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Threshold Time to Onset Serum Biochemical Changes of Turkoman Racehorses at Different Serum-Clot Contact Times and Temperatures

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Abstract

BACKGROUND: It is essential to minimize the effect of time and temperature on the serum biochemical parameters and determine the stability limits of each analyte in pre-centrifuged blood samples.

OBJECTIVES: This study aimed to investigate the stability limits of 10 analytes in Turkoman racehorses blood samples stored in different temperatures and time points.

METHODS: The whole blood samples from healthy horses (n=10) were stored for 2 h (baseline), 6, 12, 24, and 48 h at 25ºC or 4ºC. The commercial kits (Parsazmoon, Tehran, Iran) were used for the samples analysis.

RESULTS: Albumin (ALB), total bilirubin (TB), and phosphorous (P) exhibited remarkable changes at 25ºC. The storage time for as long as 12 h at 25ºC had no significant effect on urea, total protein (TP), lactate dehydrogenase (LDH), creatinine, and alkaline phosphatase (ALP). The stability of alanine transaminase (ALT) in serum samples stored at 25ºC was for 24 h and for LDH was for 48 h at 4ºC. Aspartate transaminase (AST) was the most unstable analyte at different storage times at both temperatures. Urea, TP, ALB, TB, and P were stable at 4ºC for as long as 6 h. Creatinine and ALP were affected by 24 and 48 h storage times at both temperatures. There was a significant difference (P<0.05) in AST and ALT activities between two temperatures. No significant difference was observed in creatinine, urea, and TB concentrations between two storage temperatures at any of the storage times.

CONCLUSIONS: This study showed that some analytes have acceptable stability in the clotted blood samples stored at 4°C for 6 h.

KEYWORDS: Biochemical parameters, Pre-centrifuged blood samples, Stability limits, Storage time and temperature, Turkoman racehorse

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Introduction

Laboratory medicine and clinical biochemistry are integral parts of the diagnosis in veterinary medicine. The lack of clinical diagnostic laboratories or the significant distances between horse farms and clinical laboratories in major horse breeding centers could be one of the important reasons for the delay in the transport of blood samples. The storage time and temperature of pre-centrifuged whole blood may affect some clinical biochemistry parameters. The standard guidelines for avoiding clot-induced changes in the concrete concentration of biochemistry analytes from the blood sample handling are the serum separation from whole blood as soon as possible (Hedayati *et al.,* 2020). Few studies have investigated the effect of time and temperature of serum separated from the whole blood on a limited number of serum biochemical analytes in different species such as cattle (Ehsani *et al.,* 2008; Braun *et al.,* 2015), sheep (Braun *et al.,* 2010), goats (Nagyová *et al.,* 2016), dogs (Reynold *et al.,* 2006), and pigs (Moe *et al.,* 2018). The changes in the values of total protein and electrophoretic fractions have been studied in growing foals following serum contact to the clot at different times and temperatures (Quartuccio *et al.,* 2016). The effect of serum separation time from the whole blood on serum biochemical constitutes at room temperature has only been investigated in two studies in horses (Rendle *et al.,* 2009; Ada *et al.,* 2017). Considering the pre-analytical phase changes in equine clinical medicine that may occur by handling serum samples from the field to the laboratory, optimal management of this phase seems to be essential (Braun *et al.,* 2017). Defining the stability limit for each analyte will help to identify the reason for rejecting samples before processing. Temperature and delayed whole blood centrifugation can affect the outcome, but the results have often been conflicting. Therefore, the present study was designed to investigate the stability of clinical biochemistry parameters in the clotted blood samples of Turkoman racehorses stored at different temperatures and times.

Materials and Methods

Animals

Blood samples were collected from 10 clinically healthy adult Turkoman horses from a herd in Khorasan Razavi province during August 2019 via veinpuncture of the jugular vein with 18G needles. Each sample was transferred to 10 anticoagulant-free tubes (in total, 100 blood samples were collected). The samples were delivered to the laboratory as whole blood as soon as possible. The procedures were approved by the Semnan University Ethics Committee (48/7, 01/07/2019), and care was taken to minimize the number of animals used.

Pre-analytical procedures

From each sample, five tubes were stored at room temperature (25°C) and the other 5 were stored in refrigerator (4°C). The samples from each storage temperature were centrifuged at 1800g for 15 min at 2, 6, 12, 24, and 48 h from the sampling time. The serum was then transferred to the freezer -20 °C until assay.

