Biomedical & Pharmacology Journal Vol. **9**(1), 163-168 (2016)

HbA1c External Quality Assessment: Commutable vs Noncommutable Samples

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http://dx.doi.org/10.13005/bpj/923

(Received: February 10, 2016; accepted: March 15, 2016)

ABSTRACT

HbA1c measurement is important in diagnosis and monitoring of diabetes. External quality assessment (EQA) is a way for evaluating laboratory performance in measuring HbA1c. For this, commutable quality control (QC) samples is recommended. Two commercial noncommutable QC samples were sended to 931 and 894 participant laboratories during Jully 2011 and February 2012, respectively, and Three patient commutable QC samples were also sended to 272, 231, and 886 participant laboratories during Jully 2013 and February 2014, and Jully 2014, respectively. Results of five commonly used HbA1c kits compared with total mean. With two commercial noncommutable samples, total group CVs% were 38.5% and 24.5%. With three patient commutable samples, total group CVs% were 8.0%, 6.8%, and 7.9%. In these situations mean of each kits results were in acceptable performance limits. Using commutable QC samples is essential for evaluating laboratory and kit performance in EQA.

Key words: HbA1c, External quality assessment, Commutable.

INTRODUCTION

Glycated hemoglobin (GHb), reported as HbA1c, has been used to monitoring glycemic control in patients with diabetes for many years. The critical importance of this test was not fully realized until the Diabetes Control and Complications Trial (DCCT) showed a strong relationship between HbA1c and risk for diabetes complications[1]. Recently, World Health Organization (WHO) and American Diabetes Association (ADA) have recommended HbA1c measuring as a criteria to diagnosis diabetes which increases its importance[2, 3]. So, we need methods for measurnig HbA1c with great precision an accuracy.

Many methods have been developed for the measurement of GHb on the basis of differences in charge or structure between glycated and nonglycated hemoglobins. These include ion-exchange chromatography, capillary electrophoresis, boronate affinity chromatography, immunoassay, and enzymatic methods[4].

A single sample produced widely varying results among methods and laboratories and these variations depend on species of Hb that was measured and specific method used. For example, the mean result for each method for the same sample on 1993 College of American Pathologists (CAP) proficiency survey varied from 10.7% to 17.8%. This situation often made it imposible for physicians and patients to relate their test results to DCCT-based treatmrnt goals[1].

External Quality Assessment Program (EQAP) is a survey for external quality assessment in Iran which has been performed from 2008 under

consideration of Reference Health Laboratory of of Iran. Annully two or three unknown samples are sended to participant laboratories and their results are analyzed.

According to HbA1c results of ninth and eleventh EQAP, EQAP-09 and EQAP-11, runs which were performed during July 2011 and February 2012 respectively, differences between results were too great and in some cases it was as much as 70%. So we decided to study these differences and resolve the problems. In the first step, we focused on samples which were sent to participitant laboratories. In This article, effects of sample nature on results of EQA are evaluated. For this, samples are named as "commutable" and "noncommutable". The term "commutability" was first used to describe the ability of a reference or control material to exhibit properties compareable to the properties of clinical samples when analyzed by different analytical methods. This description is now more generally defiened as the equivalence of the analytical results of different measurement procedures for a reference material and for representative samples from healthy and diseased individuals[5, 6].

MATERIAL AND METHODS

During July 2011 (EQAP-9), commercial control materials were sended to 931 participant laboratories. This practice was repeated during February 2012 (EQAP-11) by sending commertial control materials to 894 participant laboratories. EQAP for HbA1c, but not for other laboratory tests, stopped for about one years. During July 2013 (EQAP-15) and February 2014 (EQAP-17), patient-based materials were sended to 272 and 231 participant laboratories. These patient-based material were collected from venous blood of an uncontrolled diabetic patient in EDTA-containing vials. Again, during Jully 2014 (EQAP-18), patient-based material was prepared from pooled blood of diabetic patients in EDTA-containing vials and sended to 886 laboratories.

Before sending to participant laboratories, homogeneity of control material vials was assessed and confirmed. After sending. stability of these control material were assessed and confirmed. These assessments and confirmation were done according to WHO requirements[7].

