Indonesian Journal of Medical Laboratory Science and Technology

Open Access p-ISSN 2684-6748 | e-ISSN 2656-9825 IJMLST

Stability of Lyophilized Homemade Control Serum After Reconstitution on SGOT and SGPT Levels Stored in Freezer at Temperature (-2° to -4°C) and -20°C for 8 Weeks

Kadek Profit hartani[1], Anik Handayati[1]

[1]Department of Medical Laboratory Technology, Poltekkes Kemenkes Surabaya, East Jawa, Indonesia

Correspondence:
Anik Handayati,
Jl. Karang Menjangan No.18A, Airlangga, Kec. Gubeng, Surabaya, East Jawa, Indonesia
Zip Code: 60286

Email: anik_handayati@yahoo.co.id

Received: July 28, 2022
Revised: February 15, 2023
Accepted: April 16, 2023
Published: April 29, 2023

DOI: 10.33086/ijmlst.v5i1.3278

Abstract

Improving health laboratory services is closely related to strengthening the quality of health laboratories. Serum control is part of the quality assurance of health laboratories. Lyophilized homemade serum is a type of homemade control serum. This research aims to determine the stability of lyophilized homemade control serum against Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) levels. This research used an experimental method with pooled sera as research material stored at -2°C to -4°C and -20°C for 8 weeks. Pooled sera were obtained from respondents who had no history of disease, were free from HIV, AIDS, and HBsAg. The research was conducted at the Reference Laboratory and Clinical Chemistry Laboratory Politeknik Kesehatan (Poltekkes) Kementerian Kesehatan, Surabaya. The results of the study were data analysis using linear regression test. The SGOT parameter at freezer temperature (-2 to -4°C) showed that the storage time had an effect of 90.97% with an error component of 9.03%. At freezer temperature (-20°C), the result of storage time has an effect of 78.71% with an error component of 21.29%. In the SGPT parameter with freezer temperature (-2 to -4°C), the result of storage time has an effect of 96.71% with an error component of 3.29%. At freezer temperature (-20°C), the result of storage time has an effect of 91.47% with an error component of 8.53%. The results of the SGOT and SGPT examinations did not exceed the limits of ±2SD and ±3SD The CV on the SGOT and SGPT examinations also did not exceed the CCV limits, which is to be stable.

Keywords

Lyophilized Homemade Control Serum, Serum Glutamic Pyruvic Transaminase, Serum Glutamic Oxaloacetic Transaminase.

Citation: Hartani KP, Handayati A. Stability of Lyophilized Homemade Control Serum After Reconstitution on SGOT and SGPT Levels Stored in Freezer at Temperature (-2° to -4°C) and -20°C for 8 Weeks. Indones J Med Lab Sci Technol. 2023;5(1):53–67. DOI: 10.33086/ijmlst.v5i1.3278

INTRODUCTION

Clinical laboratory services are an integral part of health services needed to make a diagnosis, by determining the cause of the disease. Clinical laboratories need to be organized in a quality manner to support efforts to improve the quality of public health. Given the importance of the function of laboratory examination results, the quality of laboratory results must always be guaranteed. In accordance with government regulations, clinical laboratories are required to carry out laboratory quality assurance (1).

Laboratory quality assurance is the entire process or action taken to ensure the accuracy and accuracy of examination results. There are two types of quality assurance in laboratories, namely internal quality assurance and external quality assurance. Internal quality assurance is a prevention and control activity carried out by each laboratory on an ongoing basis to prevent and reduce the occurrence of errors or inconsistency examination results. External quality assurance is an activity held regularly by other parties outside the laboratory concerned to monitor and evaluate the performance of a laboratory in a particular field of examination (2,3). Measuring the accuracy of laboratory examination results can be carried out using the control materials (4,5).

Control materials used in laboratories to monitor the accuracy of examination results or the quality of clinical examination results (6). Control materials can be obtained from commercial or prepared from pond materials (secondary control materials). Secondary control material is made from the remaining serum of patients with normal levels and collected (pooled sera) as a substitute for commercial control materials. External quality assessment (EQA) requires stable quality control (QC) materials (7).

Nowadays, the control serum used in the laboratory in the commercial ones. This control serum is taken from animals which may not be the same as human serum and quite expensive. Meanwhile, in terms of efficiency, the homemade lyophilized human serum does not require costs to make. Besides that, to utilize the samples used in clinical chemistry examinations, there are usually only a few, while the remaining will be discarded (8). For small laboratories, health centers and educational laboratories that use the homemade lyophilized human serum as a control serum, it is hoped that it can be an alternative to control materials (9). Material quality control (QC) is critical to internal quality control (IQC) and external quality assessment (EQAS) schemes. However, many developing countries disadvantaged by unavailability and high cost of commercial control agents. Therefore, preparing homemade lyophilized human sera would be cost-effective as a quality control agent. The lyophilized serum is widely used in clinical laboratories because it is stable for

1 – 2 years when stored at 2-8°C and several years at -20°C or lower (10).

Pooled serum QC is also stable and low cost compared to commercial QC. Another use of pooled serum is to monitor a continuous flow analysis system throughout a long estimation chain, to ensure that QC is maintained. Serum pools are valuable in detecting and correcting aberrations, especially for automated analyzer techniques. The stability of the serum pool is known to have a shorter duration than the lyophilized control sera. Moreover, the serum pool only checks for precision at one level (11,12).

Examination of liver function is one of the clinical chemistry examinations that is often requested by doctors. It is because the role of the liver as an essential body organ and the central organ of metabolism. Two types of enzymes that are often associated with liver cell damage included in the aminotransferase group. In liver disease, the levels of SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase) tend to change in parallel. If liver cells are damaged, these enzymes, which are normally present in the cells, enter the bloodstream. The more liver cells that are damaged, the higher the levels of SGOT or SGPT measured in the blood (13). Previous studies demonstrated that at -20°C the maximum change was shown by SGOT and SGPT (decreased by 17% and 6%, respectively) at 6 months for the serum pool (14). ALT activity decreased after 3 days of storage at room temperature (22°C) and decreased after 7 days at 4°C (15).

