % HbA1c, 2.7 – 4.2 mmol/mol) and that of PCHp by a mean difference of 0.749 % HbA1c (8.2 mmol/mol; 95 % CI: 0.626 – 0.872 % HbA1c, 6.8 – 9.5 mmol/mol). Similarly, the median of patient samples measured with the subsequent lot was in median 0.40 % HbA1c (4.4 mmol/mol; 95 % CI: 0.30 – 0.50 % HbA1c, 3.3 – 5.5 mmol/mol) higher than that of the patient samples determined with the initial lot (Table 1). All observed differences were statistically highly significant ($p < 0.0001$). To check whether the observation was an one-time incident or not, all HbA1c measurements performed on the Cobas 8000 system since its implementation into routine diagnostics (2012) were reviewed. Only two more suitable changes of HbA1c reagent lots were identified, where at the same time hemolyzing reagent, normal and pathologic control lots did not change for a sufficiently long period of time. Statistical analysis revealed minimal changes of HbA1c levels in patient and control samples with these two reagent lot changes which were irrelevant for patient care. We thus did find no evidence that the described observation is a more frequent phenomenon.

Discussion

Our data demonstrate conclusively that the subsequent lot of A1C3 reagent yields significantly higher results compared to the initial by approximately 0.5 % HbA1c (5.5 mmol/mol). The observed increases of control and patient samples with the subsequent lot are in good agreement with the comparison studies in which only the initial, but not the subsequent lot measures lower values than the HPLC method. The bias in between initial A1C3 lot and VIIT corresponds well to the mean and median differences of the quality control and patient samples, respectively. Likewise, the larger discrepancy in the pathological compared to the normal control fits well to the comparison measurements.

Our institution's internal quality control rules were not violated upon switching from the initial to the subsequent lot. The manufacturers quality management system, e.g. suggested by ISO 13485 (Medical devices - Quality management systems - Requirements for regulatory purposes), appears not to have detected the deviation as well. No information was given to the end-users when switching the reagent lot. This is surprising as the A1C3 method is known to be NGSP certified. NGSP constitutes a comprehensive program for certification of HbA1c determination allowing standardization at the manufacturing level [7]. Lot-to-lot differences would not have been detected without the presented investigations. Clinicians modify patient treatments based on small

differences of HbA1c values [8] and changes of 0.5 % HbA1c (5.5. mmol/mol) are considered clinically relevant [9]. The described lot-to-lot variability therefore may have affected therapeutic decisions resulting in under-therapy and -diagnosis with the initial lot.

Lot-to-lot inconsistencies of immunoassays have been described for assay formats detecting antigens [10-12] as well as for those detecting antibodies [13, 14]. Immunoassays might be especially susceptible to lot-to-lot variations as the crucial formation of an antigen-antibody complex is influenced by a multitude of factors. These factors include pH and ionic strength of the buffer solution, the applied detergent, possible interfering substances in the sample and the temperature at which the assay is performed.

Remote monitoring of patient values from analyzers in numerous institutions by manufacturers may help to identify lot-to-lot differences [12]. Furthermore, rigorous validation testing by centralized institutions, assessing lot-to-lot validation results longitudinally, implementing prospective monitoring, transparency of validation failures and postvalidation issues of clinical laboratories and extended research in validation methods could improve quick discovery [15].

To the best of our knowledge, this is the first description of clinically relevant lot-to-lot variability for an immunological HbA1c assay. This is particularly important as the analyte constitutes a follow-up parameter determined repeatedly. A combined effort of manufacturers and end-users is necessary to enable clinicians to fully benefit from HbA1c's informative value and to provide the best patient care possible.

Acknowledgement

We thank Bio-Rad Laboratories for performing HbA1c measurements with the VIIT free of charge and Dr. Evangeline Thaler for carefully reviewing the manuscript.

Tables

sample	A1C3 lot	n	mean		lower 95 % CI		upper 95 % CI		p
			[%]	[mmol/mol]	[%]	[mmol/mol]	[%]	[mmol/mol]	
normal control (PCHn)	initial	27	5.18	33.1	5.13	32.6	5.23	33.7	< 0.0001[a]
	subsequent	19	5.50	36.6	5.46	36.2	5.54	37.1	
pathologic control (PCHp)	initial	28	9.57	81.1	9.52	80.6	9.62	81.6	< 0.0001[a]
	subsequent	20	10.32	89.3	10.19	87.9	10.45	90.7	
sample	**A1C3 lot**	**n**	**median**		**lower 95 % CI**		**upper 95 % CI**		**p**
			[%]	**[mmol/mol]**	**[%]**	**[mmol/mol]**	**[%]**	**[mmol/mol]**	
patient samples	initial	6463	5.40	35.5	5.40	35.5	5.50	36.6	< 0.0001[b]
	subsequent	434	5.90	41.0	5.80	39.9	6.00	42.1	

Table 1 Mean and median HbA1c values of quality control and patient samples as calculated from measurements with the A1C3 assay. Results classified according to the A1C3 reagent lot employed. [a] independent samples t-test, [b] Mann-Whitney U-Test.

Figure captions

Fig. 1 Passing & Bablok regression (A, C) and Bland-Altman plot (B, D) for the comparison of A1C3 initial lot vs. VIIT and of A1C3 subsequent lot vs. VIIT. A, C: thick line: regression line; thin line: identity line; B, D: thick line: bias; thin line: zero difference; dotted lines: 95 % limits of agreement.

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