

ARAŞTIRMA MAKALESİ

STUDIES ON THE STABILITY OF VARIOUS COMPONENTS OF CONTROL SERUMS PREPARED IN LYOPHILISED FORM

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LİYOFİLİZE FORMDA HAZIRLANMIŞ KONTROL SERUMLARININ ÇEŞİTLİ BİLEŞİMLERİNİN STABİLİTESİNİN İNCELENMESİ

ÖZET

Bu çalışmada, Türk popülasyonundan elde edilen kontrol serumları (kadın normal hasta serumu, FC, ve kadın-erkek karışık normal hasta serumu, FMC), çeşitli komponentlerin kimyasal analizleri için, liyofilize formda hazırlanmıştır. Hazırlanan her iki kontrol serumunda; albümin, glikoz, kalsiyum, kolesterol, toplam protein, trigliserid, üre, alkalın fosfatez, aspartat amino transferaz (GOT:ASAT) ve alanin amino transferaz (GPT:ALAT) komponentlerinin stabiliteleleri incelenmiştir. İnsan serumları (kadın ve kadın-erkek karışık), Türk popülasyonundan normal hasta serum havuzundan elde edilmiştir. Deneyler sırasında, liyofilize formda hazırlanan her iki kontrol serumunun 5 ay (155 gün) boyunca stabil oldukları bulunmuştur. Gene her iki kontrol serumunun dondurulduktan sonra buzunun çözülmesinden itibaren -18 ± 2 °C'de 1 ay, 6 ± 2 °C'de 24 saat ve 20 ± 2 °C'de 4 saat boyunca stabil oldukları tespit edilmiştir. Bu sonuçlar, hiçbir katkı maddesinin ilavesi olmaması durumunda, liyofilize formda hazırlanan her iki kontrol serumunun (kadın normal hasta serumu, FC, ve kadın-erkek karışık normal hasta serumu, FMC), ilgili komponentlerin konsantrasyonlarının belirlenmesinde 5 ay boyunca stabil olduklarını göstermiştir. Ancak, bundan sonraki çalışmalarda kontrol serumlarının ömürlerinin uzatılabilmesi için uygun stabilizörlerin ve anti-bakteriyel maddelerin ilavesi gerektiği sonucuna varılmıştır.

Anahtar kelimeler: Kontrol serumu hazırlanması, komponentlerin stabilitelelerinin incelenmesi

SUMMARY

In this work, the control serums based on human serums from Turkish population (female normal patient serum, FC; and female-male mixed normal patient serum, FMC) were prepared in the lyophilised form for the determination of the concentrations of the various components for the chemical assay. The stabilities of components of both control serums determined were albumin, glucose, calcium, cholesterol, total protein, triglycerides, urea, alkaline phosphatase, aspartate amino transferase (GOT:ASAT) and alanine amino transferase (GPT:ALAT). Human serums (both FC and FMC) were prepared from the pooled normal patient serums from Turkish population. During the experiments, it was found that the prepared control serums (both FC and FMC) were stable for about 5 months (155 days) in the lyophilised form. After reconstitution; the control serums (both FC and FMC) were stable for 1 month (only frizzed once) at -18 ± 2 °C, 24 hours at 6 ± 2 °C and 4 hours at 20 ± 2 °C, respectively. These results showed that prepared control serums (both FC and FMC) in lyophilised form were suitable for determination of concentrations of the components mentioned above for about 5 months. It was decided to add some suitable stabilisers and anti-bacterial material to extend the life of both control serums for the future work.

Keywords: Control serum preparation, determination of the stability of the components

INTRODUCTION

Human serums are commonly used in preparing chemical standards and quality control materials in the clinical laboratory: routinely, they are preserved by freezing or lyophilisation. Control serums used in the quality control of clinical chemistry must be stable over a comparatively long period of time to be useful and meaningful (1-6).

In this study, lyophilised control serums based on human serums from Turkish population (female normal patient serum, FC; and female-male mixed normal patient serum, FMC) were prepared without adding any stabilisers and any anti-bacterial material. There are two reasons for this, first it was to see how long these prepared control serums will stay in a lyophilised form without adding any material mentioned above. Second, it was compared the reproducibility obtained in the laboratory using prepared control serums from Turkish population in a lyophilised form when tested by the same methods specified by the commercial ones, over the same period of time. To do this, two commercial serums in the lyophilised form, Diasys Normal Control Serum (DNC) and Merck Normal Control Serum (MNC), were chosen to compare the results of prepared control serums. It was seen that the life of the both prepared control serums were much lower than the life of the commercial control serums. It is necessary to add some suitable material to extend the life of the prepared serums as it is added to the commercial ones. Also, some values obtained from prepared control serums based on human serums from Turkish population were not similar to commercial control serums. The reason for this; the control serums prepared from Turkish population differ from the commercial ones based on human serum as its inherent biological variation, its genetic structure and its nutrition which can never be exactly the same.

