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RESEARCH ARTICLE

The Stability Studies on ALT, LDH and GGT Enzymes in Control Sera Used at Clinical Biochemistry Laboratories

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Summary

The purpose of the current study is to make a stability work about some liver's function parameters such as *alanine transaminase* (ALT; EC:2.6.1.2), *L-lactate dehydrogenase* (LDH; EC:1.1.1.27) and *γ-glutamyltransferase* (GGT; EC:2.3.2.2.) enzymes that are present in five control sera belonging to different brands and suppliers. The stability of enzymes stored for a specified period at 4°C, 25°C and -30°C storage conditions, their activities were measured in autoanalyser accordance with the manufacturer's instructions for use in the commercial kit and periodic intervals. The control sera stored at 4°C, belonging to one brand, the GGT activity decreased at the rate of 21.78% in 72 h. Similarly, the control serum stored at 25°C, belonging to one brand, concerning the same parameters, the activity decreased at the rate of 19.49% as well. The control sera stored at 4°C, belonging to two brands, the LDH activities, decreased at the rate of 28.04% and 42.17% in 72 h. Similarly, the control sera stored at 25°C, belonging to three brands, concerning the same parameters, the activities decreased at the rate of 40%, 25.36% and 40.56% in 72 h. The stabilities of liver's parameters in control sera, stored at -30°C for 96 hour and at 4°C for 72 hour were satisfactory, generally. On the other hand, the stabilities of liver's parameters in control sera, stored at 25°C for 72 h were unsatisfactory in large numbers.

Keywords: *Stability, Quality control, Enzyme, Liver function test*

Klinik Biyokimya Laboratuvarlarında Kullanılan Kontrol Serumlarında, ALT, LDH ve GGT Enzimlerine İlişkin Stabilite Çalışmaları

Özet

Bu çalışmanın amacı, farklı marka ve tedarikçilere ait 5 kontrol serumunda bulunan *alanin transaminaz* (ALT; EC:2.6.1.2), *L-laktat dehidrogenaz* (LDH; EC:1.1.1.27) ve *γ-glutamiltransferaz* (GGT; EC:2.3.2.2.) gibi karaciğer fonksiyon parametresi olan bazı enzimler üzerinde bir stabilite çalışması gerçekleştirmektir. 4°C, 25°C ve -30°C'lerdeki depolama koşullarında ve belirli sürelerde bekletilen enzimlerin stabiliteleri, üretici firmanın ticari kitlerdeki kullanım talimatlarına uygun olarak ve periyodik aralıklarla, aktivitelerinin otoanalizörde ölçülmesiyle değerlendirildi. 4°C'de saklanan kontrol serumlarından, bir markaya ait GGT aktivitesi, 72 saatte %21.78 oranında azalmıştır. Benzer şekilde, 25°C'de saklanan, yine bir markaya ait kontrol serumunda da, aynı parametrenin aktivitesi %19.49 oranında azalmıştır. 4°C'de saklanan kontrol serumlarından, iki markaya ait LDH aktiviteleri, 72 saatte, %42.17 ve %28.04 oranlarında azalmıştır. Benzer şekilde, 25°C'de saklanan kontrol serumlarında, üç markaya ait aynı parametrenin aktiviteleri %40, %25.36 ve %40.56 oranlarında azalmıştır. Son olarak, 25°C'de saklanan kontrol serumlarında, iki markaya ait ALT aktiviteleri, 72 saatte, %32.6 ve %31.82 oranlarında azalmıştır. Kontrol serumlarındaki karaciğer parametrelerinin stabiliteleri, -30°C'de 96 saat ve 4°C'de 72 saat depolananlarda, genel olarak tatmin ediciydi. Diğer yandan, 25°C'de 72 saat depolanan kontrol serumlarındaki karaciğer parametrelerinin stabiliteleri, geniş ölçekte başarısızdı.