Each participant laboratory should examined sended control material as a routine patient sample according to instructions of measuring kit provider and should calibrated and controlled its measuring method by calibrator and control material, as internal quality control, provided by kit producer.

There is more than ten HbA1c kits in Iran. But in this study we focused on common kits for which the number of using laboratories was at least ten, so their statistical analysis could be valid. These included Biosystem, Nycocard, Pars Azmon. Pishtaz Teb, and Roche kits.

After measuring HbA1c, results were sended to EQAP and statistical analyses were done. First, according to used kit, results were grouped in five peer groups. Second, mean, standard deviation (SD), and coefficient variation (CV) of each peer group and also total results were calculated. In EQA, mean of each peer group is used as target value to evaluate each laboratory perfomance. For this, it is neccessary to delet outliers which are out of Mean ± 2SD or 3SD[8]. In EQAP, we used Mean ± 2.5SD. After deleting outliers, calculation of mean and SD was repeated until there was no outliers. The last calculated mean, termed as weighted mean, was used as target value. Third, one-sample t-test was performed in order to analyze differences between peer group weighted means and total weighted mean. Statistical analysis were done by SPSS 20 software. Finally, clinically acceptable mean range was calculated according to allowable maximum total error of ± 6% for EQAS programs[9]. Then acceptability of each peer group mean was investigated according to this range.

RESULTS

931, 894, 272, 231, and 886 laboratories participated in 9th, 11th. 15th, 17th, and 18th runs of EQAP, respectively. From these, 657, 664, 213, 193, and 659 laboratories used five desired Biosystem, NycoCard, Pars Azmon. Pishtaz Teb, and Roche kits, respectively. Finally after deleting

outliers, in these runs 567, 646, 195, 191, and 599 laboratories remained (table 1).

In ninth run of EQAP, 567 participated laboratories used desired kits, grouped in four peer group, including Pars Azmon, Pishtaz Teb, Biosystem, and NycoCard, with 80, 27, 212, and 248 participated laboratories, respectively. In this run, No laboratory used Roche kit. Table 2 shows target value, SD, and CV% each peer group and also total.

In eleventh run of EQAP, 646 participated laboratories used desired kits, grouped in five peer group, including Pars Azmon, Pishtaz Teb, Biosystem, Roche, and NycoCard, with 85, 44, 237, 15, and 265 participated laboratories, respectively. Table 3 shows target value, SD, and CV% each peer group and also total.

In fifteenth run of EQAP, 195 participated laboratories used desired kits, grouped in five peer group, including Pars Azmon, Pishtaz Teb, Biosystem, Roche, and NycoCard, with 35, 40, 54, 8 and 58 participated laboratories, respectively. Table 4 shows target value, SD, and CV% each peer group and also total.

In seventeenth run of EQAP, 191 participated laboratories used desired kits, grouped in five peer group, including Pars Azmon, Pishtaz Teb, Biosystem, Roche, and NycoCard, with 32, 42, 54, 9 and 54 participated laboratories, respectively. Table 5 shows target value, SD, and CV% each peer group and also total.

In eighteenth run of EQAP, 599 participated laboratories used desired kits, grouped in five peer group, including Pars Azmon, Pishtaz Teb, Biosystem, Roche, and NycoCard, with 96, 86, 229, 17 and 171 participated laboratories, respectively. Table 6 shows target value, SD, and CV% each peer group and also total.

Difference between peer group target values in EQAP-9 and EQAP11was so high and the differences between lowest and highest target values were 71% and 44% of related total target values, respectively. Also, One-sample t-test showed Significant difference between target values of all kits and total target values ($p< 0.001$) in both EQAP-9 and EQAP-11.

Difference between peer group target values in both EQAP-15, EQAP-17 and EQAP-18 was small and the differences between lowest and

Table 1: Numbers of participating laboratories

EQAP run	Participated laboratories		
	Total	used desired kits	After deleting outliers
Nine	931	657	567
Eleven	894	664	646
Fifteen	272	213	195
Seventeen	231	193	191
Eighteen	886	659	599

Table 2: Mean (Target value), standard deviation (SD), coefficient variation (CV) of HbA1c measurement kits in 9th run of EQAP

Kits	No.	Mean	SD	CV (%)
Pars Azmon	80	5.65*	0.59	10.4
Pishtaz Teb	27	5.32*	0.64	12.0
Biosystem	212	10.81*	2.86	26.5
Roche	-	-	-	-
NycoCard	248	6.07*	0.52	8.6
Total	567	7.75	2.98	38.5

* Showed significant difference with total mean ($p<0.001$).