The purpose of this study was to determine the stability of post-reconstitution homemade lyophilizate control serum stored in the freezer at -2°C to -4°C and -20°C against SGOT and SGPT parameters for 8 weeks.

MATERIALS AND METHODS

This research is an experiment study with a time series research design. The material used in this study was homemade lyophilized serum after reconstitution stored at -2°C to -4°C and -20°C for 8 weeks. The experiment was performed from November 2021 to May 2022. The selected respondents were female students of the Surabaya Ministry of Health Politeknik Kesehatan (Poltekkes) aged 18-21 years, had no history of disease, were free of HIV and HBsAg. This study used purposive sampling as sampling technique. Respondents previously asked to fill out informed consent form, for respondent who agreed with the consent. The Ethics Commission has approved this research under the Ethics Eligibility Letter No. EA/824/KEPK-Poltekkes_Sby/V/2022.

Collection of Blood

The research procedure started with injecting the respondent's blood into a red cap vacutainer tube. The blood obtained was

allowed to stand at room temperature. The vacutainer tube was centrifuge at 3000 rpm for 15 minutes. The sample criteria were not hemolyzed, not lipemic, and not icteric. The anti-HIV and HbsAg tests were performed to confirm serum sample was not infectious. The pooled serum was then homogenized using a vortex mixer and transferred into vials, each 3 mL using a volume pipette. Furthermore, the serum sample was lyophilized.

Homemade Lyophilized Control Serum Preparation

Freeze dryer used to make lyophilized serum. Firstly, serum stored in the freezer at -80°C until frozen. Then, the inside of the freeze dryer cleaned. The tool cover and tube supports were smeared with Vaseline gel. The power button on the back of the vacuum pump and the front of the freeze dryer were turned on. The display on the device would showed a temperature of -50°C. The sample tube is connected with rubber on the tube support also closed using aluminum foil. The valve connecting the sample tube to the instrument is turned. The freezer drying process can be started and considered complete when all the serum frozen into powder. Serum in the form of lyophilizate is dissolved by tapping on the bottle so that the powder falls to the bottom of bottle. Then, the powder was dissolved in 3 ml of distilled water using a volume pipette. The dissolved powder was stand 30 minutes and homogenized back and forth. After homogenized, the liquid was divided into microtube packages, 500 microliters of serum each, then stored at the appropriate temperature and storage time. 60 microtubes were obtained from 10 vials of homemade lyophilizate serum. The microtubes divided into three groups. The first group, as many as 10 micro tubes was performed duplicate examination at 5 reference laboratories to test homogeneity and determine the initial value or base line. The second group, as many as 24 microtubes stored at -2°C to -4°C, and the third group of 24 microtubes stored at -20°C. Samples in microtubes were used to test the stability of SGOT and SGPT parameters, examined by replication 3 times, once a week for 8 weeks.

Determination of SGOT and SGPT Level

The enzymatic kinetic method performed to determine of SGOT and SGPT levels. The principle of determine SGOT is that SGOT/AST in the sample catalyzes the transfer of the amino group from L-aspartate to 2-oxoglutarate to form oxaloacetate and L-glutamate. Oxaloacetate in the presence of NADH and Malate dehydrogenase (MDH) reduced to L-malate. In this reaction NADH is oxidized to NAD. The principle for determining SGPT is that Alanine aminotransferase (ALT) catalyzes the transaminase of L-alanine and 2-oxoglutarate to form L-Glutamate and pyruvate. The pyruvate formed is reduced to lactate by the

lactate dehydrogenase (LDH) enzyme and nicotinamide adenine dinucleotide (NADH) is oxidized to NAD. The amount of NADH oxidized is directly proportional to ALT activity.

The examination begins with removing the microtubes of homemade lyophilized serum samples, which have been dissolved and stored in the freezer at -2°C to -4°C and -20°C, then allowed to stand until room temperature. The sample pipette on the microtube is transferred to the sample cup and placed on the sample tray in the automatic clinical chemistry determination device. Check the selected SGOT and SGPT then click start/run to start the determination.

Statistical Analysis

The data analysis technique used in this research is the examination results of the SGOT and SGPT levels. The test is performed with a Simple Linear Regression Analysis Test with SPSS, which is tabulated (presenting data in tabular form). From the data obtained, then data analysis was carried out by calculating the average of the results of the examination (mean), standard deviation (SD), and coefficient of variation (CV). The data obtained were then performed with a linear regression test to determine the effect of storage time on homemade lyophilized serum after reconstitution on the stability of SGOT and SGPT levels.

RESULTS

This study was initiated by conducting anti-HIV and HBsAg screening tests. The aim was to detect the presence or absence of HIV and hepatitis B viruses in each respondent used the rapid test immunochromatography method. The results of the anti-HIV and HBsAg screening tests were declared non-reactive, meaning that the respondent's serum was negative for HIV and hepatitis B viruses. After the serum resulted infection-free, it was followed by the initial examination as a control test. This check aims to get a reference value or baseline value. The data obtained in this examination is used as a reference value for further research. In this examination, the sample used was homemade lyophilizate serum after being dissolved with SGOT and SGPT parameters. The results of the initial test showed in Table 1.

Table 1. Results of Initial Examination and Homogeneity Test of Homemade Lyophilized Control Serum SGOT and SGPT Parameters

	Examination Results (U/L)	
	SGOT	**SGPT**
mean	19.75	5.35
SD	2.20	0.85
CV (%)	11.16	15.86

Table 1 showed the average value of SGOT, which is 19.75; SD 2.20; and CV 11.16%, while for the average value of SGPT 5.35; SD 0.85; and CV 15.86%. The CV value on examination of SGOT and SGPT

levels at -2℃ to -4℃ storage from week 1 to week 8, does not exceed the limit of CV (CCV). Value Chosen Coefficient of Variation (CCV) for SGOT 12.5% and SGPT 17.3%, meaning that the levels of SGOT and SGPT serum lyophilizate homemade are homogeneous to variations in vials.