Anahtar sözcükler: *Stabilite, Kalite kontrol, Enzim, Karaciğer fonksiyon testi*

INTRODUCTION

The analysis of control materials in clinical laboratory, leading improved precision of detection values and to evaluation of the performances of reference methods. However, there are some possible problems concerning the preparations, transportation and storage of control sera [1,2]. The stabilities of enzymes which are present in

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control sera, are affected by multiple factors such as matrix composition (albumin, ADP, EDTA, etc.), the source of enzyme, heat, light, storage conditions and sulphydryl compounds [3-7]. According to International Federation of Clinical Chemistry (IFCC), the control sera samples are analyzed for quality control purposes rather than for calibration. It is preferred that these substances should have similar matrix with test samples. For instance, if test sample is serum, protein matrix should be chosen as control [8]. The test results for the liver's functions have to be accurate and reliable [9-11]. This could be done by quality control programs at clinical biochemistry laboratories. The accuracy of test results becomes more important when their values are at the edge of critical diagnosis and in cases where changeable therapeutic options are in question [12-14]. In control sera, the activities of the enzymes of ALT, GGT and LDH were analyzed via kits. According to Clinical Laboratory Improvement Amendments (CLIA), the activities of ALT, AST, GGT, LDH enzymes in control sera, should not exceed ±20% of targeted values [15].

The present study was performed on 5 control materials which belonged to different brands and the enzymes of ALT, GGT and LDH which were purchased from different suppliers. The stability was evaluated by storing the preparations at -30°C, 4°C and 25°C, and finally, the enzyme activities were determined periodically.

MATERIAL and METHODS

Materials

Five control materials, belonging to different brands, were used in the analysis of ALT, LDH and GGT enzymes. The analysis of control sera were done in following principle; in every storing conditions, 5 different control sera with same lot number were analysed.

1. TECO Normal Sera from TECO Diagnostic (CA, USA),
2. Lyphochek Assayed Chemistry Control from Bio-Rad Laboratories (Irvine, CA, USA),
3. XL Multical Control Sera from ERBA Diagnostic Mannheim GmBh, (Germany),
4. Control Serum 1, from Olympus (Hamburg, Germany),
5. Multisera E from Randox (Crumlin, N. Ireland).

Methods

The amounts of ALT, LDH and GGT enzymes were analyzed by using commercial kits (TECO Diagnostic, USA), according to the directions of producer, in autoanalyser (XL-600, Daman-India).

Stability Work

Lyophilized control materials were reconstituted with distilled water following the manufacturer specifications at 25°C. Some vials of the control sera which were then stored at 4°C and 25°C temperatures for at least 72 h. The others which were stored -30°C temperatures for at least 96 h.

The activities of the enzymes were measured after 30 min of reconstitution (time 0), and after 1, 2, 3, 24, 48 and 72 h following reconstitution for the vials which were stored for 4°C and 25°C. Similarly, following reconstitution of the control sera with water, the vials stored at -30°C, were analyzed at the end of 24, 48, 72 and 96 h, concerning the same parameters. The vials, stored at -30°C which were allowed to thaw for 30 min prior to analyzing periodically.

RESULTS

Fig. 1-3 show the results obtained in the stability study. The GGT activity that was in one of the control sera at 4°C for 72 h, decreased at a rate of 21.78%. Similarly, the GGT activities of control sera at 25°C decreased at rates of 19.49% and 11.94% with 2 different brands at the end of 72 h. However the control sera that was stored at -30°C, its GGT activity was stable for 96 h.

The LDH activities of control sera decreased at rates of 47.17% and 28.04%, stored at 4°C for 72 h, belonging to 3 brands. At the same time, the decreases took place in the sera, stored at 25°C, at the rates of 9.05%, 10.95%, 40%, 25.36% and 40.56%. As well as, the LDH activities of control sera with 2 different brands, at -30°C for 96 h, decreased at the rates of 10.53% and 15.28%.

The ALT activities of control sera, belonging to 2 brands, stored at 4°C for 72 h, decreased at rates of 18.52% and 24.25%. Similarly, the ALT activities of control sera, stored at 25°C, decreased at rates of 32.6% and 31.82% with 2 different brands.

DISCUSSION

Following reconstitution of lyophilized control materials with distilled water which were stored at different temperatures, their analysis were done regarding liver's parameters as mentioned previously. As well as, the control sera that are available in the market, were analyzed for quality control in the present study. Therefore, some suggestions offered for preventing economic losses, considering unsuitable storage conditions following re-constituting of the control sera processes.

