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Design of Home Made Lyophilized Serum as a Control Material for Internal and External Quality Control in Clinical Laboratories

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ABSTRACT

Important control materials are used to achieve laboratory test quality. Control materials can be obtained from commercial serum or can be made independently in the form of pooled serum. Control materials commonly used in clinical laboratories are commercial control materials in lyophilized form. However, the price is quite expensive, so it is less efficient to use in laboratories where the average number of examinations is small. Homemade lyophilized control agents can be used as an alternative to expensive factory controls. This study aims to analyze the stability of homemade lyophilized serum as a control material for the parameters of glucose, uric acid, cholesterol, triglycerides, BUN, creatinine, SGOT and SGPT stored for 7 months. This research was an experimental study with a time series research design conducted at the Clinical Chemistry Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Surabaya, in January 2022 to September 2022. Blood serum was processed using the freeze dry technique to obtain a lyophilized form. Lyophilized serum was stored at 2-8°C in the freezer, dissolved using aquabides and checked every month, for 7 months. The results of glucose, uric acid, SGOT, SGPT, creatinine, cholesterol, and triglyceride tests did not cross the CV line (CCV). The Levey Jennings graph shows that the results of glucose, uric acid, SGOT, SGPT, creatinine, BUN, cholesterol, and triglycerides were in the mean ± 2 SD and did not follow Westgard's law restrictions, meaning that the results of homemade lyophilized serum were well controlled (in control) for 7 months of storage. It can be concluded that the homemade lyophilized serum is stable over 7 months of storage.

Keywords: control material; homemade lyophilized serum; control serum stability

INTRODUCTION

The health laboratory is one of the health services for the community in helping to establish a diagnosis, monitoring treatment results, and determining the prognosis of a disease. 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 quality assurance or Quality Control (QC), including Internal Quality Control (IQC) and External Quality Assessment scheme (EQAS). [(1)]

Internal Quality Consolidation is a quality control procedure that is carried out continuously by the laboratory itself. This is done by purchasing a commercial control serum, analyzed and self-evaluated. IQC, in this case, can be a benchmark for whether the laboratory results released are reliable enough to be released to doctors. External quality assurance is a procedure or program carried out by agencies outside the laboratory to objectively assess or test, and compare the results issued by several laboratories. The main objective of external laboratory quality assurance is to enforce comparability or similarity between laboratories in an area, within a country, or between countries. In External Quality Assurance in the field of Clinical Chemistry, the organizer sends control material, namely control serum to participants, then the participants analyze the control serum together and the results are sent back to the organizer for evaluation. [(2,3)]

Control material is an important part of the implementation of Internal Quality control (IQC) and External Quality assessment scheme (EQAS). Control material is examined together with the test sample for the purpose of assessing the accuracy and precision of laboratory results. Material control is a tool in the field of quality assurance, both the implementation of internal quality control (IQC) and external quality control / External Quality assessment scheme (EQAS). [(4)]

Laboratories in developing countries, like clinical laboratories in Indonesia, face various limitations. In Indonesia, the management of the external quality control program is carried out by the government together with laboratory organizations, funded by the participants. Limited trained human resources, the absence of national standards, inadequate training facilities and laboratory equipment, and control materials that still have to be imported are the main obstacles faced by clinical laboratories in developing countries, including Indonesia. The continuous use of commercial controls is not economical in many countries because of the high cost of these controls. The high cost of commercial control materials is a limiting factor in efforts to carry out internal and external quality control in the field of clinical chemistry. [5,6]

The control material must be stable and can be inspected over a long period of time. In clinical chemistry laboratories, the stability of control serum is very important in order to be able to assess the performance of a laboratory, including the quality of equipment and reagents. Freeze-dried control serum or lyophilized control serum is the most common type of commercial control serum on the market, because it is more stable than liquid control serum. Commercial control serum lyophilized form that has never been opened and stored at refrigerator temperature, is stable for 1-2 years and can be used until the expiration date, while control serum that has been dissolved and stored at freezer temperature can still be used for up to one month. Self-made liquid form control material in the form of pooled sera is more cost-effective and can still maintain quality assurance in the laboratory [5,7]. Pooled sera can be used as a substitute for commercial control serum for internal and external quality assurance with proper storage and handling (8). However, the lyophilized form control material is more stable and durable than the liquid form, and it is easier to transport. Lyophilisate serum made by yourself without the addition of stabilizers and additives can be stable for 7 months at 2-8°C and stable for up to 9 months at -20°C. [8]

The purpose of this study was to test the stability of homemade control serum, known as homemade Lyophilized control serum, so that it can be used as an alternative to commercial control serum for internal and external quality assurance in clinical chemistry laboratories.