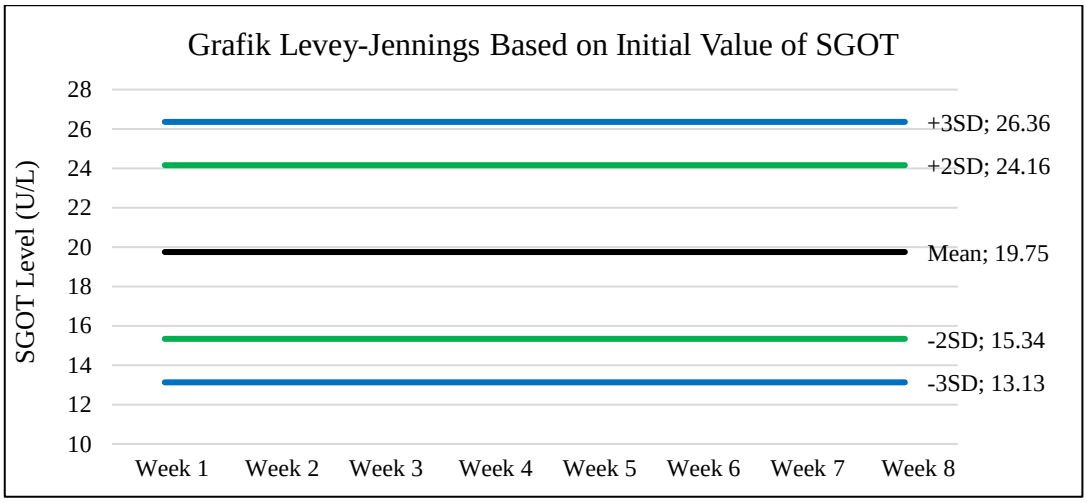

Figure 1. Levey-Jennings Graph Based on Initial Value of SGOT Levels Homemade Lyophilized Control Serum

Based on Figure 1, it shows that the warning limit (±2SD) of the control serum lyophilized homemade for SGOT examination. The upper warning limit (+2SD) is 24.16 U/L and the lower warning limit (-2SD) is 15.34 U/L. While, the control limit (±3SD) is the upper control limit is 26.36 U/L and the lower control limit is 13.13 U/L with an average value of 19.75 U/L.

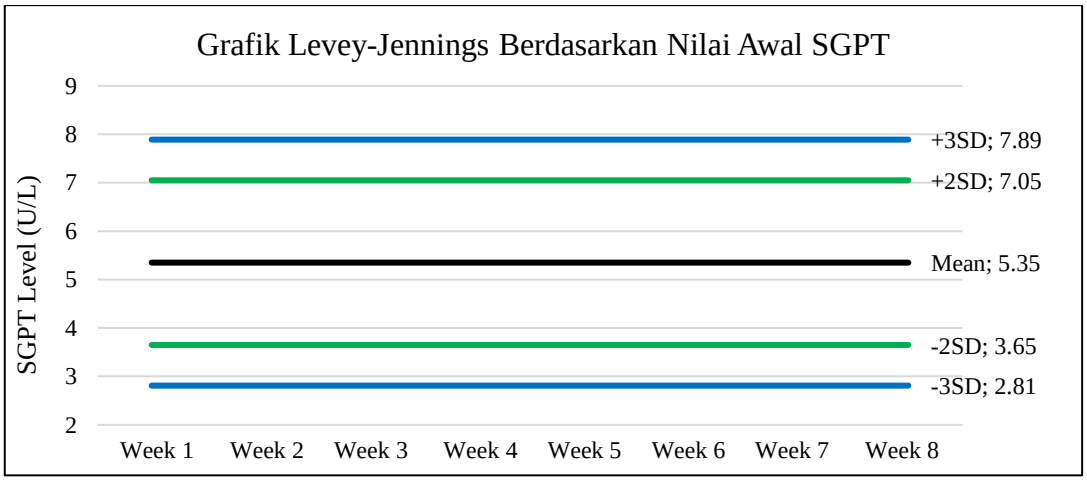

Figure 2. Levey-Jennings Graph Based on Initial Value of SGPT. Levels Homemade Lyophilized Control Serum

Based on Figure 2, it figured out that the warning limit (±2SD) of the control serum lyophilized homemade for SGPT examination, namely the upper warning limit (+2SD) is 7.05 U/L and the lower warning limit (-2SD) is 3.65 U/L. While the control limit (±3SD), the upper control limit is 7.89 U/L and the lower control limit is 2.81 U/L with an average value of 5.35 U/L

Table 2. Results of Homemade Lyophilized Control Serum Stability Examination SGOT Parameter at Temperature -2°C to -4°C

Week	1	2	3	4	5	6	7	8
Amount	102	102	102	95	94	93	92	89
Mean	20.4	20.4	20.4	19	18.8	18.6	18.4	17.8
SD	1.67	1.67	1.52	1	1.48	1.14	1.14	0.84
CV (%)	8.20	8.20	7.43	5.26	7.89	6.13	6.20	4.70

Table 2 showed the average data for weekly examination results, standard deviation, and CV stability of lyophilized homemade control serum SGOT parameters at storage temperature -2°C to -4°C for 8 weeks of storage.

Table 3. Results of Homemade Lyophilized Control Serum Stability Examination SGOT Parameter at Temperature (-20°C)

Week	1	2	3	4	5	6	7	8
Mean	19.8	20.2	19.6	20	19.6	18.8	18.6	18.2
SD	1.30	1.64	1.95	1.41	0.89	0.84	0.89	0.45
CV (%)	6.60	8.13	9.94	7.07	4.56	4.45	4.81	2.46

Table 3 showed the average data of weekly examination results, standard deviation, and CV stability of lyophilized homemade control serum SGOT parameters at storage temperature (-20 °C) for 8 weeks of storage.