In this study, the analyses were done whether the activities of ALT, LDH and GGT enzymes displayed any alterations in some of control sera, following reconstituting of the control sera, some of them stored at 4°C and 25°C at least 72 h, the others stored -30°C at least 96 h. GGT enzyme activity was found stable as well, at -30°C for 96 h. However, the GGT enzyme activities were not stable

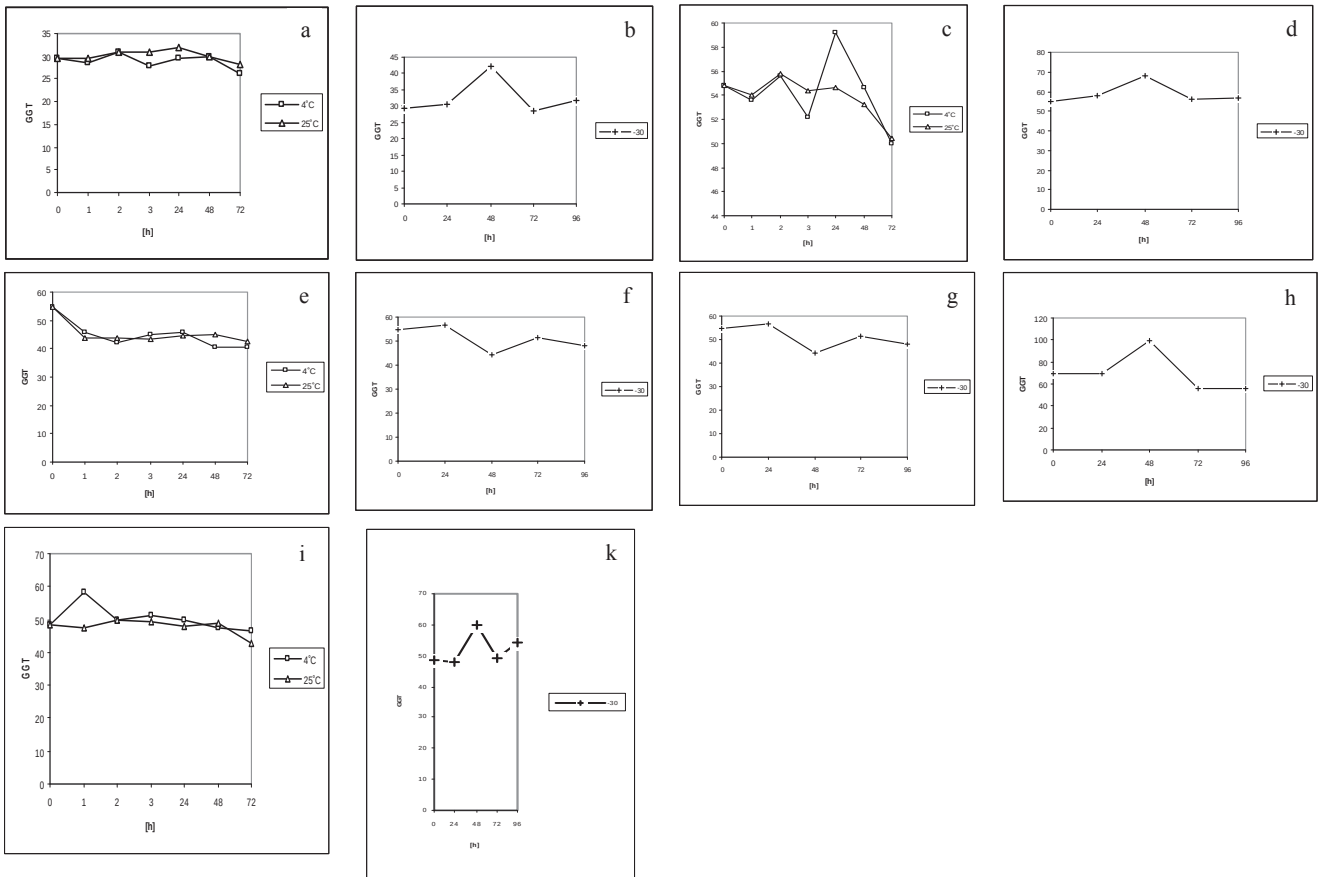

Fig 1. Stability study of GGT in

a, b; TECO Normal Sera (TECO): Control serum
c, d; Lyphochek Assayed Chemistry Control (Bio-Rad Lab): Control serum
e, f; XL Multical Control Sera (ERBA Diagnostic): Control serum
g, h; Control Serum 1 (Olympus): Control serum
i, k; Multisera E (Randox): Control serum