METHODS

The type of research used was experimental study. The design of this study was a time series control group design by analyzing the stability of homemade lyophilized serum stored for 7 months at refrigerator temperature and freezer temperature on the parameters of glucose, uric acid, cholesterol, triglycerides, BUN, creatinine, SGOT and SGPT. This research was conducted in January 2022 to September 2022 at the Clinical Chemistry Laboratory, Department of Technology, Medical Laboratory, Poltekkes Kemenkes Surabaya and in several reference laboratories.

Collection of pooled sera was carried out by taking healthy voluntary blood, as many as 10 respondents had glucose, cholesterol, uric acid, triglycerides, BUN, creatinine, SGOT and SGPT levels within the normal range. Furthermore, pooled sera screening is carried out, namely by testing pooled sera with HbsAg and HIV tests, the serum obtained must be free of infectious diseases, cannot be hemolyzed and lipemic. Serum that meets the criteria is collected and homogenized using a vortex. The serum collection was divided into 30 vials each containing 3 mL of serum and then freeze-dried using the freeze dry technique. Checking the homogeneity of the samples was carried out on 10 vials of serum lyophilisate samples which were randomly selected and examined in several reference laboratories. Homemade lyophilisate serum was stored at 2-8°C and freezer temperature, then examined levels of glucose, uric acid, cholesterol, triglycerides, BUN, creatinine, SGOT and SGPT every 0 months; 1 month ; 2 months; 3 months; 4 months; 5 months; 6 months; and 7 months. Glucose parameters were examined using the GOD-PAP method, uric acid using the uricase-PAP method, cholesterol using the CHOD-PAP method, triglycerides using the GPO-PAP method, BUN using the Urea UV method, creatinine using the Jaffe method and SGOT-SGPT using the kinetic method.

RESULTS

Table 1. Preliminary examination results (target value)

Parameter	Mean	SD	CV	CCV
Glucose	64.4	2.67	4.15	8.9
Uric acid	3.97	0.24	5.94	7.7
SGOT	19.25	1.84	9.58	12.5
SGPT	15.3	1.53	9.99	17.3
BUN	7.28	0.46	6.31	5.7
Creatinine	0.68	0.05	6.74	7.7
Triglyceride	60.9	3.41	5.61	7.6
Cholesterol	163.2	3.39	2.08	7.6

This study begins with conducting an initial examination as a preliminary test to determine the base line value or target value, which is then used as a reference value for subsequent examinations. Table 1 shows the results of calculating the average of homemade lyophilized serum for 7 months. The results of glucose, uric acid, SGOT, SGPT, creatinine, cholesterol, and triglyceride tests did not exceed the CV limit (CCV). While the BUN parameter in homemade lyophilized serum has a CV value of 6.31 and a CCV of 5.7, so that the results of examining BUN levels exceed the CV limit.

Based on table 1 the mean and standard deviation (SD) of the initial examination results as a preliminary test can be calculated as a range of mean ± 2SD and mean ± 3SD to determine the upper and lower limits in the range of ±2SD and ±3SD of the mean. The results obtained from the range can be seen in table 2 as follows:

Table 2. Mean ±2SD and Mean ±3SD

Parameter	Mean+2SD	Mean-2SD	Mean+3SD	Mean-3SD
Glucose	69.74	59.05	72.42	56.39
Uric Acid	4.44	3.50	4.68	3.73
SGOT	22.94	15.56	24.78	13.73
SGPT	18.36	12.24	19.88	10.71
BUN	8.20	6.37	8.66	5.9
Creatinine	0.77	0.58	0.81	0.53
Triglyceride	67.73	54.07	71.14	50.67
Cholesterol	169.99	156.41	173.38	153.03

Table 2 shows the range of standard deviation values for the glucose, uric acid, SGOT, SGPT, BUN, creatinine, cholesterol, and triglyceride parameter in homemade lyophilized serum based on initial examination. After the preliminary test was carried out, it was continued with stability checks on homemade lyophilized serum for 7 months. The examination was carried out by dissolving one vial once a months, the data obtained from the parameters examination results which can be seen in table 3 and 4 as follows:

Table 3. Homemade lyophilized serum test results data at a Refrigerator Temperature of 2-8ºC for 7 months