Table 3: Mean (Target value), standard deviation (SD), coefficient variation (CV) of HbA1c measurement kits in 11th run of EQAP

Kits	No.	Mean	SD	CV (%)
Pars Azmon	85	6.09	0.93	15.3
Pishtaz Teb	44	5.48*	0.70	12.8
Biosystem	237	8.56*	1.80	21.0
Roche	15	8.26*	0.93	11.3
NycoCard	265	6.33*	0.87	13.7
Total	646	7.06	1.73	24.5

* Showed significant difference with total mean ($p<0.001$).

Table 4: Mean (Target value), standard deviation (SD), coefficient variation (CV) of HbA1c measurement kits in 15th run of EQAP

Kits	No.	Mean	SD	CV (%)
Pars Azmon	35	8.90	1.11	12.5
Pishtaz Teb	40	9.06	0.48	5.3
Biosystem	54	8.57*	0.96	11.2
Roche	8	9.51*	0.53	5.6
NycoCard	58	9.39*	0.49	5.2
Total	195	9.01	0.84	9.3

* Showed significant difference with total mean (p<0.05).

Table 5: Mean (Target value), standard deviation (SD), coefficient variation (CV) of HbA1c measurement kits in 17th run of EQAP

Kits	No.	Mean	SD	CV (%)
Pars Azmon	32	9.58	0.95	9.9
Pishtaz Teb	42	9.65	0.39	4.0
Biosystem	54	9.38*	0.98	10.4
Roche	9	10.09*	0.30	3.0
NycoCard	54	9.86	0.66	6.7
Total	191	9.64	0.78	8.1

* Showed significant difference with total mean (p<0. 05).

Table 6: Mean (Target value), standard deviation (SD), coefficient variation (CV) of HbA1c measurement kits in 18th run of EQAP

Kits	No.	Mean	SD	CV (%)
Pars Azmon	96	7.18*	0.66	9.2
Pishtaz Teb	86	7.32	0.40	5.5
Biosystem	229	7.76*	0.92	11.9
Roche	17	7.83*	0.36	4.6
NycoCard	171	7.22*	0.60	8.3
Total	599	7.45	0.77	10.3

* Showed significant difference with total mean (p<0.01).

highst target values were 10%, 7%, and 9% of related total target values, respectively. Also, One-sample t-test showed no significant difference between target values of Pars Azmon and Pishtaz Teb kits and total target values in both EQAP-15 and EQAP-17. But this difference was significant in both runs for Roche and NycoCard kits and also for Biosystem kit in EQAP-15 (p<0.05). However, in exception to Pishtaz Teb kit, there was significant difference between target values of other kits and total target values in EQAP-18 (P<0.01).

One criteria for validity of using total mean as total target value is relative low CV% which for EQAS of HbA1c should be about 10% or lesser. Thus, because of very high CVs% of total HbA1c in EQAP-09 and EQAP-11, 38.5% and 24.5% respectively, total means of these groups are not valid as target values and can not be used for camparison of peer group means. But, CVs% of total HbA1c in EQAP-15, EQAP-17, and EQAP-18 are suitable, 9.3%, 8.1% and 10.3 respectively. So we can use these as target values to evaluate each kit performance.

Table 7: Acceptable bias (6% of total mean) and acceptable performance limits

EQAP	Total mean	Acceptabe bias	Acceptable performance limits
Fifteen	9.01	0.54	8.47 - 9.55
Seventeen	9.64	0.58	9.06 - 10.22
Eighteen	7.45	0.45	7.00 - 7.90

Table 7 shows accepable performance of HbA1c results in EQAP-15, EQAP-17, and EQAP-18. As can be seen, in spite of statistically significant differences between means of some kit means with corresponding total means, all of them fall in the acceptable range.

