Türk Biyokimya Dergisi
2000, Cilt: 25, Sayı: 2

MEASUREMENT OF HEMOGLOBIN A1c WITH BIO-RAD CATION EXCHANGE MICRO COLUMN TEST AND BAYER DCA 2000+ IMMUNOASSAY: COMPARISON AND TRANSFERABILITY OF RESULTS FOR TWO DIFFERENT ASSAYS

Settar KOSOVA[1] and Seçkin KARA[2]

BİO-RAD KATYON DEĞİŞ TOKUŞ MİKRO KOLON KROMATOGRAFİ TESTİ VE BAYER DCA 2000+ İMUNO TESTİ İLE HEMOGLOBİN A1c ÖLÇÜMÜ: İKİ DEĞİŞİK YÖNTEMİN KIYASLANMASI VE SONUÇLARIN DEVREDİLEBİLİRLİĞİ

Özet: *Hemoglobin A1c (Hb A1c) kan glikoz düzeyini uzun vadede gösteren çok önemli bir parametredir. Dolayısıyla, testin laboratuvarda itina ile seçilmiş ve uzun vadede istikrarlı, güvenilir ve doğru sonuçlar veren yöntem(ler)le yapılması gerekir. Bu çalışmada 65 hastanın (51 diyabetli ve 14 normal) Hb A1c testi iki yöntem kullanılarak kıyaslanmıştır. EDTA'lı venöz kan numuneleri, aynı gün BAYER DCA 2000+ ve BIO-RAD mikro kolon katyon değiş tokuş kromatografi yöntemiyle çalışılmıştır. BIO-RAD ile elde edilen sonuçlar, özellikle normal hastalarda, istatistiksel olarak anlamlı daha yüksek bulunmuştur. Lineer regresyon analizi denklemiyle:N=51, BAYER (REG-BIO-RAD)= 1.281 x BIO-RAD - 2.918, r=0.974, Sx/y=0.691, düzeltilmiş BIO-RAD değerleri ile BAYER sonuçları arasında istatistiksel anlamlı bir fark bulunmamıştır. BIO-RAD testin 24 ile 26 ° C arası çalışılması test sonuçlarını etkilememiştir. Her iki testin seri içinde ve seriler arası varyasyon katsayısı benzer ve kabul edilebilir düzeyde olup % 3.4 kadardır. BIO-RAD testinin değişik üretim serileri arasındaki fark ihmal edilebilir düzeydedir. Dolayısıyla aynı kalibrasiyon eğrisi testin farklı üretim serileri için de kullanılabilir. Buna rağmen, testin doğruluğunu kanıtlamak amacıyla her çalışma serisinde en az bir, tercihen iki kontrol (biri normal diğeri diabetik düzeyde) çalışılması gerekir.*

Anahtar Kelimeler: *Hemoglobin A1c, Kalibrasyon, Bio-Rad mikro kolon testi, Bayer DCA 2000+ testi, Sonuçların Devredilebilirliği.*

Summary: *Determination of hemoglobin A1c (Hb A1c) is considered one of the most useful parameter of long term glycemic status of diabetic patients. Within a laboratory it must be conducted with carefully chosen method(s) to ensure long term stability of precision and accuracy of the test. Sixty five patients (51 Diabetic and 14 Normals) were included in comparative study. EDTA venous blood samples were assayed the same day with BAYER DCA 2000 + HbA1c immunoassay test and BIO-RAD micro column cation exchange chromatography test. Especially in the normal range observed BIO-RAD results were statistically significantly higher than those of BAYER test. Recalculated values for BIO-RAD HbA1c results by means of linear regression analysis:N=65, BAYER (REG-BIO-RAD) = 1.281 x BIO-RAD - 2.918, r=0.974, Sx/y=0.691 were almost as same as those for BAYER test. Performing the BIO-RAD test at 24-26 ° C didn't affect the test results. Intra and inter assay coefficients of variation of both tests are comparable and at allowable range of up to 3.4 %. Lot to lot differences of BIO-RAD test are negligible so the same calibration curve can be used with other lots of assay. Nevertheless at least one, preferably two (one normal and one diabetic) controls must be run with each run of assay to verify the accuracy of assay.*