Table 4. Results of Homemade Lyophilized Control Serum Stability Examination SGPT Parameters at Temperature -2°C to -4°C

Week	1	2	3	4	5	6	7	8
mean	4.62	4.5	4.4	4.32	4.18	4	3.98	3.96
SD	0.27	0.38	0.37	0.39	0.22	0.49	0.59	0.66
CV (%)	6.0	8.46	8.50	9.02	5.18	12.37	15.01	16.71

Table 4 showed the average data of weekly examination results, standard deviation, and CV stability of lyophilized homemade control serum SGPT parameters at storage temperature -2°C to -4°C for 8 weeks of storage.

Table 5. Results of Homemade Lyophilized Control Serum Stability Examination SGPT Parameters at Temperature (-20°C)

Week	1	2	3	4	5	6	7	8
mean	4.68	4.6	4.62	4.56	4.3	4.28	4.2	4.18
SD	0.29	0.33	0.33	0.38	0.37	0.27	0.47	0.61
CV (%)	6.30	7.21	7.24	8.29	8.54	6.48	11.16	14.68

Table 5 showed the average data of weekly examination results, standard deviation, and CV stability of lyophilized homemade SGPT control serum parameters at storage temperature (-20°C) for 8 weeks of storage.

In Tables 2 and 3, it presented that the mean data, standard deviation, and CV stability of lyophilized homemade SGOT control serum parameters during 8 weeks of storage at temperature -2°C to -4°C and (-20°C) which can be displayed in graphical in Figure 3.

In Tables 4 and 5, it presented that the mean data, standard deviation, and CV stability of lyophilized homemade SGPT control serum parameters during 8 weeks of storage at temperature -2°C to -4°C and -20°C, which can be displayed in graphical in Figure 4.

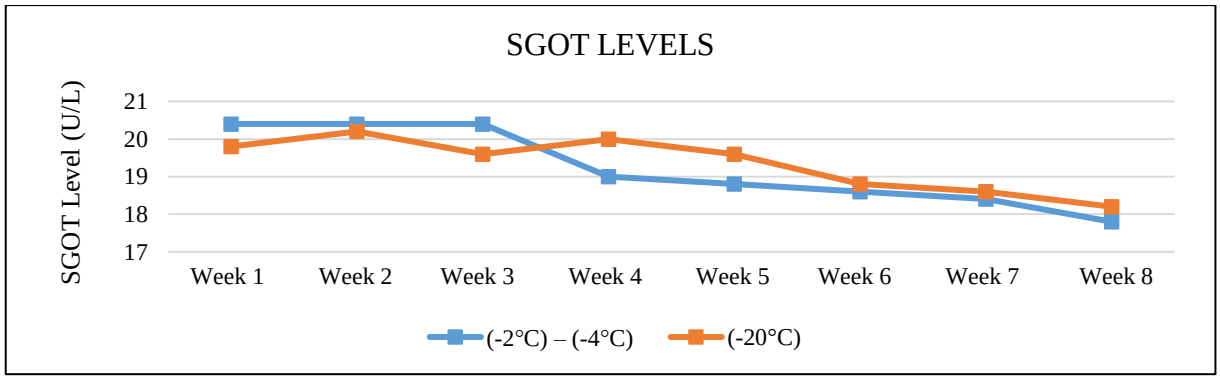

Figure 3. Graph of Weekly Average Results SGOT Levels of Homemade Lyophilized Control Serum

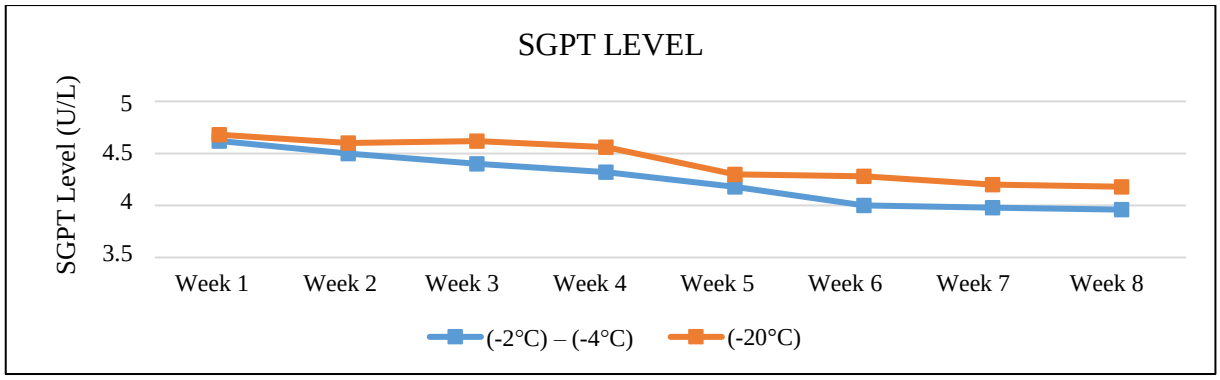

Figure 4. Graph of Weekly Average Results SGPT Levels of Homemade Lyophilized Control Serum