MATERIALS AND METHODS

Preparation of lyophilised control serum and its stability measurements:

The control serums (both FC and FMC) were prepared from pooled human plasma. FC and FMC serums were obtained from a sample of 50 Turkish women, and 100 Turkish men and women aged (20-40) from the Marmara Region, respectively. The source materials were tested by an FDA (Food and Drug Administration) approved method for the presence of the antibody to HIV and HCV as well as for hepatitis B surface antigen and found to be negative. The prepared control serums (both FC and FMC) were lyophilised (3-4) by using Lyovac GT2 equipment. The stabilities of the components of both control serums were determined with Selectra 2 Autoanalyser by using automated methods. The values were measured by monitoring absorbance change. The methods and wave lengths for each component were given in Table 1.

Table 1. Components and used methods for chemical assay

Component	Method	Wave length (nm)
Albumin, g/dl	Bromcresol Green	546
Glucose, mg/dl	GOD-PAP	510
Calcium, mg/dl	Arsenazo III	630
Cholesterol, mg/dl	CHOD-PAP	505
Total Protein, g/dl	BIURET	546
Triglycerides, mg/dl	GPO-PAP	505
Urea, mg/dl	UREASE UV	340
Alk. Phos., U/L	DGKC	405
GOT, U/L	IFCC/NVKC	340
GPT, U/L	IFCC/NVKC	340

In addition to the prepared control serums, two other commercial serums (in the lyophilised form) mentioned before, were chosen to compare the results. Two lots of commercial lyophilised serum in 5 ml samples were purchased for this study. These commercial serums are Diasys Normal Control Serum (DNC) and Merck Normal Control Serum (MNC), respectively. The labelled vials, for each lot, were placed in separate containers. These containers were stored in a standard refrigerator.

Daily testing was performed by the same people, methods and equipment over a 5 month period. Three readings were made from each sample taken daily. Average values of the daily readings varied. This may have been caused by the following reasons; 1) distilled water was used to dissolve lyophilised samples instead of using buffer solution, 2) contamination of the samples during the readings, 3) some control parameters of autoanalyser vary during the analysis such as heat, autopipet, optic, filter, detector, voltage and power etc..

Statistical analysis:

The results were also analysed and evaluated using a basic statistical method (7). When the determinations for any specific substance were obtained, the mean value and standard deviation were calculated. For this, the following equations were used:

$$SD = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

x_i = concentration values

\bar{x} = mean value

n = number of values

SD = standard deviation

The method of quality control generally adopted has been the inclusion of an aliquot of a reference serum pool in the series of analyses to determine whether the

assay value obtained falls within the control limits for the procedure. These may have been set at ± 2 or ± 3 standard deviations from the mean value, which was established by repetitive assays when the serum was prepared or obtained.

It is also expressed in each case as percentage confidence limits which are calculated as ± 1 standard deviations (*SD*) from the mean, divided by the mean, and multiplied by 100. It was chosen to show all acceptable ranges in the tables as a percentage in order to provide uniformity and to facilitate the interpretation of the data.

$$\%CV = \frac{SD}{\bar{x}} \cdot 100$$

CV = confidence limit value

RESULTS AND DISCUSSIONS

Stability of prepared control serums

The values of albumin, glucose, calcium, cholesterol, total protein, triglycerides, urea, alkaline phosphatase, GOT and GPT for each of the serums used in this study (DNC, MNC, FMC and FC lyophilised control serums) are measured by using Selectra 2 Autoanalyser.

The statistical values for each component were given in Tables 2-5. In these tables, different standard deviation values were obtained for each constituent. The assay value obtained for each constituent falls within the control limit, $\pm 2(SD)$, for this procedure. However, it can be seen that the statistical values obtained for each component (albumin, glucose, calcium, cholesterol, total protein, triglycerides, urea, alkaline phosphatase, GOT and GPT) of prepared FMC and FC serums were comparable to commercial serums (DNC and MNC) in a lyophilised form. %CV values of all constituents for DNC and MNC lyophilised control serums (in Tables 2 and 3) were much lower than the values obtained for FMC and FC lyophilised control serums (in Tables 4 and 5). For example, %CV values of glucose of DNC, MNC, FMC and FC serums were obtained as 2.1, 1.83, 7.54 and 4.57 mg/dl, respectively. Also, %CV values of all constituents except GOT for FMC lyophilised control serum were higher than the values obtained for FC lyophilised control serum, as can be seen in Tables 4 and 5. %CV values of GOT of FMC and FC serums were obtained as 6.72 and 11.70 U/L, respectively.