Key Words: *Hemoglobin A1c, Calibration, Bio-Rad micro column test, BAYER DCA 2000+ assay, Transferability of Results.*

INTRODUCTION

Glycated hemoglobins (Glycohemoglobins) are valuable indicators of long-term (previous 2-3 months) glycemic control of diabetic patients (1). There is a wide variety of methods for measurement of glycohemoglobins with different principals and equipment used (2). Thus the results from one method to the other might not be the same. The diversity of methods, combined with the fact that there is still no consensus on a reference method or reference material(s) makes it difficult to compare the results from one laboratory to the other. Measuring of glycohemoglobin in the form of Hemoglobin A1c (Hb A1c) is widely accepted

1 Bursa Kızılay Tıp Merkez Laboratoruvarı
2 Bursa Kızılay Tıp Merkezi Diyabet Polikliniği

although determination of other or total glycohemoglobins is also in use (3). In our laboratory we routinely perform Hb A1c assay in a BAYER DCA 2000+ analyser (immunoassay). For the reason that time to time we experience difficulties in supplying BAYER kit we wanted to establish another method with BIO-RAD disposable microcolumn assay (atmospheric pressure cation-exchange chromatography) for which the results would be directly comparable to our DCA 2000+ system.

MATERIALS AND METHODS

In this study a total of 65 patients (51 diabetic and 14 non diabetic patients i.e. normal) were included. All the patients had full oral consent for this study. EDTA blood was collected by venipuncture and the test was performed the same day with both methods.

In the BIO-RAD Hb A1c micro column test (4), 100 μl of whole blood is mixed with 1,5 ml of hemolising reagent. One hundred μl of hemolisate is applied to a weakly acidic cation-exchange resin in a disposable column. One and a half ml of a low ionic strength borate/phosphate buffer is then passed through the column. This buffer elutes hemoglobin A1a and A1b fractions. Second elution is done with 4,0 ml of phosphate buffer of a higher ionic strength which elutes Hb A1c. Hemoglobin Ao, Hb S or Hb C remain in column. Another tube is prepared for total hemoglobin. The relative concentration of Hb A1c is calculated from the photometric measurements of both tubes. The test is completed in about one hour. Optionally three different levels of calibrators are available from the manufacturer which can be run in each series (which is the procedure recommended by the manufacturer). Samples exhibiting visual lactescence or bilirubin levels of up to 10 mg/dl do not interfere with this test (4).

BIO-RAD HbA1c micro column assay was performed according to manufacturer recommendations except that the measuring wavelength was 405 nm instead of 415 nm. According to the manufacturer optimal temperature of the test is 24° Celsius (° C) with 20-28 ° C allowable. We performed the assay at 24-26 ° C.

BAYER DCA 2000+ methodology test (5) is a latex immunoagglutination inhibition test. One μl of whole blood is used. Hb A1c in specimen competes with an agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA1c). Increasing concentrations of sample HbA1c causes a decrease in scattering of light at 531 nm. Total Hemoglobin is measured by thiocyan-methemoglobin method at 531 nm. Instrument is factory calibrated. For each lot the values for the calibration parameters are read from the calibration card provided in the kit. The values of calibration parameters are derived from the calibration with a HPLC method as a reference method. The test is completed in just 6 minutes. Results less than 2.5% and higher than 14.0% are reported reported as < 2.5 % and >14.0 % respectively. Triglyceride levels of up to 1347 mg/dl or bilirubin, up to a level of 20 mg/dl and rheumatoid factor , up to 1:5120 titre, do not interfere with this test (5).

In this study BAYER Hb A1c test was considered the reference method. Observed results of BIO-RAD Hb A1c were calibrated against the BAYER Hb A1c values by means of linear regression analysis. The recalculated values were then assigned as REG-BIO-RAD Hb A1c.