The collected serum samples were used for the measurement of analytes such as ALB (Colorimetric in the presence of bromocresol green), TP (Biuret reaction), urea (Urease-GLDH), creatinine (JAFFE method), TB (DCA-dichloroanylin), P (phosphomolybdate UV), LDH, ALP (DGKC), ALT and AST (IFCC). The concentration and activity of the serum analytes were determined by an automated analyzer (BS Mindray 1200, China) one week after freezing using commercial kits (Parsazmoon, Tehran, Iran) according to the manufacture's instruction.

Data statistical analysis

The analytes concentrations were expressed as means ± standard deviation (SD). To identify the threshold time of the onset of serum biochemical changes at 4°C or 25°C following delayed whole blood separation, a repeated-measures analysis of variance (ANOVA) was performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). A multivariate general linear model was used to determine the statistically significant difference between two storage temperatures (4°C and 25°C) at each time point (2, 6, 12, 24, and 48 h). The significance was determined at the level of less than 0.05.

Results

The concentration stability of ALB, TP, urea, creatinine, TB, and P, and the activities of LDH, ALT,

AST, and ALP were evaluated when the centrifugation of whole blood stored at 4°C or 25°C was delayed for 6, 12, 24, or 48 h as compared to the separation within 2 h after whole blood collection (baseline).

The AST activity increased significantly (P<0.05) at 6, 12, 24, 48 h in comparison with 2 h (baseline) at both storage temperatures (4°C and 25°C) (Figure 1).

Serum concentration of creatinine and ALP activity increased significantly (P<0.05) at 24 and 48 h compared to 2, 6, and 12 h after blood sampling at both storage temperatures (Figures 2 and 3).

Serum TP concentrations increased significantly (P<0.05) at 24 and 48 h in comparison with 2, 6, and 12 h after blood sampling at 25°C. In the samples stored at 4°C, TP levels at 12, 24, and 48 h were significantly different (P<0.05) from the 2 and 6 h (Figure 4).

For urea, the storage effect at 4°C was not significant for up to 12 h. Storing the samples at 25°C for 6 h resulted in not significant effect but urea concentrations increased significantly at 12, 24, and 48 h after sampling (Figure 5).

Our results indicated that ALB, TB, and P levels were stable at 25ºC and 4ºC for as long as 2 h and 6 h (Figures 6, 7, and 8).

The effect of storage at 25ºC and 4ºC on ALT activity for at most 24 h and 48 h did not result in any significant changes (Figure 9).

In samples stored at 4°C, the LDH activity changed significantly at 48 h (P<0.05) compared to the 2, 6, and 12 h. The activity of LDH increased significantly (P<0.05) at 24 and 48 h compared to the 2, 6, and 12 h at 25°C (Figure 10).

There was a significant difference (P<0.05) in AST and ALT activities between two temperatures at different storage times other than 2 h. There was no significant difference in creatinine, urea, and TB concentrations between two storage temperatures at different storage times.

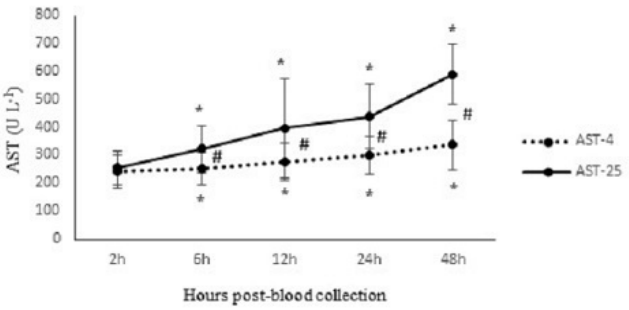

Figure 1. Line plots of average serum AST activity ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2 h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). #statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

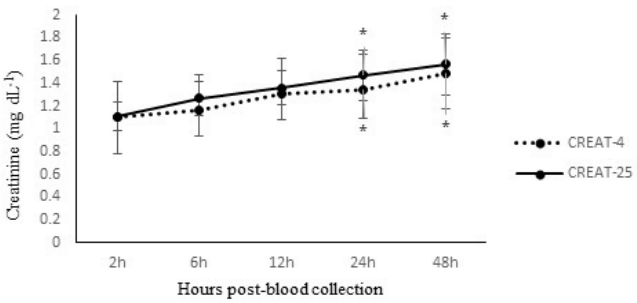

Figure 2. Line plots of average serum creatinine concentration ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2 h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). # Statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