Mean of CVs% were 14.4%, 14.8%, 8.0%, 6.8%, and 7.9% in EQAP-9, EQAP-11, EQAP-15, EQAP-17, and EQAP-18, respectively. these shows that mean of CVs% were about two fold higher in

EQAP-9 and EQAP-11 relative to EQAP-15, EQAP-17, and EQAP-18.

DISCUSSION

Internal quality control (IQA) and external quality assessment (EQA) are complemntary activities for reducing analytical errors in clinical laboratories. IQA is necessary for daily monitoring of the precision and accuracy of the analytical method, and EQA is important for maintaning long-term accuracy of analytical methods[10].

EQA organizers often use commercially QC materials specifically prepared to ease transportation and storage, having relatively low cost, and exhibits a low vial to vial variability. For this, control materials are commercially prepared by adding preservatives and other substances which may have adverse effects on the physicochemical properties of samples[11]. As a consequence, QC materials are frequently noncommutable with clinical patient sample and they may produce significantly different results with different assays[11].

Commutable samples are typically prepared by pooling clinical patient samples with minimal processing or additives to avoid any alteration of the sample matrix. When commutable PT samples can be prepared, the results reflect what would be expected if patient samples were sent to each of the different laboratories. Thus, harmonization or agreement among different laboratories and methods can be correctly evaluated. Although preparing commutable materials for use in large EQA programs is challenging, use of these materials adds substantial value to the information obtained from the results[11].

Our study showed that when uncommutable QC material were used, we cann't compare results of different methods with each other, and also varations of the results with the same method is very much; these findings were reflected in siginficantly different weighted mean values and high CV% of the results, respectively. Conversely, when commutable QC materials were used, differences between weighted mean values and CV% results were much lower. So, we can compare results of different methods with each other.

Different multiple studies had shown using commutable materials in EQAS is necessary for comparing laboratory results for different analytes. In 1993. Noito et al showed the effects of noncommutability materials on intrpretation of proficiency testing (PT) results. In their study, pooled patient sera and PT samples were assayed by the duPont Dimension Analyzer and by Abell-Kendall reference method for cholesterol. The Abell-Kendall method is known to be unaffected by matrix-induced changes in PT samples. The patient samples showed excellent agreement between two methods (average bias = 0.2%). However, the PT samples had a large negative bias (-9.5%) between methods, caused by a matrix-related bias with the duPont method that was not present with the reference method[11].

Gould et al studied the commutability of six UK National External Quality Assurance Schemes (UKNEQAS) samples and two reference serum preparations using five methods for the measurement of albumin. They showed that commutability is important in the investigation of between- method differences in EQAS[12].

In 2007, Carobene et al evaluated the performance of the laboratories participating in two Italian EQAS, presenting similar characterstics in terms of number of participants, type of EQAS samples, and program organization. They had no information about commutability of EQAS samles, but they concluded noncommutability of materials can introduce an additional bias[13].

Dominici et al studied the feasibility of using commercial control materials in a EQAS for serum carcinoembryonic antigen (CEA) measurement. They assessed the commutability of 12 commercial control materials using five automated immunochemical systems. They also compared the intermethod behavior of the materials with that of 12–14 patient serum pools. They showed the use of noncommutable materials has negative effects on EQAS results and concluded the materials planned to be used in EQAS must be commutable[14].

Freshly collected pooled serum and whole blood materials have been used successfully in some EQA/PT programs and their use is increasing. Such materials must be collected and processed carefully to presserve native properties[6]. In 1996, the CAP began a fresh blood survey for HbA1c that eliminated matrix effects due to the use of processed blood samples[1]. In Iran, we have been used fresh pooled blood for HbA1c in EQAP from 2013 which has led to good results for studying agreements between results of differernt methods and kits. We should extend using commutable materials to analytes other than HbA1c in EQAP and other EQAS.

This study showes that peer group mean of HbA1c results fall in acceptable perfirmance limits and bias% of means. But it tells us nothing about performance of individual laboratories and also nothing about performance of these kits when CV% and bias% of each kit are considered. By method evaluation and sigma metrics determination, we could evaluate performace of different HbA1c kits better, which need more studies.

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