Quality control samples of normal and diabetic EDTA blood samples were obtained by analysing these specimens on BAYER DCA 2000+. Control samples were stored up to two weeks at 4 ° C and run with each series of BIO-RAD micro column assay to verify stability of the calibration curve and accuracy of the test.

For testing the between-group comparisons t-paired test was used. Statistical significance of the two-tailed test for less than 0.05 was considered significant. Pearson's r was used for the analysis of correlation. All values are expressed as Mean ± Standard Error of Mean (SEM).

RESULTS

Intra assay precision was studied by assaying two different samples (one Normal and one Diabetic) in a

single run performing 10 replicates of each sample. Inter-assay precision was studied with two other samples (one Normal and one Diabetic) which were assayed in 10 different runs within 2 weeks at different ambient temperatures (24-26 ° C). Results are shown in Table I.

Initially we tried to calibrate the BIO-RAD test with optionally available three level calibrators from the manufacturer. The obtained results deviated significantly from the results of the BAYER test. We postulated that there might be no difference in test results when different lots of BIO-RAD test were assayed. To test lot to lot variability of BIO-RAD Hb A1c test 10 different samples (3 normal and 7 diabetic) were analysed the same day with two different lots. Indeed, there was no statistical significant difference between the two lots of the assay: Mean Hb A1c lot A: 8.79 vs. lot B: 8.70; mean difference was 0.097,standard error of difference 0.1219 and p=0.4467.

In order to calibrate BIO-RAD test with BAYER test, 65 patients' results for both methods were included in linear regression analysis. Fourteen patients were considered normal (for BAYER HbA1c up to 5.7 %). Our previous (unpublished) non parametric analysis of non diabetic patients with BAYER assay indicated a reference interval of 4.2 % to 5.7% (which is similar to the data found by the manufacturer) (5). The calculated linear regression equation was found to be : BAYER(REG-BIO-RAD) = 1.281 x BIO-RAD - 2.918; standard deviations of slope and intercept were 0.038 and 0.359 respectively; correlation coefficient, r = 0.9740 and the standard error of estimate, Sx/y =0.691. The scatter plot of all patients data (N=65) is shown in Figure 1. In six cases where BAYER Hb A1c levels were displayed as >14.0% we found REG-BIO-RAD Hb A1c levels of 13.8±0.53 and the range of 12.4 to15.5 %.

One patient was not included in linear regression analysis because she had disproportionately high observed HbA1c (18.5 %) values determined with BIO-RAD micro column assay compared to BAYER DCA 2000+ test (7.4 %). This discrepancy was resolved later by performing hemoglobin electrophoresis with BIO-RAD HPLC Variant System[§]. The patient had Hemoglobin F value of 10.2% (Normal value is up to 2.0 %). Corrected BIO-RAD HbA1c is 18.5%-10.2%=8.3%; Regression derived REG-BIO-RAD recalculated is 7.7%, which is close to BAYER result of 7.4 %.

The summarised data of % HbA1c values for BAYER, observed BIO-RAD and REG-BIO-RAD in all patients (A), Diabetics (B) and Normals (C) are shown in Table II. The effect of temperature on test results are shown in tables III, IV and V.

DISCUSSION

Even though available methods measure different glycohemoglobin species they can be calibrated and usually there is a good correlation between the methods. Any of these methods alone will give clinically useful results given the method is precise and accurate (6, 7). The Intra- and inter-assay precision of BAYER and BIO-RAD tests are comparable. Imprecision of both methods is in

Table I. Mean (% Hemoglobin A1c) and Coefficient of variation (CV) (%) data for BAYER and (observed) BIO-RAD assays.

