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Stability of Homemade Lyophilized Serum for use as a Quality Control Material for Cholesterol and Triglyceride Parameters

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ABSTRACT
Important control materials are used to achieve the quality of laboratory examinations. Control materials can be obtained from commercial serum or can be made yourself in the form of pooled sera. The control material that is usually used in clinical laboratories is a commercial control material in the form of lyophilisate. However, the price is quite expensive, so it is less efficient to use in laboratories that have a small average number of examinations. The self-made lyophilized control material can be used as an alternative to replace the manufacturer's control which is quite expensive. This study aimed to analyze the stability of homemade serum lyophilisate as a control material for cholesterol and triglyceride parameters for 8 weeks. This research is an experimental study using a time series design research conducted at the Clinical Chemistry Laboratory, Department of Medical Laboratory Technology, Poltekkes, Ministry of Health, Surabaya, in October 2021-May 2022. Blood serum is processed using the freeze dried technique to obtain a lyophilized form. Lyophilized serum was stored at 2-8°C and dissolved using aquabides every week to check cholesterol and triglyceride levels. In the Levey-Jennings chart cholesterol and triglyceride levels did not deviate from 2SD during storage. Regression test showed that storage time had an effect of 0.4% on cholesterol levels and 55% effect on triglyceride levels. The results of the study concluded that the homemade serum lyophilisate was stable for 8 weeks of storage.
Keywords: Internal Quality Assurance (IQA); Homemade lyophilized serum; Cholesterol levels; Triglyceride levels

BACKGROUND
Clinical laboratories have an important meaning in terms of diagnostics. Data from laboratory tests are important information used to establish a disease diagnosis (Siregar et al., 2018). The implementation of a clinical laboratory is said to be good if it carries out activities to improve and strengthen the quality of laboratory examination results. Laboratory quality assurance activities include Internal Quality Assurance (PMI) and External Quality Assurance (PME), one of which is carried out by examining control serum (Permenkes, 2013).

An important control serum is used to achieve the quality of laboratory tests. Control serum can be obtained commercially or can be made yourself in the form of pooled sera. Control serum commonly used in clinical laboratories is control serum in liquid form and lyophilized or freeze-dried (Siregar et al., 2018). However, many developing countries are disadvantaged by the unavailability and high cost of commercial control materials. In addition, this control material is derived from bovine serum which may not be the same as human serum (Jamtsho, 2013).

Homemade control material in the form of pooled sera is more cost-effective and can still maintain quality assurance in the laboratory (Kulkarni et al., 2020). Pooled sera can be used as a substitute for commercial control serum for internal and external quality assurance with proper storage and handling (Handayati et al., 2014). However, the lyophilized form control material is more stable and durable than the liquid form, and it is easier to transport. Homemade lyophilized human serum used as a Quality Control (QC) material will save costs for use in developing countries. Self-made lyophilized serum without the addition of stabilizers and additives can be stable for 7 months at a temperature of 2-8°C and stable for up to 9 months at a temperature of -20°C (Jamtsho, 2013).

The control material that is usually used in clinical laboratories is a commercial control material in the form of lyophilisate. However, this control material is sometimes made from bovine serum which may not be the same as human serum, besides that the price is also very expensive, making it less efficient to use for laboratories that have a small average number of examinations. The stability of homemade human serum lyophilisate can be determined by measuring cholesterol and triglyceride parameters, this parameter was chosen because it is a parameter that is often requested for routine examination in clinical chemistry laboratories. This study aimed to analyze the stability of homemade serum lyophilisate as a control material for cholesterol and triglyceride parameters stored at 2-8°C for 8 weeks.

RESEARCH METHODS

The method used in this research is experiment with time series design research. This research was conducted in October 2021 – May 2022 at the Clinical Chemistry Laboratory, Department of Medical Laboratory Technology, Poltekkes, Ministry of Health, Surabaya and in several reference laboratories. Serum samples were collected from 10 respondents who had cholesterol and triglyceride levels in the normal range, the serum obtained was not hemolyzed, not lipemic, not icteric and free of infectious diseases such as HIV and HBsAg. Serum that met the criteria was collected and homogenized using a vortex. The serum collection was separated in 15 vials each containing 3 mL of serum and then freeze-dried using the freeze dry technique. Sample homogeneity examination was carried out on a number of vials containing serum lyophilisate which were selected at random and examined in several reference laboratories. The homemade lyophilized serum was stored at 2-8°C and checked for cholesterol and triglyceride levels every 0 weeks; 1 week; 2 weeks; 3 weeks; 4 weeks; 5 weeks; 6 weeks; 7 weeks; and 8 weeks. Cholesterol parameters were checked using the CHOD-PAP method, read with a photometer at a wavelength of 500 nm with units of mg/dL. Triglyceride parameters were checked using the GPO-PAP method, read with a photometer at a wavelength of 500 nm with units of mg/dL.

