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Daftar Isi Artikel

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Biochemistry

Bone Marrow-derived Mesenchymal Stem Cells Reverse Hepatic Fibrosis, Improved Vascularity, and Attenuate the Apoptosis in Carbon Tetrachloride-induced Hepatic Fibrosis Experimental Rats

Zainab Altaib, Walaa S. Sabbah, Eman K. Rashwan, Ashraf Albrakati, Abeer Mostafa (Author) 698-706

PDF

Benefits of Nigella sativa Extract Protecting Ovary Due to Cisplatin Chemotherapy

Khairani Sukatendel, M. Fidel Ganis Siregar, Muharam Natadisastra, Iqbal Pahlevi Adiputra Nasution, Syafruddin Ilyas, M. Rhiza Tala, Putri Chairani Eyanoer, Poppy Anjelisa Z. Hasibuan (Author) 680-687

PDF

Acute Toxicity Study of Porang (Amorphophallus oncophyllus) Flour Macerated with Strobilanthes crispus in Wistar Rats

Rizka Qurrota A'yun, Uswatun Hasanah, Hamam Hadi, Mustofa Mustofa, Eva Nurinda, Yulinda Kurniasari, Veriani Aprilia (Author) 976-981

PDF

Brain Derived Neurotrophic Factor and Serotonin Levels in Autistic Children: Do They Differ in Obesity?

Ehab R. Abdelraouf, Hend Rashad, Ayman Kilany, Hala M. Zeidan, Mohamed Elhadidy, Adel Hashish, Neveen Hassan Nashaat, Fateheya M. Metwally (Author) 959-963

PDF

Ethanol Extract of Carica papaya Leaf Can Increase Breast Milk in Lactating Rat

Yanti Herawati, Umi Kalsum, I Wayan Arsana Wiyasa, Lelly Yuniarti, Teguh Wahju Sardjono (Author) 520-526

PDF

Physicochemical Characteristics of Chicken Eggshell Flour Produced by Hydrochloric Acid and Acetic Acid Extraction

Rosnah Rosnah, Nurpudji A. Taslim, Andi Makbul Aman, Irfan Idris, Suryani As'ad, Agussalim Bukhari, Elly Wahyudin (Author) 428-432

PDF

Effect of the Ethanol Extract of Mimosa Leaves on the Blood Glucose, Malondialdehyde, and Histopathological Characteristics of Wistar Rats

S. Wahjuni, I. A. Raka Astiti Asih, Desmon Tutu Bili, Ni Made Puspawati, Ahmad Fudholi (Author) 1296-1301

PDF

Effect of Non-alcoholic Fatty Liver Disease on Some of Bone Biomarkers in Men

Dalal Al-Akabi, Faris S. Kata (Author) 924-927

PDF

Evaluation of Lipid Peroxidation under Immobilization Stress in Irradiated Animals in Experiment

Assem K. Okassova, Oralbek Z. Ilderbayev, Akmaral Zh. Nursafina, Gulmira M. Zharmakhanova, Bibigul B. Rakhimova, Bayan T. Yessilbaeva, Bayan N. Dyussenbekova (Author)

PDF

Features of the Course of Non-alcoholic Fatty Liver Disease in Experimental Animals at High Altitudes

Nurgul Toktogulova, Rustam Tuhvatshin (Author) 1092-1096

PDF

Insulin-like Growth Factor Initiates Hepatocellular Carcinoma in Chronic Hepatitis C Virus Patients through Induction of Long Non-coding Ribonucleic Acids AF085935

Role of LncRNA AF085935 in HCC development

Abeer Mostafa, Noha El-Sayed Ibrahim, Dina Sabry, Wael Fathy, Amany Y. Elkazaz (Author) 222-228

PDF

Antiproliferative Effect of Mesenchymal Stem Cells on Human Breast Carcinoma: New Insight on FOXO/lncRNA-AF085935 Axis

