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A cost effective method for stabilizing commercial QC for the measurement of HbA1c using TOSOH G8 analyser

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Abstract

Haemoglobin A1c (HbA1c) represents a key biomarker in diabetes diagnosis and management, as it is indicative of glucose control during the preceding 2-3 months. Laboratories undertaking the measurement of HbA1c utilise Quality Controls (QC) to monitor precision, accuracy and reproducibility of results generated which is an integral part of any health care laboratory. Commercial companies supply lyophilised QC materials with target mean and acceptable range. After reconstitution it is stable for 7 days as per the control inserts. The stability of reconstituted lyophilised QC material beyond 7 days is not well established and there are lacunae in the literature about the stability of reconstituted QC material at temperatures below 0°C. The purpose of this study was to develop a simple method to extend the stability of the constituted commercial HbA1c lyophilised QC material in an attempt to maximise its usage, reduce wastage and make significant cost savings. Clinical laboratories could easily adopt this proposed method as it is easy to implement, and would extend the stability of their reconstituted lyophilised Quality Control (QC) material for a much longer period.

Keywords: HbA1c, Quality control, TOSOH G8, Stability, Cost saving.

1. Introduction

HbA1c has been the gold standard marker for the monitoring of the control of blood glucose during the preceding 2-3 months. Several methods such as colorimetric, electrophoresis, column chromatography and immune-agglutination have been used to measure HbA1c. The assay performance has been validated by using the commercial QC which is readily available in the market. The commercial QC is also used to continuously ensure reliability and accuracy of these methods prior to releasing test results. Laboratories have the responsibility to maintain the required level of quality but must also employ strategies to reduce cost to the services through the effective use of commercial QC material. This study therefore seeks to make substantial cost savings by exploring ways to maximise the Biorad Lyphochek Diabetes Control usage on the Tosoh G8 analysers for Haemoglobin A1c (HBA1c) analysis through stability studies. The Biorad QC is human whole blood based, with a 3 year shelf life and 7 day reconstituted stability at 2- 8^0C when stored tightly capped according to the manufacturer's instructions for use. A reconstituted vial contains 500µl of QC material, 10µl of which is required to run on an analyser daily. The reconstituted vial of QC material expires in 7 days and hence it can be estimated that approximately 70µl of the QC is used up weekly and 430µl discarded due to expiration. The study proposes that significant cost reduction can be made by eliminating this wastage.

QC techniques are used to monitor day-to-day reliability of clinical biochemistry laboratory performance. Application of these techniques help reduce errors and give both the laboratory and the clinician confidence in the results [1]. The advantage of liquid QC is that there is bottle to bottle homogeneity and hence no wastage of material due to aliquoting. This results in increased savings on labour cost and elimination of frequent reconstitution such as occurs when lyophilized materials are used [2]. Reconstitution of lyophilized QC may introduce some error, due to inaccurate addition of the exact volume of deionised

water and further inevitable vial-to-vial filling variation, recorded as being up to 0.6% [3].

The usefulness of measuring HbA1c to assess glycemic control in patients with diabetes mellitus is now well established by the United Kingdom Prevention of Diabetes Study, following attempts on many fronts to improve the quality of HbA1c testing [4]. The National Glycohemoglobin Standardization (NGSP) program has achieved remarkable success in decreasing the imprecision in HbA1c testing [5]. Estimates of imprecision are usually based on the repeated analysis of quality control specimens. These estimates are dependent on the matrix of the control specimen which may differ significantly from actual patient specimens [6].

In Clinical chemistry, maximum stability of analytes in the QC material is desirable and the initial stability studies of commercial QC material is customarily performed by manufacturers using a wide variety of statistical methods during product development and also during the period following distribution when the products are in the field[7]. Users and evaluators of such materials periodically report on the stability of control material in clinical chemistry, review criteria employed to define instability, and present an approach to evaluate stability of analytes involving both statistical and clinical criteria [7].

A study on the effect of a single freeze/thaw cycle on HBA1c concentrations measured by commercially available HPLC method revealed that storage for up to 12 weeks at -80^0 C with a single freeze/thaw cycle does not affect HBA1c concentrations measured with HPLC method on Bio-Rad D-10 analyzer [8]. Several studies evaluated repeatability of HBA1c concentration on different instruments after freezing the blood samples which might be useful if prolonged storage is required (i.e. for research or evaluation of new methods). For prolonged storage (several months or years) recommended storage temperatures are -70 °C or lower [9]. HBA1c concentration is stable at 4^{0}C usually for 2–3 weeks, while at room temperature relevant changes in HBA1c concentration are observed within 1–3 days [10].