	BAYER DCA 2000+		BIO-RAD microcolumn assay	
	Mean	CV	Mean	CV
Intra-assay precision (N=10)	4.9	2.3	6.1	2.4
	10.1	2.1	10.3	2.0
İnter-assay precision (N=10)I	5.4	3.1	6.5	3.4
	11.0	2.7	10.9	2.9

accordance with suggestion of Phillipou and Phillips (8) that desirable analytical coefficient of variation (CV) for Hb A1c should be about 3 % or less.

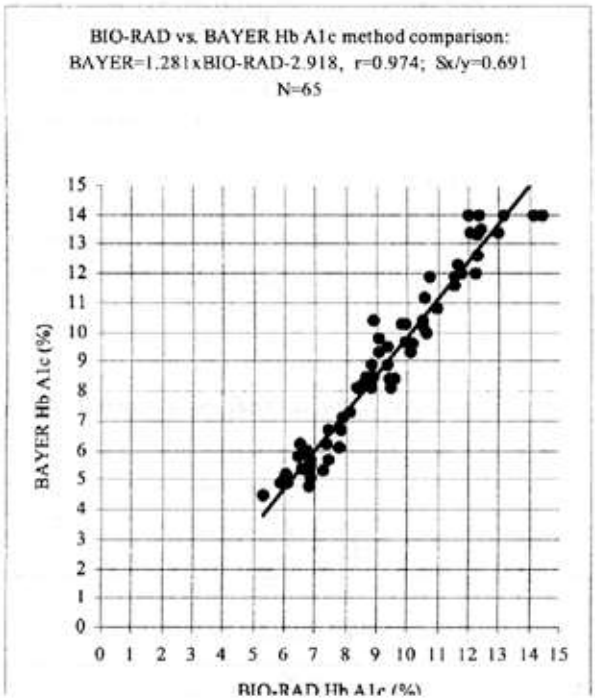

Figure 1. The scatter plot of Hemoglobin Alc results determined with BAYER dCA 2000+ and BIO-RAD micro column test

In diabetics there is no statistical significant difference between the observed BIO-RAD and BAYER Hb A1c results (p=0.89), but there is statistically significant difference in the normal range (p=0.000). There are also statistically significant different results between observed BIO-RAD and BAYER results for all patients (p=0.013). Especially in the normal and borderline high levels of Hb A1c, interference from carbamylated hemoglobin may partly explain the higher values for actual BIO-RAD values, because carbamylated hemoglobin co-elutes with hemoglobin A1c in cation exchange chromatography methods (9,10).

Actual results of HbA1c measurements with disposable columns are to be corrected by means of calibration. We used linear regression analysis model for multi point calibration of actual BIO-RAD HbA1c values with BAYER HbA1c results. There was no statistically significant difference between linear regression analysis corrected results of BIO-RAD (REG-BIO-RAD) and BAYER results for Normals, diabetics and for all patients. The negligible lot to lot variability of BIO-RAD assay allows that the calibration of one lot to be used with other lots of BIO-RAD test. Until now we have used several different lots of BIO-RAD micro column test with the same linear regression equation and we have seen no deterioration in our quality control results. Nevertheless, at least one (preferably two, one normal and one diabetic) controls should be assayed in every run of the test to verify the test's precision and accuracy.

Table II. *Comparison of % HbA1c values (Mean ± SEM) independently of ambient temperature for BAYER vs. (observed) BIO-RAD and REG-BIO-RAD for (A) all patients, (B) Diabetics and (C) Normals.*

A: All patients (Normals and Diabetics): N=65

BAYER	9.01±0.37	
BIO-RAD	9.31±0.28	P=0.013 *
REG-BIO-RAD	9.00±0.36	P=0.95

B: Diabetics: N=51

BAYER	10.06±0.35	
BIO-RAD	10.08±0.27	P=0.89
REG-BIO-RAD	9.99±0.35	P=0.49

C: Normals: N=14

BAYER	5.16±0.09	
BIO-RAD	6.49±0.16	P=0.000 *
REG-BIO-RAD	5.39±0.21	P=0.135

Table III. *Comparison of % HbA1c values (Mean SEM) at 24o, 25o and 26oC for BAYER vs. (observed) BIO-RAD and REG-BIO-RAD for all patients*