Sahar H. Ahmed, Abeer Mostafa, Amany A. Abou-Elalla (Author) 748-752

PDF

The Effect of Uncomplicated Knee Arthroscopy on C-reactive Protein and Erythrocyte Sedimentation Rate

Andrew Hannah, Graeme Ethan Hancock , James Edward Stoddard, Paul Mark Sutton (Author) 299-302

PDF

Evaluation of Proximate and Mineral Composition of Biscuit Formulated Using Chayote (Sechium edule) and Mung Bean (Vigna radiata) Flours

Jamaludin M. Sakung, Siti Nuryanti, Afadil Afadil, Sri Hastuti Virgianti Pulukadang, Maryam Maryam, Mar'atun Mar'atun (Author) 373-377

PDF

Bovine Serum as an Alternative to Control Serum for Total Protein Levels

Bambang Supriyanta, Martha Atik Martsiningsih, Steven Soenjono, Audrey Amy Andreansyah, Budi Setiawan (Author) 1292-1295

PDF

Organoleptic Properties, Proximate Compositions, and Antioxidant Activity of Carrot – Navel Orange Marmalade

Dzul Fadly, Nur Afni Rahmatiya Abdul, Yuges Saputri Muttalib, Bohari Bohari (Author) 488-492

PDF

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Bovine Serum as an Alternative to Control Serum for Total Protein Levels

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Abstract

BACKGROUND: Total protein describes the liver's ability to synthesize protein and metabolize substances in the blood.

AIM: We aimed to investigate whether cow serum can be used as a control agent because the analyte in bovine blood serum is almost the same as that of humans.

METHODS: Homogeneity and stability of bovine serum by adding 7.5% ethylene glycol preservative after being stored for 12 weeks at −20°C. This study uses a one-group pretest-posttest design. The alternative serum is derived from cow blood waste at a slaughterhouse in Giwangan, Yogyakarta, Indonesia. Data analysis was based on ISO 13528:2005 regarding stability and homogeneity of control serum.

RESULTS: From the examination of the total protein level in the serum, the value of Xr–Yr = 0.23817; this value meets the test criteria |Xr–Mr| 0.3σ, that is 0.23817 ≤ 0.91585.

CONCLUSION: Based on the results, serum from bovine can be an alternative to assess total protein levels and is still stable and homogeneous even though it is stored for 12 weeks.

Introduction

The assessment of total protein is needed to monitor the risk of liver and kidney disease. On the liver by describing the ability to synthesize proteins and metabolize substances contained in the blood [1].

Quality control is needed to detect analytical errors that affect laboratory test results [2]. There are two types of control materials, namely, modified control materials and commercial control materials. Commercial control serum is widely used in laboratories today [3] but commercial control materials are relatively more expensive for developing countries [4].

Control materials can be made from human, animal, or pure chemicals that can be traced to Standard Reference Materials. Bovine blood can be used as a control material by processing bovine blood into the serum. Bovine blood is blood waste from slaughtering animals that have not been used optimally. Utilization of bovine serum as a control material because the total protein analyte contained in bovine blood serum is the same as in human serum [5]. The selection of control material using bovine serum aims to avoid the risk of hepatitis infection and HIV-causing agents from human serum [6]. The use of control serum derived from bovine blood can be an alternative because of its low cost.

In its manufacture, the control material must have homogeneous and stable conditions during the storage period [5]. Stability is carried out to ensure that the test sample does not experience significant changes at any level. The stability of the control serum can be used to assess laboratory performance, including the quality of equipment and reagents. Therefore, the stability of the control serum is crucial [7].

The use of pooled serum with the addition of ethanediol can maintain the stability of the total protein content for 30 days at a temperature of −20°C [8]. The study was conducted using a bovine serum with a storage period of 12 weeks. This needs to be done to know the length of time the use of control serum.