RESULTS AND DISCUSSION

This study begins with conducting an initial examination as a preliminary test to determine the basic value which is then used as a reference value for subsequent examinations, and the results obtained can be seen in table 1 as follows:

Table 1. Initial examination of cholesterol and triglyceride levels

Parameter	Mean	SD	CV	CCV
Cholesterol	163,2	3,39	2,07	7,6
Triglyceride	60,9	3,41	5,6	7,6

Table 1 shows the results of the average calculation of cholesterol levels in homemade lyophilized serum stored at 2-8°C for 8 weeks is 163.2 mg/dL, standard deviation of

cholesterol levels is 3.39 mg/dL, and CV (Coefficient Variation) cholesterol level is 2.07%. Meanwhile, the results of the average calculation of triglyceride levels in homemade lyophilized serum stored at 2-8°C for 8 weeks was 60.9 mg/dL, the standard deviation of triglyceride levels was 3.41 mg/dL, and CV (Coefficient of Variation) triglyceride levels was 5.6%. The value of CCV (Chosen Coefficient of Variation) of cholesterol and triglyceride parameters in External Quality Assurance for clinical chemistry is 7.6%, so that the triglyceride level in homemade lyophilized serum stored at 2-8°C for 8 weeks does not exceed the CCV limit.

After the preliminary test was carried out, it was continued with stability checks on homemade serum lyophilisate stored at 2-8°C for 8 weeks. The examination was carried out by dissolving one vial once a week, then continued with the examination of cholesterol and triglyceride levels, the data obtained from the cholesterol and triglyceride examination results which can be seen in table 2 as follows:

Table 2. Cholesterol level examination for 8 weeks

Time (week)	1	2	3	4	5	6	7	8
Mean	163,5	169	168,5	163,16	162,83	164,16	166,66	166,5
SD	1,64	3,40	3,39	2,22	1,72	2,63	2,87	3,08
CV%	1	2,01	2,01	1,36	1,05	1,60	1,72	1,85

Table 2 above shows the results of checking cholesterol levels in homemade serum lyophilisate stored at 2-8°C for 8 weeks, the coefficient of variation (CV) value on cholesterol levels during storage from week 1 to week 8 did not exceed maximum limit for cholesterol CCV. Meanwhile, the results of examination of triglyceride levels can be seen in table 3 as follows:

Table 3. Triglyceride level examination for 8 weeks

Time (week)	1	2	3	4	5	6	7	8
Mean	61,83	64,66	62,33	60,83	63,16	65,5	67,33	67,16
SD	2,71	1,86	3,72	3,18	2,71	4,23	1,96	2,71
CV%	4,38	2,87	5,97	5,24	4,29	6,45	2,92	4,04

Table 3 above shows the results of examination of triglyceride levels in homemade lyophilized serum stored at a temperature of 2-8°C for 8 weeks, the coefficient of variation (CV) value on examination of triglyceride levels from week 1 to week 8 does not exceed the specified limit. The maximum CCV of triglycerides. Based on table 3, the average results of cholesterol levels in homemade serum lyophilisate for 8 weeks of storage made in the form of a Levey-Jennings graph can be seen in Figure 1 as follows:

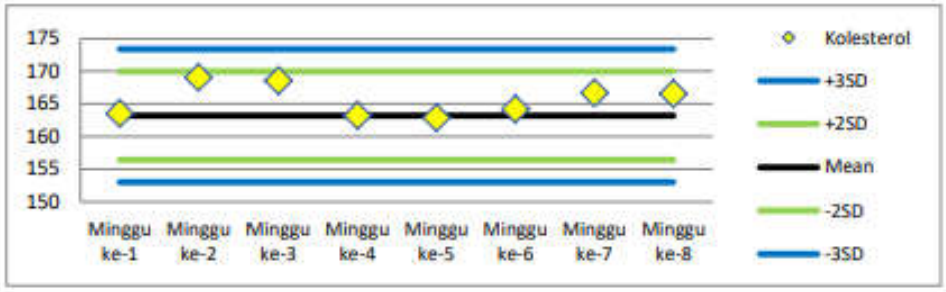

Figure 1. Levey-Jenning chart of cholesterol levels

Figure 1 shows a Levey-Jennings graph of cholesterol levels in serum lyophilized for 8 weeks of storage, the average results of examination of cholesterol levels from week 1 to week 8 of storage did not deviate from the limit of ±2SD, this indicates that cholesterol levels in serum lyophilisate Homemade is stable for 8 weeks of storage. Meanwhile, the average results of examination of triglyceride levels in lyophilisate can be seen in Figure 2 as follows:

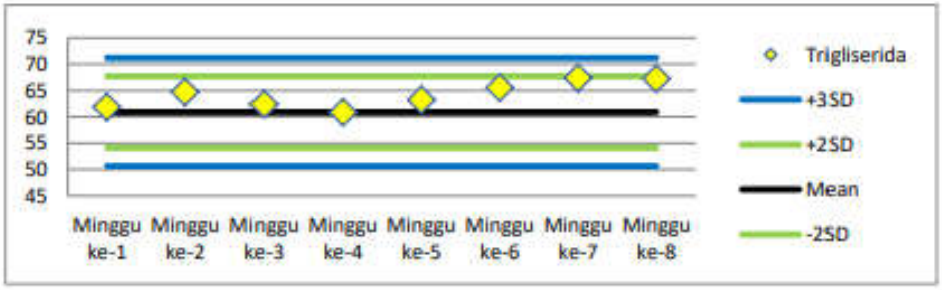

Figure 2. Levey-Jenning graph of triglyceride levels

Figure 2 shows a Levey-Jennings graph of triglyceride levels in lyophilized serum during 8 weeks of storage, the mean results of examination of triglyceride levels from week 1 to week 8 of storage did not deviate from the ±2SD limit, this indicates that triglyceride levels in serum lyophilisate Homemade is stable for 8 weeks of storage.

Regression test is used to predict the influence of the independent variable on the dependent variable. In this study, storage time acted as an independent variable, while cholesterol and triglyceride levels acted as dependent variables. The results of the examination of cholesterol levels in homemade lyophilized serum stored at a temperature of 2-8°C can be displayed in the form of a linear regression test graph which can be seen in Figure 3 as follows:

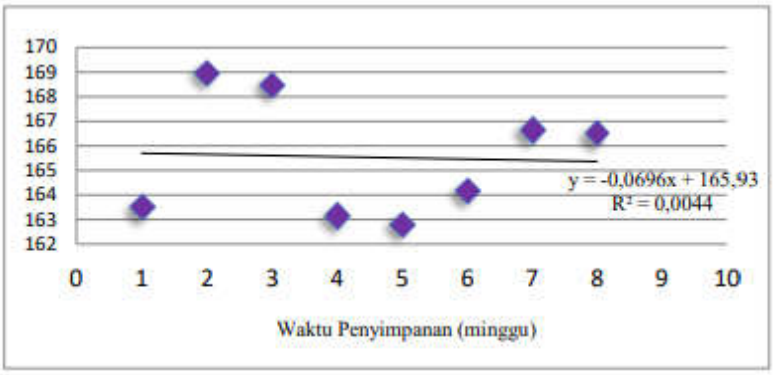

Figure 3. Linear regression graph of cholesterol levels

Figure 3 shows the results of the linear regression equation on cholesterol levels, namely y = -0.0696x+165.93; R^2 = 0.0044. The regression coefficient value has a negative value of (-0.0696), this value indicates that there is a negative (opposite direction) effect between storage time and cholesterol levels. The value of the coefficient of determination (R^2) is 0.0044 or 0.4%, meaning that the storage time has an effect of 0.4% on the stability of cholesterol levels. Meanwhile, the results of examination of triglyceride levels in homemade lyophilized serum stored at a temperature of 2-8°C can be displayed in the form of a linear regression test graph which can be seen in Figure 4 as follows:

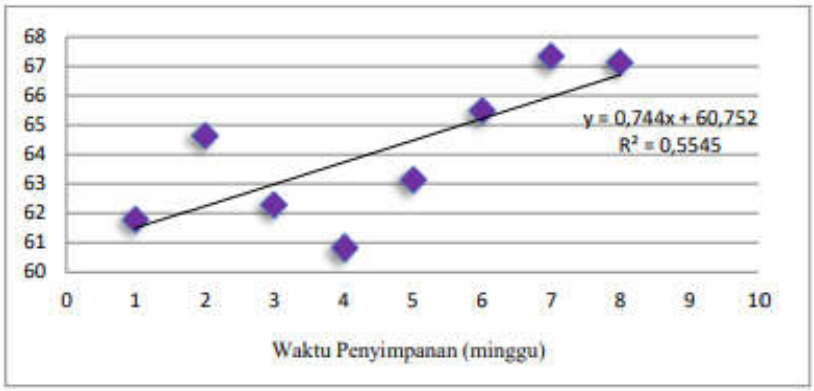

Figure 4. Linear regression graph of triglyceride levels

Figure 4 shows the results of the linear regression equation on triglyceride levels, namely y = 0.744x+60,752; R^2 = 0.5545. The regression coefficient value has a positive value of (0.744), this value indicates that there is a positive (unidirectional) effect between storage time and triglyceride levels. The value of the coefficient of determination (R^2) is 0.5545 or 55%, meaning that the storage time has an effect of 55% on the stability of the content triglycerides.