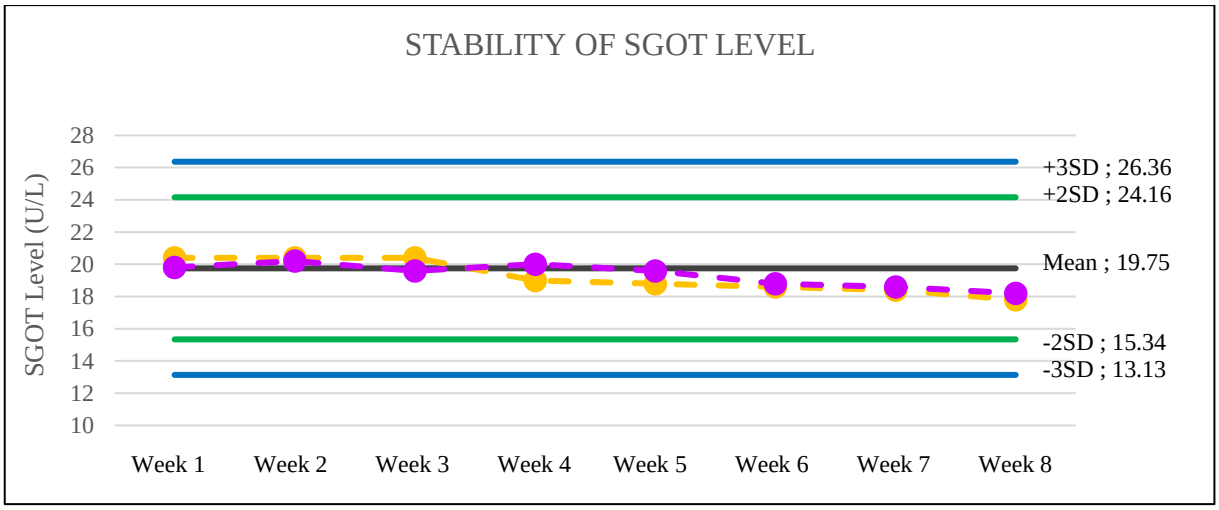

Figure 5. Results of Examination of Homemade Lyophilized Serum Parameters SGOT 8 Weeks of Storage on the Levey-Jennings Chart

Based on Figure 5, it displayed that the results of the examination of the stability of the homemade lyophilized control serum on the levels of SGOT stored in the freezer at a temperature of -2°C to -4°C and temperature (-20°C) with a storage time of 8 weeks were stable because they did not exceed the warning limit (±2SD) and control limit (±3SD).

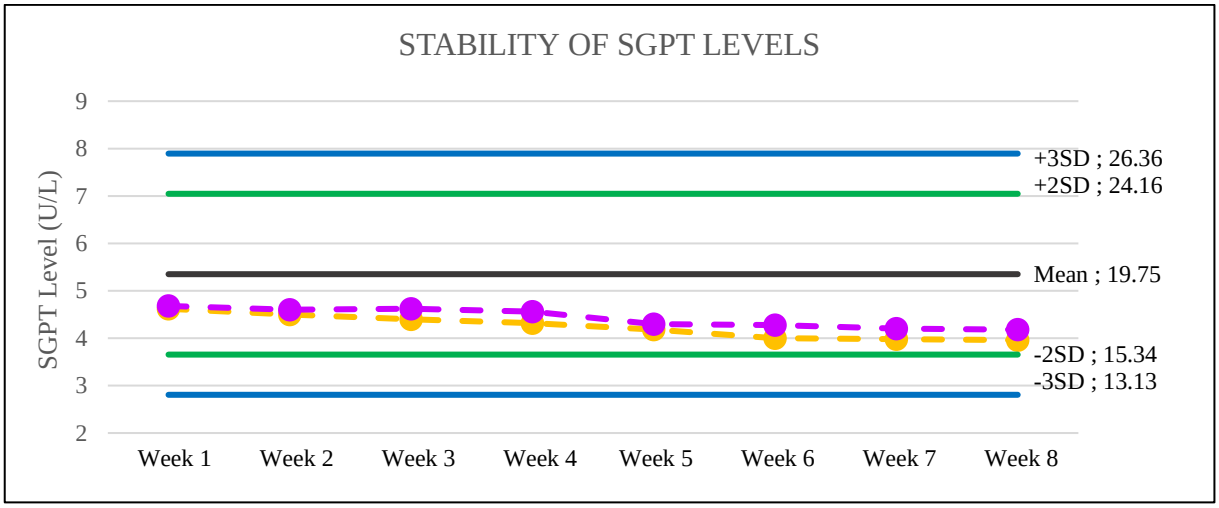

Figure 6. Results of Examination of Homemade Lyophilized Serum Parameters of SGPT 8 Weeks of Storage on the Levey-Jennings Chart

Based on Figure 6, it displayed that the results of the examination of the stability of the homemade lyophilized control serum on the levels of SGPT stored in the freezer at a temperature of -2°C to -4°C and temperature (-20°C) with a storage time of 8 weeks were stable because they did not exceed the warning limit (±2SD) and control limit (±3SD).

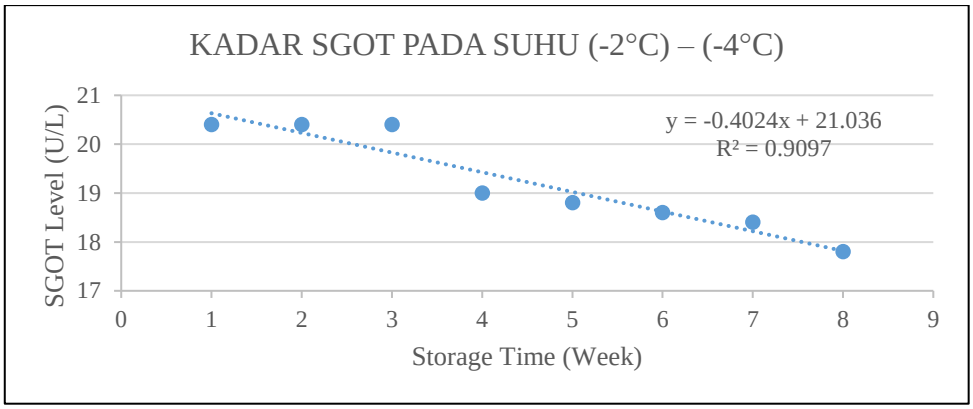

Figure 7. Graph of SGOT Linear Regression Test at Temperature (-2) to (-4) °C

Based on Figure 7, it presented that the SGOT level of homemade lyophilized control serum stored in a freezer temperature -2°C to -4°C for 8 weeks obtained the linear equation y = -0.4024x + 21.036 with R^2 = 0.9097. Value analysis R^2 (R Square) or the coefficient of determination is 0.9097, which means that the storage time has an effect of 90.97% on the stability of SGOT stored at -2°C to -4°C. While the rest (100% - 90.97% = 9.03%) influenced by other variables outside this regression equation or variables that are not examined (error component).