The vials were reconstituted and stored at 4°C (□), 25°C (Δ) and -30°C (+). The vials stored at -30°C which were allowed to thaw for 30 min before analysing. The values are the mean of duplicates. The activity of GGT analysed at each time and compared to the value which was obtained at 0 time

Şekil 1. GGT'nin stabilite çalışmasında,

Bu Eppendorf tüplerinin içerikleri sulandırıldıktan sonra, 4°C (□), 25°C (Δ) ve -30°C (+)'lerde depolandılar. -30°C'de depolanan Eppendorf tüpleri, analizlerinden 30 dakika önce çözdürüldüler. Değerler, dublikelerin ortalamasıdır. GGT aktivitesi, her defasında yeniden analiz edildi ve 0 zamanda elde edilen değerle karşılaştırıldı

at 4°C and 25°C, belong to 1 and 2 brands, respectively. The reason behind the increases in enzyme activities after 48th hours might be the suppressive effects of stabiliser substances on enzyme activity until this period that are frequently exist in control sera. Following this period, the effects of stabilisers became weaker, therefore, the enzyme activities increased.

The control sera, stored at -30°C and treated as freezing-thawing processes periodically, were found as stable generally, concerning liver's parameters. However, the enzymes in control sera stored at -30°C, when compared with the enzymes stored in the other conditions, they were much more stable at 96 h. On the other hand, the LDH and ALT enzymes were not stable at the end of 96 h, belong to 2 and 1 brands, orderly. The LDH enzymes in control sera were detected as non-stable at 25°C and 96 h. Similarly, the LDH enzymes in control sera were not stable either, at 4°C and -30°C, belong to 3 and 2 brands, respectively.

The ALT enzymes activities in control sera were determined as non-stable at 4°C and 25°C, belong to 2 brands for both temperatures. Similarly, ALT enzyme activities at 25°C were not stable either, belong to 3 brands. Overall, during the study, the control sera, stored at -30°C and 4°C were determined as stable generally, concerning liver parameters. However, the control sera, stored at 25°C, were not found as stable generally for liver parameters.

It has been reported that the creatin kinases activities of 11 different commercial brands of control sera, stored at different temperatures, were found as stable during 72 h at 4°C [3]. In similar with 2 exceptions, they found out