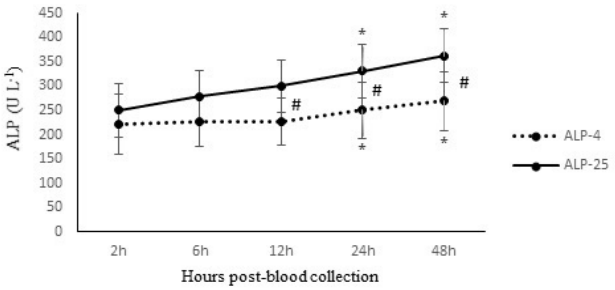

Figure 3. Line plots of average serum ALP activity ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline (2 h) and different times at both storage temperatures. #statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

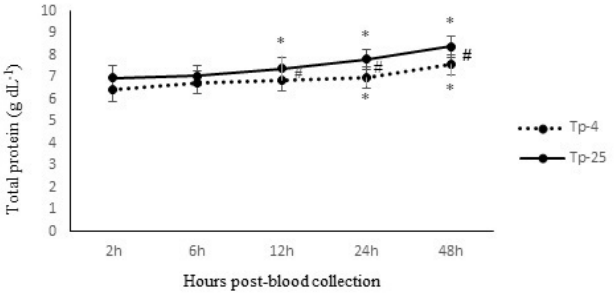

Figure 4. Line plots of average serum total protein concentration ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). # Statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

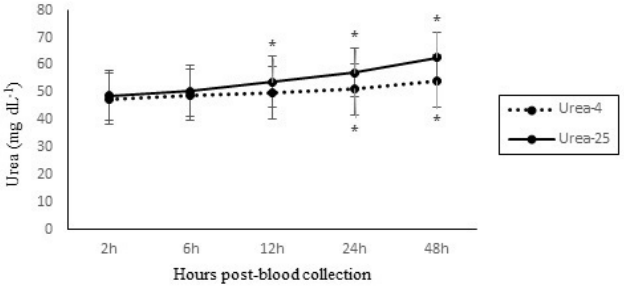

Figure 5. Line plots of average serum urea concentration ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2 h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). #statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48h).

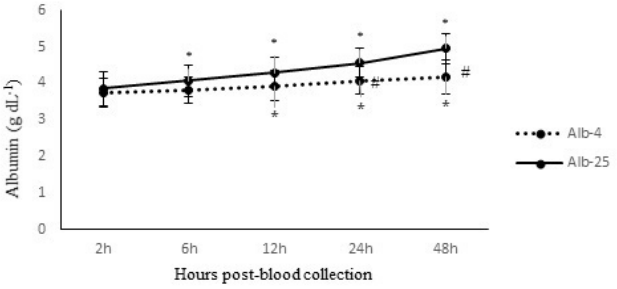

Figure 6. Line plots of average serum albumin concentration ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). #statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

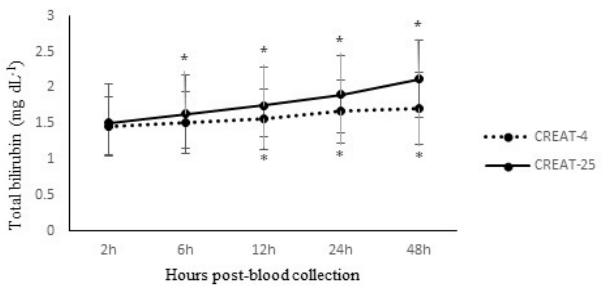

Figure 7. Line plots of average serum total bilirubin concentration ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48h) at each of the storage temperatures (4°C and 25°C). #statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

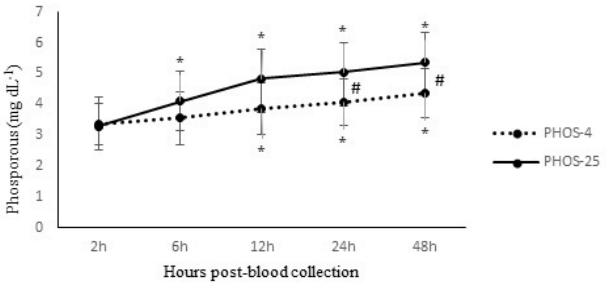

Figure 8. Line plots of average serum phosphorous concentration ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2 h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). # Statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2h, 6h, 12h, 24h, and 48h).