However, the data derived from this testing suggests that the lyophilised serums from Turkish population give good results due to the assay values obtained fall within the control limits for the procedure. These control limits have been set at ± 1 and ± 2 standard deviations from the mean value.

Some of the values obtained from prepared control serums based on human serums (FMC and FC) from Turkish population were not similar to commercial control serums. In the present study, for example, the values of cholesterol and triglyceride were obtained as 193.1 and 121.30 mg/dl for FMC and 177 and 130.8 mg/dl for FC, respectively. In the literature (8), cholesterol levels in a random sample of Turkish adults were given as 185 mg/dl for men and 192 mg/dl for women and triglyceride levels 200 mg/dl for both genders. Again, these values for commercial ones, DNC and MNC, were given as 104.5 and 115.86 mg/dl and 100.9 and 104.7 mg/dl, respectively. The reason for this is that the control serum prepared from Turkish population differs from the commercial ones based on human serum as its inherent biological variation, its genetic structure and its nutrition which can never be exactly the

same. However, except cholesterol and triglyceride levels, no data for other components could be found from the literature for Turks for comparison.

Another interesting result is that alkalen phosphatase value (47.75 and 45 U/L for FMC and FC) obtained from this study was much lower than the commercial ones. Even in commercial ones, they have different alkalen phosphatase levels as 432.8 and 287 U/L for DNC and MNC, respectively. It was concluded again that lifestyle factors affect the levels of the components in Turks as in other populations.

As can be seen in Table 4 and 5, there is no significant differences in the values of components except cholesterol, triglycerides levels between prepared control serums FMC and FC.

About the life of the control serums, the DNC and MNC commercial control serums in a lyophilised form were stable for 2 and 3 years, respectively, as specified by their manufacturers. But, in this study, the values for each constituent prepared from Turkish population were consistent for about 5 month, followed by a gradual loss of activity in glucose, amounting to about 39% after 5 month. Last and previous readings of glucose were 52 mg/dl and 85 mg/dl, respectively. The reason for this is that there is growth of some microorganisms in the samples. It is necessary to add some anti-bacterial material such as sodium azaide into the samples as the commercial serums contain it.

Table 2. Statistical values of DNC lyophilised control serum

Components	Min. value	Max. value	Mean value	±1(SD)	±2(SD)	% CV
Albumin, g/dl	3.40	3.60	3.53	0.08	0.16	2.26
Glucose, mg/dl	112.0	121.0	117.4	2.47	4.94	2.10
Calcium, mg/dl	8.20	9.0	8.58	0.24	0.48	2.79
Cholesterol, mg/dl	103.0	107.0	104.5	0.91	1.82	0.87
Total Protein, g/dl	4.8	5.0	4.9	0.06	0.12	1.22
Triglycerides, mg/dl	113.0	118.0	115.86	1.77	3.54	1.53
Urea, mg/dl	48.60	54.40	51.81	1.41	2.82	2.72
Alkalen Phos., U/L	394.0	456.0	432.8	14.81	29.62	3.42
GOT, U/L	58.6	60.8	60.2	0.31	0.62	0.51
GPT, U/L	46.2	48.8	47.8	0.16	0.32	0.33

Table 3. Statistical values of MNC lyophilised control serum

Components	Min. value	Max. value	Mean value	±1(SD)	±2(SD)	% CV
Albumin, g/dl	4.0	4.2	4.1	0.05	0.10	1.22
Glucose, mg/dl	102.0	109.0	104.3	1.91	3.82	1.83
Calcium, mg/dl	8.20	9.10	8.78	0.21	0.42	2.39
Cholesterol, mg/dl	99.0	103.0	100.9	1.33	2.66	1.32
Total Protein, g/dl	6.10	6.20	6.12	0.04	0.08	0.65
Triglycerides, mg/dl	102.0	107.0	104.7	1.71	3.42	1.63
Urea, mg/dl	47.20	51.10	50.17	0.89	1.78	1.77
Alkalen Phos., U/L	280.0	298.0	287.0	5.32	10.64	1.85
GOT, U/L	59.30	62.40	60.82	0.93	1.86	1.53
GPT, U/L	43.1	44.2	43.5	0.26	0.52	0.59