Based on the results of the stability test of homemade lyophilized serum stored at a temperature of 2-8°C for 8 weeks on cholesterol and triglyceride levels, the Coefficient of variation (CV) of cholesterol and triglyceride levels did not exceed the limits of the CCV cholesterol and triglycerides, which was 7.6 %. Thus, the results of examination of cholesterol and triglyceride levels in homemade serum lyophilisate during 8 weeks of storage had variations in results that were not much different.

The Levey Jennings chart shows that the distribution of cholesterol and triglyceride test results is in the ± 2SD area for 8 weeks of storage and does not follow the Westgard law prohibition, meaning that the results of cholesterol and triglyceride levels in homemade serum lyophilisate are well controlled (in control) for 8 weeks. storage. This indicates that the freeze-dried process was running well and the serum was stored at the appropriate temperature. The results of the examination of cholesterol and triglyceride levels in homemade lyophilized serum stored at 2-8°C for 8 weeks showed varying results. Examination of cholesterol and triglyceride levels in homemade serum lyophilisate increased and decreased levels during the 8-week storage period, this was concluded due to variations in results because the distribution of data still showed in the ±2SD area on the Levey-Jennings chart.

Based on the data obtained, data analysis was carried out using linear regression test on serum lyophilisate and the results of the cholesterol level regression equation showed that there was a negative (opposite direction) effect between storage time and cholesterol levels. The value of the coefficient of determination (R^2) is 0.0044 or 0%, meaning that the storage time has an effect of 0.4% on the stability of cholesterol levels. Meanwhile, the results of the linear regression test of triglyceride levels in homemade serum lyophilisate showed that there was a positive (unidirectional) effect between storage time and triglyceride levels. The value of the coefficient of determination (R^2) is 0.5545 or 55%, meaning that storage time has an effect of 55% on the stability of triglyceride levels. The coefficient of determination was used to measure the effect of storage time on the stability of cholesterol and triglyceride levels. The value of the coefficient of determination is between 0 and 1, if R^2 is getting closer to 1, it means that the independent variable (storage time) has more effect on the dependent variable (cholesterol and triglyceride levels).

The results of this study are in accordance with previous studies, that there was no effect of storage time on cholesterol levels in pooled sera stored at a temperature of -7º to -4ºC, the average cholesterol levels in pooled sera were quite stable for 8 weeks of storage (Handayati et al., 2014). Other studies concluded that cholesterol and triglyceride levels in pooled sera stored at -20°C were stable up to 30 days of storage (Kachhawa et al., 2017).

The stability of cholesterol and triglyceride levels is not only influenced by storage time, but there are other factors that can affect their levels. Other influencing factors are the cleanliness of all the tools used, improper pipetting, staff skills, air bubbles in the equipment, imperfect homogeneity, inappropriate incubation time and temperature (Permenkes, 2013). In addition, contamination of blood cells in serum samples can also affect the results, because the blood cells undergoing hemolysis during storage time will affect the reading of the results resulting in increased cholesterol levels (Hartini & Suryani, 2016).

Control serum that is usually used in clinical laboratories is commercial control serum in the form of lyophilisate, commercial control serum that has never been opened and stored at 2-8°C can still be used until the expiration date specified by the manufacturer, while control serum that has been dissolved and stored at -15°C can be used for up to one month, with the condition that it must be stored in the original bottle and in a dark place (Handayati et al., 2014). Self-made lyophilized serum without the addition of stabilizers and additives can be stable for 7 months at a temperature of 2-8° C and stable for up to 9 months at -20°C for glucose, BUN, keatinine, AST, ALT, ALP, TP, bilirubin, and albumin parameters (Jamtsho, 2013).

CONCLUSION AND RECOMMENDATION

Based on the results of the research and data analysis conducted, it was found that the CV value of cholesterol and triglyceride levels did not exceed the maximum CCV limit, the Levey-Jennings chart shows that cholesterol and triglyceride levels did not deviate from the ±2SD limit and did not follow the prohibition of the Westgard-Multirule law. Thus, it can be concluded that the lyophilized serum made by itself using the freeze dry technique stored at 2-8°C can be used as an alternative to commercial control serum for cholesterol and triglyceride testing because it has good accuracy and precision for 8 weeks.

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