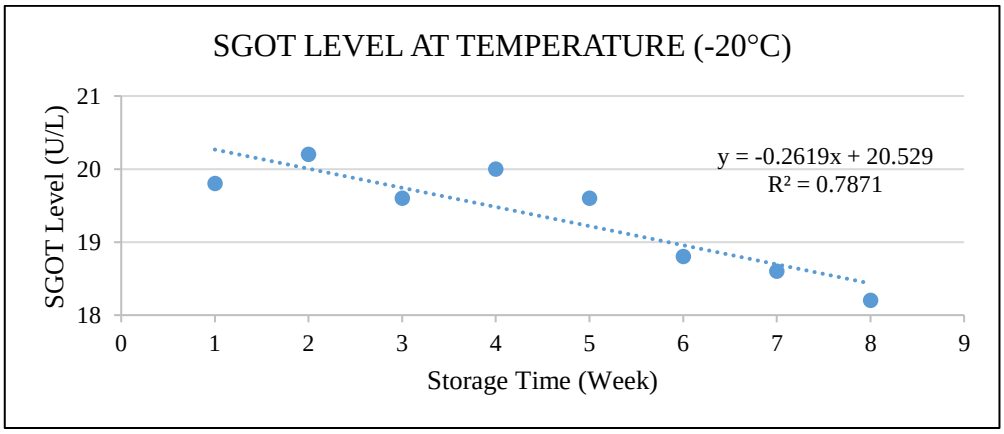

Figure 8. Graph of SGOT Linear Regression Test at Temperature (-20°C)

Based on Figure 8, it displayed that the SGOT level of homemade lyophilized control serum stored in a freezer temperature (-20°C) for 8 weeks obtained a linear equation y = -0.2619x + 20.529 with R^2 = 0.7871. Value analysis R^2 (R Square) or the coefficient of determination is 0.7871, which means that the storage time has an effect of 78.71% on the stability of SGOT stored at a temperature of (-20°C). While, the rest (100% - 78.71% = 21.29%) influenced by other variables outside this regression equation or variables that are not examined (error component).

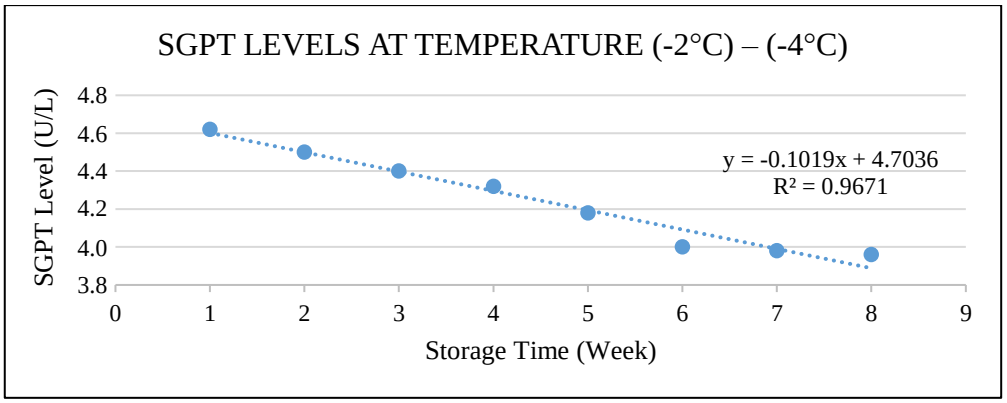

Figure 9. Graph of SGPT Linear Regression Test at Temperature -2°C to -4°C

Based on Figure 9, it presented that the SGPT level of homemade lyophilized control serum stored in a freezer temperature (-2) to (-4) °C for 8 weeks obtained a linear equation y = -0.1019x + 4.7036 with R^2 = 0.9671. Value analysis R^2 (R Square) or the coefficient of determination is 0.9671, which means that the storage time take effect 96.71% of the stability of SGPT stored at temperature -2°C to -4°C. While, the rest (100% - 96.71% = 3.29%) influenced by other variables outside this regression equation or variables that are not examined (error component).

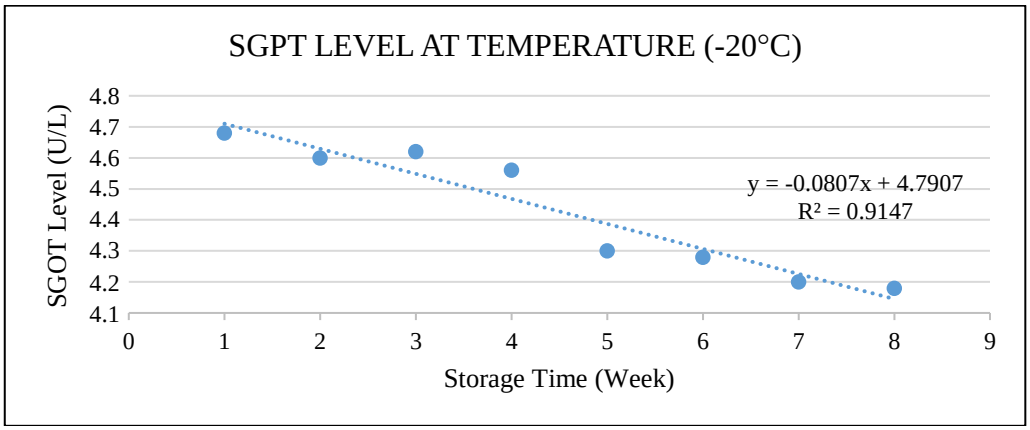

Figure 10. Graph of SGPT Linear Regression Test at Temperature -20°C

Based on Figure 10, it displayed that the SGPT level of homemade lyophilized control serum stored in a freezer temperature (-20°C) for 8 weeks obtained a linear equation y = -0.0807x + 4.7907 with R^2 = 0.9147. Value analysis R^2 or the coefficient of determination is 0.9147, which means that the storage time has an effect of 91.47% on the stability of SGPT stored at a temperature of -20 °C. While, the rest (100% - 91.47% = 8.53%) is influenced by other variables outside this regression equation or variables that are not examined (error component).