Table 4. Statistical values of FMC lyophilised control serum

Components	Min. value	Max. value	Mean value	±1(SD)	±2(SD)	% CV
Albumin, g/dl	4.60	5.70	5.17	0.31	0.62	5.99
Glucose, mg/dl	70.0	88.0	78.0	5.87	11.74	7.54
Calcium, mg/dl	8.20	9.60	9.25	0.42	0.84	4.55
Cholesterol, mg/dl	172.0	208.0	193.1	10.06	20.12	5.21
Total Protein, g/dl	7.20	8.80	8.18	0.45	0.90	5.51
Triglycerides, mg/dl	112.0	126.0	121.30	3.98	7.96	3.28
Urea, mg/dl	22.60	31.0	26.35	2.16	4.32	8.19
Alkalen Phos., U/L	38.0	59.0	47.75	6.85	13.70	14.34
GOT, U/L	14.10	17.20	15.44	1.04	2.08	6.72
GPT, U/L	9.0	16.0	12.30	2.32	4.64	19.31

Table 5. Statistical values of FC lyophilised control serum

Components	Min. value	Max. value	Mean value	±1(SD)	±2(SD)	% CV
Albumin, g/dl	4.7	4.7	4.7	0.0	0.0	0.0
Glucose, mg/dl	69.0	76.0	72.80	3.11	6.22	4.57
Calcium, mg/dl	9.10	9.90	9.63	0.29	0.58	3.05
Cholesterol, mg/dl	172.0	180.0	177.0	2.75	5.50	1.55
Total Protein, g/dl	7.10	7.20	7.13	0.05	0.10	0.72
Triglycerides, mg/dl	129.00	134.0	130.80	1.94	3.88	1.48
Urea, mg/dl	22.80	25.70	24.18	1.07	2.14	4.44
Alkalen Phos., U/L	37.0	52.0	45.0	5.17	10.34	11.50
GOT, U/L	11.80	15.80	13.80	1.61	3.22	11.70
GPT, U/L	10.0	12.0	11.22	0.77	1.54	6.91

Stability of reconstituted control serum (FMC)

Lyophilised samples of FMC were reconstituted and stored at -18 ± 2 °C, 6 ± 2 °C and 20 ± 2 °C, respectively. When reconstituted, the powder dissolves readily (typically within 50 min) and remains clear. The reconstituted samples were again analysed for the chemical constituents by continuous-flow analysis with the Selectra 2 Autoanalyser.

The values for albumin, glucose, calcium, cholesterol, total protein, triglycerides, urea, alkaline phosphatase, GOT and GPT of FMC serum were consistent for about 1 month when stored at -18 ± 2 °C. At day 32, the reconstituted control serum colour changed to green. In Table 6, the statistical values were given for FMC prepared serum after reconstitution during one month when stored at -18 ± 2 °C. The highest %CV value was obtained as 15.72 U/L for GOT and the lowest %CV value was obtained as 5.90 g/dl for albumin. The stability of the measured constituents in the reconstituted prepared serum (FMC) (see Table 6) was similar to that obtained with the untreated sample (see Table 4).

In Table 7, the statistical values were given for FMC prepared serum after reconstitution during 24 hours when stored at 6 ± 2 °C. After 24 hours, the reconstituted

control serum colour changed to green. The highest %CV value was obtained as 22.28 U/L for GPT and the lowest %CV value was obtained as 2.96 g/dl for total protein. The stability of the measured constituents in the reconstituted prepared serum (FMC) (see Table 7) was similar to that obtained with the untreated sample (see Table 4).

In Table 8, the statistical values were given FMC prepared serum after reconstitution during 4 hours when stored at 20 ± 2 °C. After 4 hours, the reconstituted control serum colour changed to green. The highest %CV value was obtained as 17.35 mg/dl for urea and the lowest %CV value was obtained 2.43 mg/dl for calcium. The stability of the measured constituents in the reconstituted prepared serum (FMC) (see Table 8) was again similar to that obtained with the untreated sample (see Table 4).

After reconstitution; DNC commercial control serum was stable for 1 month (only frizzed once) at -18 ± 2 °C, 5 days at 6 ± 2 °C and 24 hours at 20 ± 2 °C as specified by the manufacturer. Also, after reconstitution; MNC commercial control serum was stable for 1 month (only frizzed once) at -18 ± 2 °C, 10 days at 6 ± 2 °C and 2 days at 20 ± 2 °C as again specified by the manufacturer. Reconstitution times again differ between the prepared and commercial ones. The reason for this was that the control serums based on human serums from Turkish population were prepared without adding any stabilisers and any anti-bacterial material.