Methods

This study uses an analytical survey method. The research design used is one group pretest-posttest, with the calculation of ISO 13528 in 2005 [9]. Bovine serum was declared homogeneous if Ss ≤ 0.3σ standard deviation for the proficiency assessment (SDPA). Determination of the value of SDPA is calculated based on ISO 13528:2005 by Horwitz and modified by Thompson. The total protein level of bovine serum was assessed using the TMS 30i tool with the Biuret method. This method is based on colorimetry (spectrophotometry), in which proteins from purple polypeptides are associated with copper ions in a strongly alkaline solution [10].

Table 1: Total cow serum protein levels before and after storing for 12 weeks at −20°C

Treatment (week)	N (Duplo)	Total protein				
		Maximum	Minimum	Average	Standard Deviation	Coevisien Variation (%)
0	10	5.65	6.17	6.02	0.14	2.25
12	3	5.76	5.81	5.78	0.02	0.39

This study used bovine blood waste obtained from the Giwangan Slaughterhouse, Yogyakarta. Bovine blood waste comes from bovine blood, which can stand for a while after the flow is not strong and is accommodated in a 15 ml centrifuge tube. The blood obtained was then centrifuged to collect serum that met the criteria, namely not hemolysis, not lipemic, and not icteric because it will affect the measurement results [11]. In this study, 80 ml of serum samples were obtained, which were then added 6.03 ml of ethylene glycol.

Table 2: Homogeneity test

Sample	Result		X_t	X_t-X_r	$(X_t-X_r)^2$	W_t	W_t^2
	1st	2nd					
14	6.12	6.11	6.115	0.0935	0.00874225	0.01	0.00
11	6.02	6.03	6.025	0.0035	0.00001225	−0.01	0.00
33	5.67	5.65	5.660	−0.3615	0.13068225	0.02	0.00
9	6.03	5.93	5.980	−0.0415	0.00172225	0.1	0.01
1	6.05	6.06	6.055	0.0335	0.00112225	−0.01	0.00
17	6.05	6.07	6.060	0.0385	0.00148225	−0.02	0.00
16	6.08	6.09	6.085	0.0635	0.00403225	−0.01	0.00
4	6.17	6.17	6.170	0.1485	0.02205225	0	0.00
40	6.06	6.06	6.060	0.0385	0.00148225	0	0.00
61	6.01	6.00	6.005	−0.0165	0.00027225	0.01	0.00
		Σ	60.215	Σ	0.17160250	Σ	0.01
		X_r	6.0215				
				S_x	0.13808	S_w	0.02377
				S_x^2	0.01907	S_w^2	0.00057
						$S_w^2/2$	0.00028
						$S_x^2-(S_w^2/2)$	0.01878
						S_s	0.13706

X_t: The average total protein content of the 1st and 2nd examinations (g/dl), X_r: The average total protein content of the 1st and 2nd examinations of the 1st to 10th samples (g/dl), W_t: The difference between the total protein levels of the 1st and 2nd examinations, which were absolute (g/dl), Σ: Total, S_x: Sample mean standard deviation, S_w: Standard deviation within samples, S_s: Standard deviation between samples.

Evaluation of total protein levels was carried out before storage and after 12 weeks of storage. A homogeneity test was carried out before storage, and ten samples were taken in duplicate. Examination of total protein levels after 12 weeks was carried out as a stability test which took three samples in duplicate.

This study used bovine serum with 7.5% ethylene glycol, stored for 12 weeks at −20°C. Ethylene glycol is one of the recommended preservatives for control materials because it has antifreeze and antibacterial properties [6]. According to research by Fauziah *et al*. [12] the stability of glucose levels in pooled sera with 7.5% ethylene glycol was statistically and clinically stable until day 30. The stability of glucose levels in pooled sera with 1% sodium azide was statistically stable until day 8, while clinically stable until day 25 [12]. The storage stability of the control serum at −20°C was relatively better [13]. This research has received approval from the Ethics Committee of the Health Polytechnic of the Ministry of Health of Yogyakarta by obtaining an ethical clearance letter e-KEPK/POLKESYO/0174/II/2021.