A: Ambient temperature: 24° C; N=30:

BAYER	8.77±0.49	
BIO-RAD	9.24±0.38	P=0.0041*
REG-BIO-RAD	8.92±0.49	P=0.222

B: Ambient temperature: 25° C; N=22:

BAYER	10.05±0.75	
BIO-RAD	9.99±0.55	P=0.805
REG-BIO-RAD	9.88±0.70	P=0.3379

C: Ambient temperature: 26° C; N=13

BAYER	7.77±0.73	
BIO-RAD	8.29±0.57	P=0.0410*
REG-BIO-RAD	7.70±0.73	P=0.5466

Table IV: *Comparison of % HbA1c values (Mean ± SEM) at 24°, 25° and 26° C for BAYER vs. (observed) BIO-RAD and REG- BIO-RAD for Normal patients.*

A: Ambient temperature: 24° C; N=5:

BAYER	5.24±0.17	
BIO-RAD	6.76±0.20	P=0.0013*
REG-BIO-RAD	5.74±0.25	P=0.0907

B: Ambient temperature: 25° C; N=5:

BAYER	5.24±0.15	
BIO-RAD	6.55±0.27	P=0.0006*
REG-BIO-RAD	5.48±0.35	P=0.3090

C: Ambient temperature: 26° C; N=4

BAYER	4.95±0.15	
BIO-RAD	6.05±0.32	P=0.0161*
REG-BIO-RAD	4.83±0.41	P=0.7281

Table V: *Comparison of % HbA1c values (Mean ± SEM) at 24°, 25° and 26° C for BAYER vs. (observed) BIO-RAD and REG-BIO-RAD for the Diabetics.*

A: Ambient temperature: 24° C; N=25:

BAYER	9.48±0.47	
BIO-RAD	9.74±0.38	P=0.0837
REG-BIO-RAD	9.56±0.49	P=0.5682

B: Ambient temperature: 25° C; N=17:

BAYER	11.47±0.63	
BIO-RAD	11.00±0.47	P=0.0779
REG-BIO-RAD	11.18±0.60	P=0.1876

C: Ambient temperature: 26° C; N=9

BAYER	9.02±0.60	
BIO-RAD	9.28±0.53	P=0.0405*
REG-BIO-RAD	8.97±0.67	P=0.6624

It is known that cation-exchange chromatography methods are more or less effected by temperature. But, we have seen no difference in test results if the test is conducted at 24 - 26 ° C. This makes the test more resistant to minor laboratory ambient temperature fluctuations.

The antibody of BAYER DCA 2000+ test is specific for the first few amino acid residues of the glycated amino-end of the ß-chain of Hb A ($\alpha 2\beta 2$). Any glycohemoglobin molecule having this same structure will be measured in the assay. Hb S and Hb C (point mutations of the ß-chain at the 6 position) and Hb E (point mutation at the 26 position of the ß-chain) variants do not affect this assay. Hb F ($\alpha 2\gamma 2$) molecule is not recognised by this assay since amino end of γ chain is different from that of ß chain (Val-His-Leu vs. Gly-His-Phe). According to manufacturer in cases of up to 10 % of Hb F content Bayer assay will accurately indicate the levels of Hb A1c. In the cases where Hb F is significantly higher than 10 % Hb A1c values will be lower than expected because a greater proportion of the glycated hemoglobin will be in the form of glycated hemoglobin F (5).

High levels of hemoglobin F (New-born, some young children 1-12 months, pregnant women and patients with ß-thalasemia or hereditary persistence of Hb F) in BIO-RAD atmospheric pressure ion-exchange chromatography method will exhibit falsely high Hb A1c values because it co-elutes with the Hb A1c fraction (4, 11). After the final elution the band patterns on the eluted columns should be interpreted with caution. These samples should be further evaluated to identify the abnormal hemoglobin(s) which may be present. The above mentioned patient with high levels of Hb F had normal band pattern after chromatography.