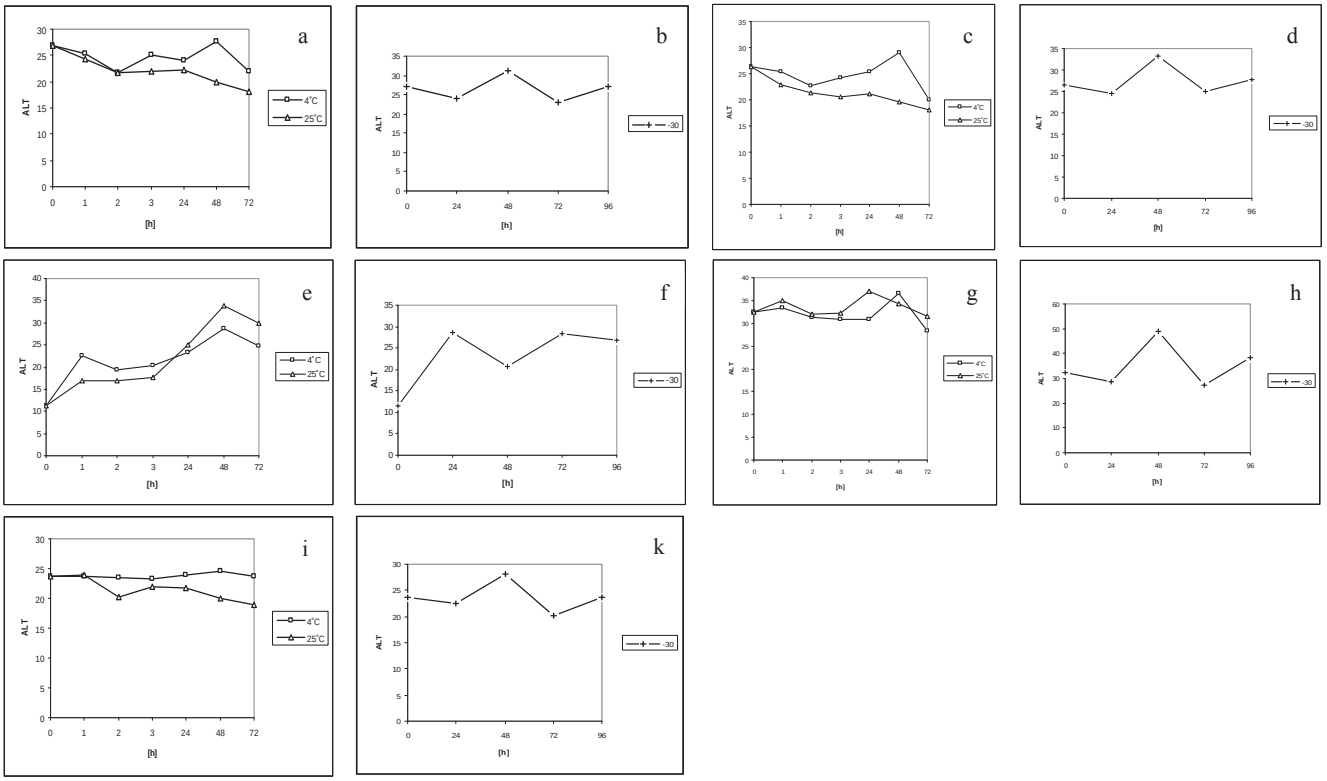

Fig 2. Stability study of ALT in

a, b; TECO Normal Sera (TECO): Control serum
c, d; Lyphochek Assayed Chemistry Control (Bio-Rad Lab): Control serum
e, f; XL Multical Control Sera (ERBA Diagnostic): Control serum
g, h; Control Serum 1 (Olympus): Control serum
i, k; Multisera E (Randox): Control serum

The vials were reconstituted and stored at 4°C (□), 25°C (Δ) and -30°C (+). The vials stored at -30°C which were allowed to thaw for 30 min before analysing. The values are the mean of duplicates. The activity of ALT analysed at each time and compared to the value which was obtained at 0 time

Şekil 2. ALT'nin stabilite çalışmasında,

Bu Eppendorf tüplerinin içerikleri sulandırıldıktan sonra, 4°C (□), 25°C (Δ) ve -30°C (+)'lerde depolandılar. -30°C'de depolanan Eppendorf tüpleri, analizlerinden 30 dakika önce çözdürüldüler. Değerler, dublikelerin ortalamasıdır. ALT aktivitesi, her defasında yeniden analiz edildi ve 0 zamanda elde edilen değerle karşılaştırıldı

that the creatin kinases which belonged to all commercial brands were stable at least during 3 h at 27°C. However, they also detected that the creatin kinase activities of control sera, stored at 37°C, decreased rapidly but only one of them stayed stable at such a high temperature.

In the another study, the activities of ALP, AST, GGT and LDH in lyophilized control sera were determined during 6 months of routine work in 16 different laboratories. 24.07% of results were determined out of targeted values [16].

The similar study was conducted that the amounts of albumin, total bilirubin and direct bilirubin in lyophilized control sera were detected during 6 months of routine work in 16 different laboratories every 15 days periodically [17]. 29.79% of results were determined out of targeted values. In spite of using control sera in 15 min following reconstituting, the reasons for the big percentage of out of targeted results could be related to the transportation and storage periods and the measurement capacities of laboratory equipments [17].

The findings suggested that the lyophilized control sera which were reconstituted with distilled water and aliquoted in order to store at 4°C for 72 h and -30°C for 96 h, it was more convenient concerning some of the liver parameters in these control sera. However, activities of the liver's parameters in control sera which were stored at 25°C for 72 h decreased in large numbers.

One of the study has revealed that after reconstitution with water at room temperature and storage at 37°C, the control sera materials were stable during the routine working day [18]. Another study from Wilson et al.[19] has reported that alkaline phosphatase (ALP) activities in human sera stored at 37°C, 4°C and frozen temperatures showed different profiles. The enzyme stored frozen temperatures showed better activity even slightly