DISCUSSION

In the calculation of the average level of SGOT stored at a temperature of -2°C to -4°C and temperature (-20°C) entered on the Levey-Jennings' chart, there was no mean result from week 1 to week 8 that exceeds the warning limit (±2SD) and control limit (±3SD). At the level of SGPT stored at a temperature of -2°C to -4°C and temperature (-20°C) result average calculation included in the Levey-Jennings' chart, there was no mean result from week 1 to week 8 that crossed the warning limit (±2SD) and control limit (±3SD). These results indicate that homemade lyophilized control serum stored at (-2) to (-4) °C and (-20°C) for 8 weeks were stable for SGOT and SGPT parameters.

The results of CV calculations obtained on SGOT examination stored for 8 weeks at freezer temperature, namely (-2) to (-4) °C and temperature (-20°C), which showed different results (Table 4 and 5). The results of the CV calculation do not exceed the CCV limit, it is stable and the inspection method used is compatible for the SGOT parameter. CV calculation results on SGPT inspection at freezer temperature -2°C to -4°C and temperature (-20°C), which showed different results (Table 4 and 5). The results of the CV calculation on the SGPT examination did not exceed the CCV limit, so it was stable for the SGPT parameters and the examination method used was compatible.

The linear regression test showed the value of the coefficient of determination (R Square), which serves to find out what percentage of the influence is given by the independent variables (temperature and storage time) simultaneously on the dependent variable (SGOT and SGPT levels). R^2 has a value between 0 – 1 with the provision that the closer to the number one, the better. If the r square is 0.6, it means 60% of the distribution of the dependent variable is explained by the independent variable. The remaining 40% is explained by variables outside the independent variable (error component). If the value of the r square is small, it means that the error component is high.

Previous research stated there was no significant difference between SGOT and SGPT examination stored at -20°C. It concludes that prepared homemade lyophilized human serum can be used up to 9 months if stored at -20°C and 7 months at 2-8°C (2). The SGOT and SGPT levels decreased consistently in the time interval of sample storage, but the rate of change of the decrease in levels was considered more influential than storage temperature (16).

The previous results studies in the collected serum examined enzyme parameters, including SGOT and SGPT, which were stable for up to 30 days at a temperature of -20°C. The variation was not clinically significant. Decreased SGPT levels

can cause by loss of enzyme activity on long storage and interference by lactate dehydrogenase (LDH) in ALT (17).

All common clinical chemistry analytes examined, aside from serum amylase, showed adequate stability following up to 30 days of storage at -20°C. These results indicate that deep freezing at -20°C could serve as an effective tool for additional analyses at later time points along with research purposes, which require that samples be stored for longer until batch analysis can be conducted (18).

In this stability test, the factors that can affect the stability of SGOT and SGPT are storage temperature and storage time. The enzymatic reactions in SGOT and SGPT influenced by several factors, one of which is the influence of temperature, both the specimen storage temperature and the working temperature. At high temperatures, the protein portion of the enzyme begins to break down (denature) by that inhibited the reaction (18).

Storage temperature is an essential factor that causes the stability of the control material. The control serum stability temperature when it is stored at -20℃ is relatively better than storage in the refrigerator (2).

The working reagent factor and the storage time of the reagents can also affect the results of the examination, due to how the working reagents are used when mixing, and the working reagents that have been mixed and stored, even though the expiration date on the reagents have not exceeded the time limit stated in the reagent KIT or guidelines work (19).

Another factor that can affect the measurement of the level of examination parameters is turbidity because turbidity in the serum can affect the measurement absorbance. The process of making homemade lyophilized control serum can also affect the results of the examination. Incomplete centrifugation and contamination are unavoidable but can be reduced to a minimum (20). The type of freezer used in the laboratory to determine the level of stability of pooled sera. Most small laboratories use household refrigerators whose freezer temperature is higher than the freezer for serum storage (21).

Each laboratory has standard procedures and different quality reagents and instruments, so further research is needed if you want to use the homemade lyophilized serum as a commercial control serum with different types of parameters and for a longer time. Many things can affect the results of control serum or lyophilized homemade at the pre-analytical stage, which consists of patient preparation, specimen identification, specimen collection and storage, specimen handling, specimen

delivery, specimen processing and preparation, and analysis consisting of specimen examination, equipment maintenance and calibration, reagent quality test, accuracy test, and post-analytic, which consists of writing, interpreting, and reporting results (3).

The advantage of using homemade lyophilized serum is that it is easy to obtain and relatively inexpensive, while commercial control serum is expensive. However, the stability of commercial control serum is longer than the homemade lyophilized serum, especially in certain analytes. With proper storage and handling homemade lyophilized serum can be used instead of commercial control serum for internal and external quality assurance (20).

CONCLUSIONS

There is an effect of storage time on the stability of SGOT and SGPT levels stored in the freezer at -2°C to -4°C and -20°C for 8 weeks, but the results of SGOT and SGPT stored for 8 weeks still meet the requirements Levey-Jennings control chart.