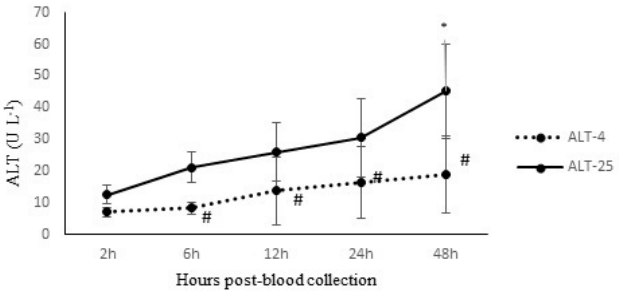

Figure 9. Line plots of average serum ALT activity ± standard deviation at each time point in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P<0.05) between baseline time (2 h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). # Statistically significant difference (P<0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

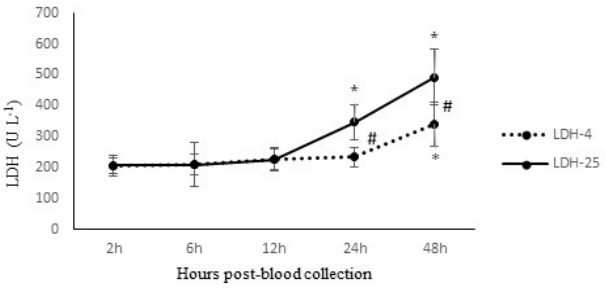

Figure 10. Line plots of average serum LDH activity ± standard deviation at each time points in whole blood samples stored at 4°C and 25°C. *statistically significant difference (P< 0.05) between baseline time (2 h) and other serum-clot contact times (6 h, 12 h, 24 h, and 48 h) at each of the storage temperatures (4°C and 25°C). # Statistically significant difference (P< 0.05) between two storage temperatures (4°C and 25°C) at each serum-clot contact times (2 h, 6 h, 12 h, 24 h, and 48 h).

Discussion

The stability limits of clinical biochemistry analytes was investigated in pre-centrifuged blood samples of Turkoman racehorses stored in different temperatures and times.

The present study demonstrated that ALT activity can be measured reliably in whole blood samples kept at 4°C and room temperature for at least 24 h before serum separation. Moreover, the concentrations of urea, creatinine, and TB were not affected by the storage temperatures in whole blood, even up to 48 h after serum separation. The ALB, urea, TB, LDH, ALT, and P exhibited faster changes at room temperature than 4°C. However, AST, ALP, and creatinine changes occurred concurrently at room temp-erature and 4°C. Only the TP concentration at 4°C began to change earlier than room temperature. The most sensitive analyte to storage temperatures and serum-clot contact duration time in the present study was determined to be AST. For such parameter detection, the sample should be transferred immedi-ately to the laboratory for serum separation and analysis.

Only two previous studies have investigated the stability of the selected clinical biochemistry analytes in equine blood kept unseparated beyond 24 h. Rendle *et al.* (2009) collected blood from 20 horses into plain serum tubes and left aliquots of whole blood to stand at 20-25°C for up to 72 h. They found a significant increase in ALB, TP, urea, creatinine, AST, and LDH at 20-25°C, which is in agreement with the present study. However, they found TB to decrease at these temperatures, whereas we found this to increase after 6 and 12 h at 25°C and 4°C, respectively. Decrease in TB concentration in the study of Rindel *et al.* (2009) could be partly related to the dilutional effect of hemolysis and the effect of photo-oxidation. In another study, Moe *et al.* (2018) evaluated the effects of storage times (6, 12, 24, 48, and 72 h) and temperatures (4°C and 25°C) on biochemical analytes in porcine un-centrifuged blood samples. They investigated ALB, GLDH, Mg, P, and TP, which significantly increased over time. Ca, GGT, GLDH, Glu, Mg, and P concentrations were significantly different between two temperatures, and the level of all analytes at room temperature was higher than the chilled temperature, which is consistent with our study. The different analytical methods used in the two studies, is one of the most important reasons for the discrepancies in the results.

The results of the Ehsani *et al.* (2008) study stated that serum phosphorous, magnesium, urea, cholesterol, β-hydroxybutyrate (BHBA), triglyceride, albumin, total protein, and GGT concentrations in dairy cows were not affected by the storage at 4°C and 25°C for as long as 24 h. Duration of the storage time had a significant effect on serum levels of Ca, glucose, CK, total bilirubin, and AST. The effect of two different storage temperatures was significant on serum concentrations of Ca, glucose, CK, total bilirubin, and BHBA.