With increasing automation in clinical laboratories, the requirements for quality control material have greatly increased in order to monitor performance. The constant use of commercial control material is not economically feasible for many countries because of non-availability or the high cost of these materials. Extensive use of Home Made Quality Control sera in clinical laboratories have saved about 69% of the amount spent on the commercial material without any compromise in quality of the laboratory performance [11]. The IQC prepared for HbA1c using 15 %(V/V) ethanediol as preservative and pooled whole blood samples as raw material showed very good stability for a period of 90 days. The HbA1c control prepared in pooled whole blood in a study was stable for up to 6 months as in the case of serum stabilised with the same 15%(V/V) ethanediol [12].

A previous study was carried out using three whole blood samples at three HbA1c levels which were collected and stored at -70^{0}C, -20^{0}C, 4^{0}C, room temperature (17-23^{0}C), and 37^{0}C and an aliquot from each temperature analysed using different analysers. The Bio-Rad Variant II showed stability at -20^{0}C for 28 days. The CLC 385/330, Tosoh G7, and Bio-Rad Variant II high performance liquid chromatography methods showed better stability than the Tosoh 2.2 Plus and Bio-Rad Variant [13]. Storage at 4^{0}C was stable for two weeks, without major sample degradation. An earlier degradation occurred at -20^{0}C. The temperatures of 4^{0}C and -80^{0}C are of interest for whole blood storage before HbA1c measurement, for short and long term preservations respectively [14].

Before implementation, all QC systems require estimates of the mean and standard deviation (SD) using a reasonable number of analytical runs which will accurately reflect between run variability of the assay. A general rule would be that the QC should be analysed a minimum of 20 times on different days by different users [15]. The world health organization (WHO) document LAB/81.4 encourages the local production of lyophilised and liquid control in laboratories [16]. Previous studies shows that internal QC prepared for HbA1c using ethanediol as preservative and pooled whole blood samples as raw material had a very good stability for a period of 90 days stored at -20^{0}C. Laboratories undertaking the measurement of HbA1c could easily adopt this method to prepare their own internal QC as the method is very simple to implement and the cost involved in preparing QC is negligible [17].

2. Materials and methods

Using a volumetric pipette, each vial of Bio-Rad QC was reconstituted with deionised water and allowed to stand for approximately 5 to 10 minutes as per the manufactures instructions. 100µl of the QC was then aliquoted into micro tubes which were then frozen immediately. An aliquot of the QC was defrosted every week and run on three different TOSOHG8 analysers daily and the data collected. The repeatability and intermediate precision of the QC aliquots was assessed in comparison with the expected normal QC performance over a period of 7 weeks. A minimum of 20 replicates of each of the two levels of QC material was run to determine repeatability. The two levels of QC were also added to the standard QC panel and analysed multiple times over multiple days by different users to determine intermediate precision. A minimum of 20 data points for each QC on each instrument

were obtained. The coefficients of variations (%CV) were also calculated for each data set.

In this study we have compared the stability of lyophilised Biorad HbA1c QC which was reconstituted and stored at 2-8^{0}C (control -I) and the reconstituted lyophilised Biorad HbA1c control which was aliquoted frozen at -20^{0}C and then thawed and used (control – II). The data was compared for the three different TOSOH G8 analysers for Intermediate precision and Repeatability. The total QC usage and cost savings were also calculated to estimate the financial effects. The data is given in the table below.

Control I: Reconstituted lyophilised Biorad HbA1c control stored at 2-8^{0}C.

Control II: Reconstituted lyophilised Biorad HbA1c control aliquoted and stored at-20^{0}C and the thawed stored at 2-8^{0}C.