Results and Discussion

Based on the results of the study, the results are as in the following Tables 1-3.

Table 3: CV Horwitz result for homogeneity test

CV Horwitz	$2^{1-0,5 \text{ Log } C}$
Average Concentration	6.0215
Unit	Percent
Concentration Faction (C)	0.0602
Log C	−1.22030
0,5 Log C	−0.61015
1-0,5 Log C	1.61015
$2^{1-0,5 \text{ Log } C}$	3.05283
$CV_{Horwitz}$	3.05283
0.3σ (0.3×CV Horwitz)	0.91585

Based on ISO 13528:2005, bovine serum samples are considered homogeneous if Ss is 0.3σ. The SDPA determined by CV Horwitz is shown in Table 3 of 3.05283, and the value of 0.3σ is 0.91585. In Table 2, the homogeneity test results obtained an Ss value of 0.13706, so that the sample was declared homogeneous because it was following the criteria of ISO 13528:2005 Ss 0.3σ, namely 0.13706 ≤ 0.91585. To determine the stability of bovine serum was stored at 4C for 12 weeks. After being stored at at −20°C for 12 weeks, total protein was examined using the Biuret method. The results of the examination are in Table 4.

Table 4: Data on examination of total protein levels and calculation of ISO 13528 stability test in 2005

No Sample	Result		Y_t
	1	2	
22	5.77	5.76	5.77
54	5.81	5.8	5.81
65	5.8	5.76	5.78
		Y_r	5.7833

According to ISO 13528:2015, the control material is stable if the value of |Xr–Yr| 0.3σ. Based on the examination of the total protein content in Table 4, the Yr value of 5.7833 was obtained. Value of |Xr–Yr| = 0.2382, so that the value meets the stability test criteria according to |Xr–Yr| 0.3σ, that is, 0.2382 ≤ 0.91585.

The homogeneity and stability test results show a good CV (bovine serum CV calculation results = 2.25%) because it is still below the maximum CV according to the 2013 Minister of Health of Indonesia Regulation, 3%. The precision value is used to indicate how close the results of repeated examinations are with the same sample. A decrease in the CV value yields an increase in the accuracy of the system/ method.

The average total protein content in Table 3 decreased by 0.24 g/dl or 3.96%. The decrease in total protein content can be caused by the fast-freezing process and the slow thawing process, damaging the protein [14]. The refreezing cycle can also affect the total protein content. Refreezing cycles can result in changing trends, therefore limiting freeze or thaw cycles is highly recommended [15].

In a previous study, pooled sera added with ethanediol (ethylene glycol) stored at 4–8°C was stable for 18 days [16]. In contrast to this study, which used bovine serum with the addition of ethylene glycol 7.5%, which was stored for 12 weeks at a temperature of −20°C to the total protein content. The temperature of −20°C is relatively better for storage stability of control serum compared to refrigeration [13].

According to a previous study, the total protein content in pooled sera without preservatives changed significantly at −20°C after three months of storage [17]. In contrast to the research conducted, bovine serum was added with 7.5% ethylene glycol preservative, stored at −20°C for 12 weeks, and remained stable. Ethylene glycol has antifreeze and antibacterial properties, which allow maintaining the stability of bovine serum [6]. The increase in total protein content can be associated with the release of protein from glycoproteins and increased bacterial activity producing several enzymes and other microbial products that contribute to increasing protein levels [18]. The use of ethylene glycol preservative was more stable than sodium azide preservative where clinically pooled sera added with 7.5% ethylene glycol was stable for up to 30 days, while 1% sodium azide added was stable for up to 25 days [12].

The limitation of this study was that there was no control using commercial control serum, so there was no comparison test to validate the results of alternative control serum tests.

Conclusion

Based on the research that has been done, the total protein content in bovine serum with the addition of 7.5% ethylene glycol preservative, which was stored for 12 weeks at −20°C, was homogeneous and stable.

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