Conclusion

Serum levels of all analytes at room temperature were higher than the chilled temperature. Among the analytes reviewed in the present study, albumin, total bilirubin, AST, and IP were the most sensitive analytes at room temperature, and of course, to evaluate these analytes, the serum should be separated from the whole blood in less than 6 h. The creatinine concentration and ALT, ALP, and LDH activities remain stable at both 4 and 25°C for at least 12 h of serum-clot contact times. The limitation of the present study was the relatively small sample size. Also, the effect of pre-analytical factors on hematological parameters was not studied.

Acknowledgments

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Conflict of Interest

The authors declared no conflict of interests.

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مجله طب دامی ایران، ۱۴۰۰، دوره ۱۵، شماره ۴، ۴۳۲-۴۳۹

زمان آستانه شروع تغییرات بیوشیمیایی اسب‌های مسابقه‌ای ترکمن در زمان‌ها و دماهای مختلف تماس سرم - لخته

ثمره کمالی صادقیان[۱]، محمود احمدی همدانی[۱*]، محمد حسن یوسفی[۲]، رضا نارنجی ثانی[۱]

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(دریافت مقاله: ۲۵ فروردین ماه ۱۴۰۰، پذیرش نهایی: ۰۷ تیر ماه ۱۴۰۰)

زمینه مطالعه: به‌منظور به حداقل رساندن تأثیر دما و زمان بر برخی پارامترهای بیوشیمیایی سرم، تعیین حد پایداری هر آنالیت در نمونه‌های خون پیش سانتریفیوژ، ضروری است.

هدف: این مطالعه با هدف تعیین حد پایداری ۱۰ آنالیت در نمونه‌های خون اسب مسابقه نژاد ترکمن ذخیره شده در دما و زمان‌های مختلف، صورت گرفت.

روش کار: نمونه‌های خون کامل اسبهای سالم (۱۰ رأس) در دمای ۲۵ یا ۴ درجه سلسیوس برای ۲ ساعت (زمان پایه)، ۶، ۱۲ ، ۲۴ و ۴۸ ساعت ذخیره شدند. از کیت‌های تجاری (پارس آزمون، تهران، ایران) برای تجزیه و تحلیل نمونه‌ها استفاده شد.

نتایج: آلبومین (ALB)، بیلی روبین تام (TB) و فسفر (P)، تغییرات چشمگیری را در دمای ۲۵ درجه سلسیوس نشان دادند. مدت زمان نگهداری اثر معنی‌داری بر اوره، پروتئین تام (TP)، لاکتات دهیدروژناز (LDH)، کراتینین و آلکالین فسفاتاز (ALP) در دمای ۲۵ درجه سلسیوس به مدت ۱۲ ساعت نداشت. پایداری آلانین ترانس آمیناز (ALT) در سرم ذخیره شده در دمای ۲۵ درجه سلسیوس ۲۴ ساعت و در دمای ۴ درجه سلسیوس ۴۸ ساعت مانند لاکتات دهیدروژناز بود. آسپارتات ترانس آمیناز (AST) در زمان‌های مختلف ذخیره‌سازی در هر دو دما، ناپایدارترین آنالیت بود. اوره، پروتئین تام، آلبومین، بیلی روبین تام، و فسفر تا ۶ ساعت در دمای ۴ درجه سلسیوس پایدار بودند. کراتینین و آلکالین فسفاتاز(ALP) تحت تأثیر زمان ذخیره‌سازی ۲۴ و ۴۸ ساعت در هر دو دما قرار گرفتند. تفاوت معنی‌داری ($P< ۰/۰۵$) در فعالیت‌های آسپارتات ترانس آمیناز و آلانین ترانس آمیناز بین دو دما وجود داشت. در غلظت کراتینین، اوره و بیلی روبین تام تفاوت معنی‌داری بین دو دمای ذخیره‌سازی در هیچ یک از زمان‌های ذخیره‌سازی وجود نداشت.

نتیجه‌گیری نهایی: این مطالعه نشان داد که برخی آنالیت‌ها در نمونه‌های خون لخته شده که به مدت ۶ ساعت در دمای ۴ درجه سلسیوس نگهداری می‌شوند پایداری قابل قبولی دارند.

واژه‌های کلیدی: پارامترهای بیوشیمی، نمونه خون پیش سانتریفیوژ، حد پایداری، زمان و درجه حرارت ذخیره‌سازی، اسب مسابقه نژاد ترکمن

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