3. Results

Table I: Comparison of Intermediate Precision on all three analysers

Intermediate Precision		Control – I			Control - II		
Lot Number	**Instrument**	**Mean**	**SD**	**%CV**	**Mean**	**SD**	**%CV**
33941	Tosoh G8 -1	34.5	1.0	3.3	34.2	0.77	2.24
	Tosoh G8 -2	35.3	1.1	3.0	34.7	0.59	1.69
	TOSOH G8 -3	34.7	1.5	4.3	34.4	1.04	3.03
33942	Tosoh G8 -1	79.2	1.5	1.8	79.2	1.54	1.95
	Tosoh G8 -2	80.1	1.6	2.0	79.2	1.55	1.96
	TOSOH G8 -3	79.4	1.2	1.6	79.2	0.99	1.25

Table 1 show the intermediate precision Mean, SD and %CV of control- I and control - II of the QC material included in this study. The %CV shows the results obtained during the study period i.e. variations in inter-day results. Comparing both %CV for control I & II, control II shows less %CV than control I, indicating a better precision using the reconstituted lyophilised control which was aliquoted, frozen, thawed and used for a week. These data strongly supports that lyophilised control which is frozen and used shows better stability and %CV when compared with lyophilised reconstituted ones stored at 2-8^{0}C.

Table II: Comparison of Repeatability (Precision and Accuracy) for all three analysers

Repeatability			Results obtained for Control – I				Results obtained for Control - II			
Lot Number	**Instrument**	**Ref Mean**	**Mean**	**SD**	**%CV**	**%bias**	**Mean**	**SD**	**%CV**	**%bias**
33941	Tosoh G8 -1	34.0	36.5	0.22	0.62	-6.8	34.3	0.53	1.55	-0.87
	Tosoh G8 -2		35.1	0.31	0.88	-3.1	35.4	0.51	1.43	-3.95
	TOSOH G8 -3		34.9	0.22	0.64	-2.6	33.7	0.45	1.33	0.88
33942	Tosoh G8 -1	79.0	81.9	0.31	0.38	-3.6	79.3	0.55	0.69	-0.38
	Tosoh G8 -2		81.1	0.22	0.28	-2.6	79.8	0.42	0.53	-1.0
	TOSOH G8 -3		79.8	0.41	0.51	-1.0	78.1	0.31	0.39	1.1

Table II shows the repeatability data for the two controls used in this study. The %CV for control I and II shows good precision even though control I was better when compared to the control II. However taking into account the accuracy, control II shows less %bias than control I. The acceptable CV for the stability studies was set at less than 5% as indicated in the manufacturer's instructions [18]. This data strongly supports that the obtained %CV was less than the manufacturer's indication for this study.

Table III: Cost saving analysis

	Normal usage (Control I)	**Recommended Usage (Control II)**
Duration of use per box of QC	3 Weeks	15 weeks
% of QC material used per month	100%	19.2%
% of Cost saved per month	0%	80.8%
% of cost saved per year	0%	80.8%

Table III shows % decrease in the usage of QC material by implementing the recommended method of QC aliquoting and storage. 80.8% cost was saved by the method used in this study, due to the extended usage of the QC material which was 5 times more than the manufacturers recommendation.

4. Discussion

Clinical laboratory depends upon the maintenance of accuracy and precision, the two watch words upon which the total reliability of the laboratory results can be guaranteed. Many studies have been carried out in the past

decade for QC preparation and stability[4]. Commercial organisation supply lyophilised QC material stable for 7 days stored at 2-8°C when reconstituted. The stability of the QC beyond 7 days has not been established yet. The %CV for control II shows good precision when compared to the control I as the previous studies have mentioned earlier [12]. The lyophilized commercial QC when reconstituted and frozen at -20°C, thawed and used on weekly basis, its stability lasted for 5 weeks has been proven. This shows an advantage over normal usage as the study indicated an approximately 80% potential cost savings due to the extended usage of the Commercial QC material. The production and use of lyophilised QC are much encouraged by WHO [16] and many studies have been carried out for preparation and stability. Once judged to be satisfactory, not only does stability studies help save cost but also permit the detection of any QC stability issues and atypical trends. The data generated and conclusions reached in a commercial QC stability studies should be documented prior to implementation in the laboratory.

5. Conclusion

The outcome of the study confirms that the commercial control used in the clinical laboratories when reconstituted and frozen at -20^{0}C thawed and used daily shows a less %CV confirming a better stability. The commercial control which was reconstituted and stored at 2-8^{0}C was less stable. This procedure will certainly help laboratories to use controls for a much longer period of time to reduce the wastage of commercial controls and save cost. This study gives the guidelines for clinical laboratories to extend the usage of lyophilised commercial controls with a 7 day reconstitution stability to 5 weeks which is more cost effective. However further studies can be done to assess the stability of lyophilised controls for periods longer than 5 weeks.

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