AUTHOR CONTRIBUTIONS

Kadek Profit: original draft writing and reviewing and data curation, investigation and data collection. Anik Handayati: conceptualization, methodology and investigation.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Hedayati M, Razavi SA, Boroomand S, Kheradmand Kia S. The impact of pre-analytical variations on biochemical analytes stability: A systematic review. Journal of Clinical Laboratory Analysis. John Wiley and Sons Inc; 2020; 34. DOI: 10.1002/jcla.23551
2. Carey RB, Bhattacharyya S, Kehl SC, Matukas LM, Pentella MA, Salfinger M, Schuetz AN. Practical guidance for clinical microbiology laboratories: implementing a quality management system in the medical microbiology laboratory. Clinical microbiology reviews. 2018; 31(3), e00062-17. DOI: 10.1128/CMR.00062-17
3. Kaur V, Kare PK, Madaan H. Quality Control in a Clinical Laboratory In book: Advances in Biochemistry & Applications in Medicine. 1st ed. Vol. 4. USA: Open Access eBooks; 2018; 1–9.
4. Broadhurst D, Goodacre R, Reinke SN, Kuligowski J, Wilson ID, Lewis MR, Dunn WB. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. Metabolomics. 2018; 14: 1-17. DOI: 10.1007/s11306-018-1367-3
5. Oosterhuis WP, Bayat H, Armbruster D, Coskun A, Freeman KP, Kallner A, Theodorsson E. The use of error and uncertainty methods in the medical laboratory. Clinical Chemistry and Laboratory Medicine (CCLM). 2018; 56(2): 209-219. DOI: 10.1515/cclm-2017-0341
6. Salma FD, Rahayu IG, Kurnaeni N, Rinaldi SF. Cost-Effectiveness Analysis (Cea) Commercial Control Material And Patient Pool Serum. J Health Res. 2017;(11):298–3. DOI:10.34011/juriskesbdg.v11i1.797
7. Lee A, Kim HJ. Production and Performance Evaluation of External Quality Assessment Materials for Human Epididymis Protein 4 Assay. Journal of Laboratory Medicine and Quality Assurance. 2019; 41(3): 153-160. DOI: 10.15263/jlmqa.2019.41.3.153
8. Danilenko U, Vesper HW, Myers GL, Clapshaw PA, Camara JE, Miller WG. An updated protocol based on CLSI document C37 for preparation of

off-the-clot serum from individual units for use alone or to prepare commutable pooled serum reference materials. Clinical Chemistry and Laboratory Medicine (CCLM). 2020; 58(3): 368-374. DOI: 10.1515/cclm-2019-0732

9. Lestari WS, Karwiti W, Latifah A, Listiani Y, Harianja SH. Sera Pooled Stability as a SGPT Control Material With Storage Time and Temperature Variation. J Med Lab Sci. 2022;2(1):33–9. DOI: 10.36086/medlabscience.v2i1
10. Handayati A. Design of Home Made Lyophilized Serum as a Control Material for Internal and External Quality Control in Clinical Laboratories. Health Notions' 2022; 6(12): 502-507. DOI: 10.33846/hn61203
11. Wu AH, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Llanos J, Apple FS. Clinical laboratory practice recommendations for the use of cardiac troponin in acute coronary syndrome: expert opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. Clinical Chemistry. 2018; 64(4): 645-655. DOI: 10.1373/clinchem.2017.277186
12. West T, Kirmess KM, Meyer MR, Holubasch MS, Knapik SS, Hu Y, Yarasheski KE. A blood-based diagnostic test incorporating plasma Aβ42/40 ratio, ApoE proteotype, and age accurately identifies brain amyloid status: findings from a multi cohort validity analysis. Molecular neurodegeneration. 2021; 16(1): 1-12. DOI: https://doi.org/10.1186/s13024-021-00451-6
13. Tambse V. Study of the Stability of various Biochemical Analytes in Pooled Sera preserved at 4-8oC. Asian Journal of Biomedical and Pharmaceutical Sciences. 2015;05(48): 38–9. DOI: 10.15272/ajbps.v5i48.722
14. Prasad P, Kumar R, Kumar S. Effect of Storage on the Stability of Enzyme Activities in Pooled Serum. Int J Res Rev. 2019;6(7):121–6. https://www.ijrrjournal.com/IJRR_Vol.6_Issue.7_July2019/IJRR0017.pdf. DOI: 10.52403/ijrr
15. Gómez-Rioja R, Segovia Amaro M, Diaz-Garzón J, Bauçà JM, Martinez Espartosa D, Fernández-Calle P. Analytical Quality Commission of the Spanish Society of Laboratory Medicine (SEQCML). A protocol for testing the stability of biochemical analytes. Technical document. Clinical Chemistry and Laboratory Medicine (CCLM). 2019; 57(12): 1829-1836. DOI: 10.1515/cclm-2019-0586
16. Kulkarni S, Pierre SA, Kaliaperumal R. Efficacy of Pooled Serum Internal Quality Control in Comparison with Commercial Internal Quality Control in Clinical Biochemistry Laboratory. J Lab Physicians. 2020;12(3): 191–5. DOI: 10.1055/s-0040-1721151
17. Alegre E, Varo N, Fernández-Calle P, Calleja S, González Á. Impact of ultra-low temperature long-term storage on the preanalytical variability of twenty-one common biochemical analytes. Clinical Chemistry and Laboratory Medicine (CCLM). 2022; 60(7): 1003-1010. DOI: 10.1515/cclm-2022-0063
18. Dupuy AM, Cristol JP, Vincent B, Bargnoux AS, Mendes M, Philibert P, Badiou S. Stability of routine biochemical analytes in whole blood and plasma/serum: focus on potassium stability from lithium heparin. Clinical Chemistry and Laboratory Medicine (CCLM). 2018; 56(3): 413-421. DOI: 10.1515/cclm-2017-0292
19. Warsyidah AA. Stability Test of Working Reagent Storage After Mixing at Room Temperature Against Blood Urea Examination. Media Laboran. 2016;6:1–4. https://uit.e-journal.id/MedLAb/article/view/299
20. Pawlik-Sobecka L, Sołkiewicz K, Kokot I, Kiraga A, Płaczkowska S, Schlichtinger AM, Kratz EM. The influence of serum sample storage conditions on selected laboratory parameters related to oxidative stress: a preliminary study. Diagnostics. 2020; 10(1): 51. DOI: 10.3390/diagnostics10010051
21. Maulidiyanti ETS The Effect of Storage Time on Pooled Sera in Freezers on the Quality of Clinical Chemical Examination. J Med Lab Sci. 2021;4(2):78–82. DOI: 10.21070/medicra.v4i2.1613