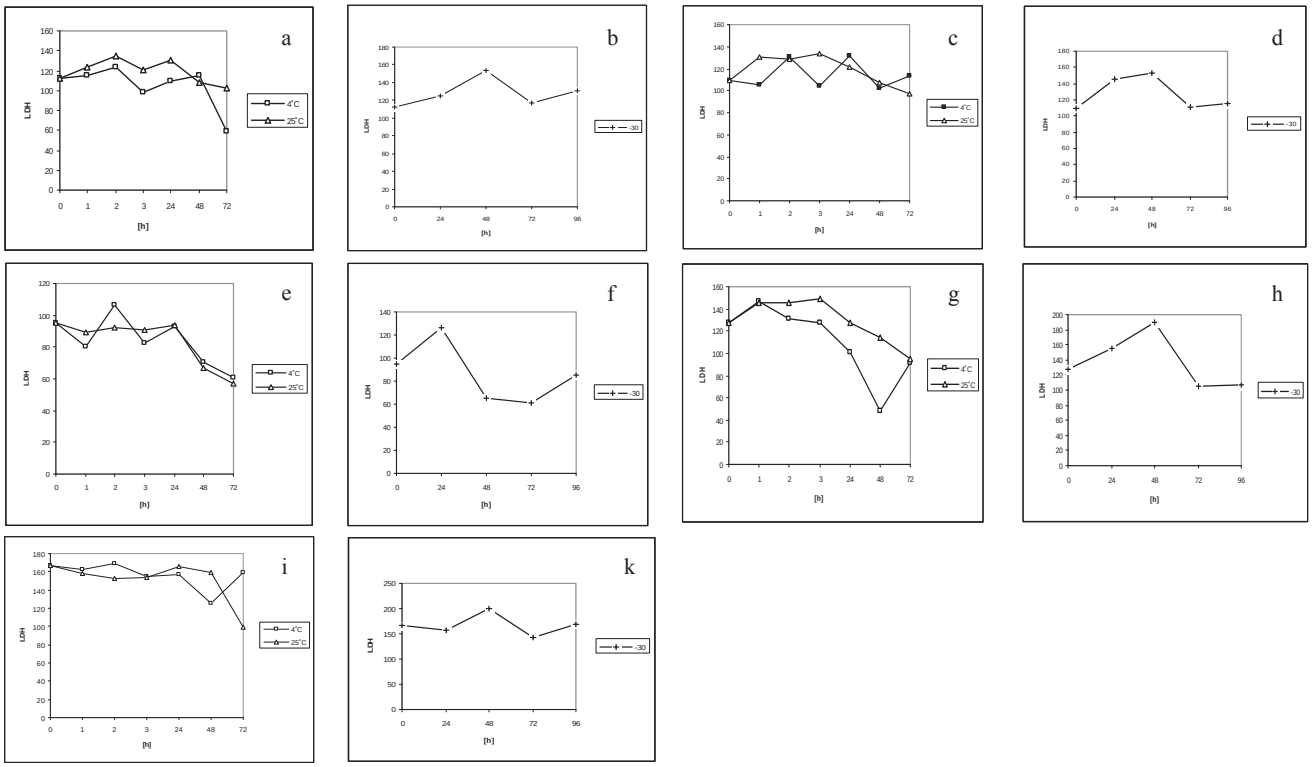

Fig 3. Stability study of LDH in

a, b; TECO Normal Sera (TECO): Control serum
c, d; Lyphochek Assayed Chemistry Control (Bio-Rad Lab): Control serum
e, f; XL Multical Control Sera (ERBA Diagnostic): Control serum
g, h; Control Serum 1 (Olympus): Control serum
i, k; Multisera E (Randox): Control serum

The vials were reconstituted and stored at 4°C (□), 25°C (Δ) and -30°C (+). The vials stored at -30°C which were allowed to thaw for 30 min before analysing. The values are the mean of duplicates. The activity of LDH analysed at each time and compared to the value which was obtained at 0 time

Şekil 3. LDH'nın stabilite çalışmasında,

Bu Eppendorf tüplerinin içerikleri sulandırıldıktan sonra, 4°C (□), 25°C (Δ) ve -30°C (+)'lerde depolandılar. -30ºC'de depolanan Eppendorf tüpleri, analizlerinden 30 dakika önce çözdürüldüler. Değerler, dublikelerin ortalamasıdır. LDH aktivitesi, her defasında yeniden analiz edildi ve 0 zamanda elde edilen değerle karşılaştırıldı

decreased after 34 days. Similarly, the specimens stored at 4°C and room temperature, there were the increases the enzyme activities after 5 days. In contrast, the enzyme activity stored at 37°C obviously decreased after 4 days in speciments. Both of these studes are in agrement with our data.

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