Neither method is affected by labile Shift Base but, both methods are affected by abnormal red cell survival. Samples from patients with haemolytic anaemia will exhibit decreased Haemoglobin A1c values due to the shortened life span of the red cells. Post-splenectomised patients may exhibit increased Hb A1c values due to a somewhat longer life span of the red cells. BAYER test is not affected by carbamylated (effect of urea) or acetylated (effect of acetylsalicylate) hemoglobin. Disposable ion-exchange columns may exhibit higher values because HbA1c co-elutes with carbamylated and acetylated hemoglobins. This is especially important in end-stage renal disease where high levels of urea may exhibit falsely higher levels of Hb A1c with disposable columns (10).

CONCLUSION

Inter method and inter laboratory differences present great obstacle to the standardisation of glycohemoglobin measurements. To achieve a long term clinical usefulness of glycohemoglobin results within a laboratory, independently of method(s) used, is possible if the method(s) are performed with carefully controlled precision, calibration and accuracy.

If conducted at 24-26 °C BIO-RAD Hb A1c micro column test can be effectively calibrated with BAYER DCA 2000+ assay using fresh donated venous blood from low normal to high abnormal levels of Hb A1c. Linear regression analysis corrected results are almost as same as those for BAYER assay. This way transferability of assay results and clinical usefulness is achieved .

REFERENCES

1. D.E. Goldstein, KM Parker, J.D. England (1982) Clinical application of glycosylated hemoglobin measurements. Diabetes 31 (Suppl 3), 70-78.
2. C.W. Weykamp, T.J. Penders, F.A.J. Muskiet, W. Van der Slik (1994) Effect of calibration on dispersion of glycohemoglobin values determined by 111 laboratories using 21 methods. Clin Chem 40, 138-144.
3. G.S. Bodor, R.R. Little, N. Garrett, W. Brown, D.E. Goldstein, M.H. Nahm (1992) Standardisation of glycohemoglobin determinations in the clinical laboratory: Three years of experience. Clin Chem 38, 2414-2418.
4. BIO-RAD; Hemoglobin A1c micro column test. Catalog Number 1928001/1928092, 100 tests, January 1993 edition.

5. BAYER DCA 2000+ Hemoglobin A1c Reagent Kit.

6. R.R. Little, H.-M. Wiedmayer, J.D. England, A.L. Wilke, C L. Rohlfing, F.H. Wians, Jr., J.M. Jacobson, Zellmer, D.E. Goldstein (1992) Interlaboratory Standardization of measurements of glycohemoglobins. Clin Chem 38, 2472-2478.

7. R.R. Little, J.D. England, H.-M. Wiedmeyer, E.M. McKenzie, R. Mitra, P.M. Erhart, J.B. Durham, D.E. Goldstein (1986) Interlaboratory standardization of glycohemoglobin determinations. Clin Chem 32, 358- 360.

8. G. Phillipou, P. J. Phillips (1993) Intraindividual variation of glycohemoglobin: Implications for interpretation and analytical goals. Clin Chem 39, 2305-2308.

9. C.W. Weykamp, T.J. Penders, C.W.M. Siebelder, F.A.J. Muskiet, W. Van der Slik (1993) Interference of carbamylated and acetylated hemoglobins in assays of glycohemoglobin by HPLC, electrophoresis, affinity chromatography and enzyme immunoassay. Clin Chem 39, 138-142

10. P. Bannon, F. Lessard, R. Lepage, L. Joly, L. Dufresne (1984) Glycated hemoglobin in uremic patients as measured by affinity and ion-exchange chromatography. Clin Chem 30, 485-486.

11. C.W. Weykamp, T.J. Penders, F.A.J. Muskiet, W. Van der Slik (1993) Influence of hemoglobin variants and derivatives on glycohemoglobin determinations, as investigated by 102 laboratories using 16 methods. Clin Chem 39, 1717-1723.