Table 6. Statistical values of FMC lyophilised control serum after reconstitution (kept at -18 ± 2 °C)

Components	Min. value	Max. value	Mean value	± 1 (SD)	± 2 (SD)	% CV
Albumin, g/dl	4.80	5.80	5.21	0.31	0.62	5.90
Glucose, mg/dl	74.0	92.0	82.14	6.91	13.82	8.41
Calcium, mg/dl	8.50	9.90	9.14	0.57	1.14	6.28
Cholesterol, mg/dl	166.0	229.0	192.8	21.94	43.88	11.37
Total Protein, g/dl	7.10	9.40	7.98	0.90	1.80	11.32
Triglycerides, mg/dl	109.0	142.0	123.57	10.63	21.26	8.60
Urea, mg/dl	23.0	30.10	26.57	2.45	4.90	9.22
Alkalen Phos., U/L	48.0	65.0	57.3	6.55	13.10	11.43
GOT, U/L	14.0	20.4	16.63	2.61	5.22	15.72
GPT, U/L	11.0	14.5	12.36	1.28	2.56	10.37

Table 7. Statistical values of FMC lyophilised control serum after reconstitution (kept at 6 ± 2 °C)

Components	Min. value	Max. value	Mean value	± 1 (SD)	± 2 (SD)	% CV
Albumin, g/dl	4.30	5.10	4.66	0.29	0.58	6.31
Glucose, mg/dl	70.0	87.0	76.16	6.49	12.98	8.53
Calcium, mg/dl	8.60	9.40	9.05	0.34	0.68	3.75
Cholesterol, mg/dl	170.0	202.0	189.3	11.41	22.82	6.03
Total Protein, g/dl	8.0	8.70	8.38	0.25	0.50	2.96
Triglycerides, mg/dl	90.0	137.0	114.60	16.46	32.92	14.36
Urea, mg/dl	22.0	30.0	26.50	2.81	5.62	10.61
Alkalen Phos., U/L	43.0	57.0	50.25	7.14	14.28	14.22
GOT, U/L	13.0	17.0	14.76	1.29	2.58	8.74
GPT, U/L	8.60	14.50	10.96	2.44	4.88	22.28

Table 8. Statistical values of FMC lyophilised control serum after reconstitution (kept at 20±2°C)

Components	Min. value	Max. value	Mean value	±1(SD)	±2(SD)	% CV
Albumin, g/dl	4.10	5.30	4.70	0.47	0.94	10.16
Glucose, mg/dl	70.0	85.0	78.50	5.32	10.64	6.77
Calcium, mg/dl	8.60	9.20	8.86	0.21	0.42	2.43
Cholesterol, mg/dl	160.0	197.0	179.2	14.52	29.04	8.10
Total Protein, g/dl	7.40	8.60	8.06	0.50	1.00	6.25
Triglycerides, mg/dl	97.0	138.0	119.16	14.24	28.48	11.95
Urea, mg/dl	19.0	28.0	23.56	4.09	8.18	17.35
Alkalen Phos., U/L	40.0	59.0	51.16	7.14	14.28	13.95
GOT, U/L	13.0	15.0	13.90	0.78	1.56	5.66
GPT, U/L	9.0	11.0	10.0	0.84	1.68	8.46

CONCLUSIONS

It is generally recognised that there is a need for a reference human serum standard particularly towards improving the reliability of specific serum protein assays in laboratories that use quantitative diagnostic tests. For this purpose, it has been carefully prepared from the pooled normal patient serums from Turkish population.

In the examination of commercial control serums, they were analysed for a number of constituents and compared results with the values obtained in this study. The same automated methods specified by the manufacturer were used for determination of the constituents for the commercial and prepared ones. In general, when the same method was used, the value obtained fell within the acceptable range (well within ±2 standard deviation). The control serum prepared from Turkish population differs from the commercial ones as its inherent biological variation, its genetic structure and its nutrition which can never be exactly same. Lifestyle factors affect the levels of the components in Turks as in other populations.

To sum up, both prepared serums (FC and FMC) will be useful as a base material for the preparation of calibration materials or quality control materials for many procedures. The prepared control serums (FC and FMC) in the lyophilised form were found suitable for about 5 months. So that, it was decided to add some suitable stabilisers such as sodium azide to extend the life of the both control serums (either FMC or FC) for the future work.

NOMENCLATURE

FC: female normal patient serum
 FMC: female-male mixed normal patient serum
 DNC: diasys normal control serum
 MNC: merck normal control serum
 GOT: Aspartate amino transferase (ASAT)
 GPT: Alanine amino transferase (ALAT)
 SD: standard deviation
 CV: confidence limit value

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