Parameter	Result	Storage Time (month)						
		1	2	3	4	5	6	7
Glucose	64.4	66	64	63	62	62	63	63
Uric Acid	3.97	4	4	4	4	3.9	4	3.9
SGOT	19.25	21	19	19	19	19	21	19
SGPT	15.3	16	14	16	17	17	17	15
BUN	7.28	6.8	7.1	6.7	7	6.9	7	7
Creatinine	0.68	0.64	0.61	0.62	0.62	0.59	0.62	0.61
Triglyceride	60.9	67	66	66	65	65	63	65
Cholesterol	163.2	166	165	164	164	163	169	167

Table 4. Home made lyophilized serum test results data at a Freezer Temperature of 0ºC for 7 months

Parameter	Result	Storage Time (month)						
		1	2	3	4	5	6	7
Glucose	64.4	68	63	63	63	65	61	60
Uric Acid	3.97	4.1	4.2	4.2	4.2	4.1	4.2	4.2
SGOT	19.25	20	20	20	18	18	18	18
SGPT	15.3	15	15	15	12	12	15	15
BUN	7.28	6.5	6.6	6.7	6.5	6.4	6.4	6.5
Creatinine	0.68	0.65	0.65	0.58	0.59	0.59	0.61	0.61
Triglyceride	60.9	65	64	65	65	64	67	65
Cholesterol	163.2	160	159	165	165	161	164	165

Based on table 2, the results of the examination of homemade lyophilized serum for 7 months can be made in the form of a Levey-Jenning graph to evaluate the presence or absence of deviations which can be seen in the following figure:

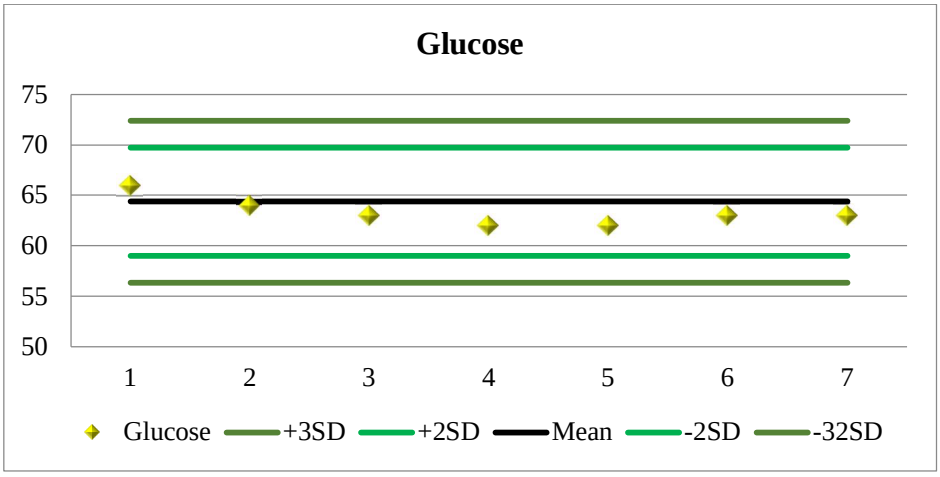

Figure 1. Levey jenning graph of glucose levels at refrigerator temperature 2-8ºC

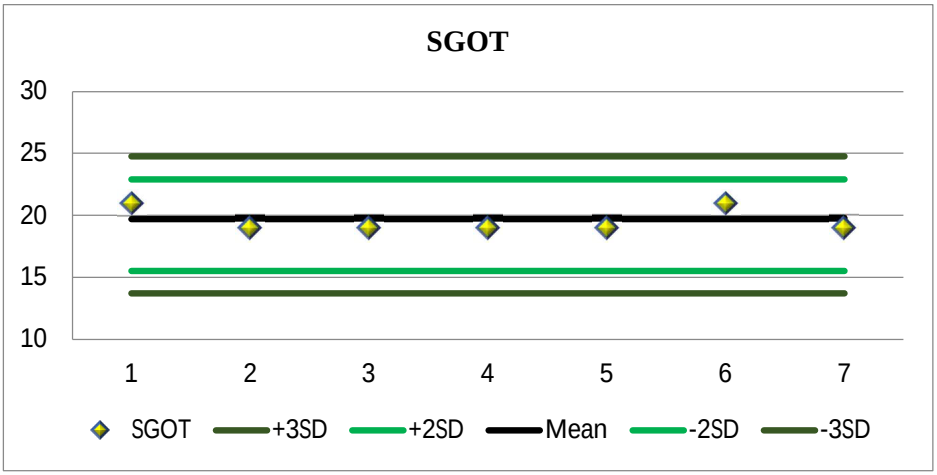

Figure 2. Levey jenning graph of SGOT levels at refrigerator temperature 2-8ºC

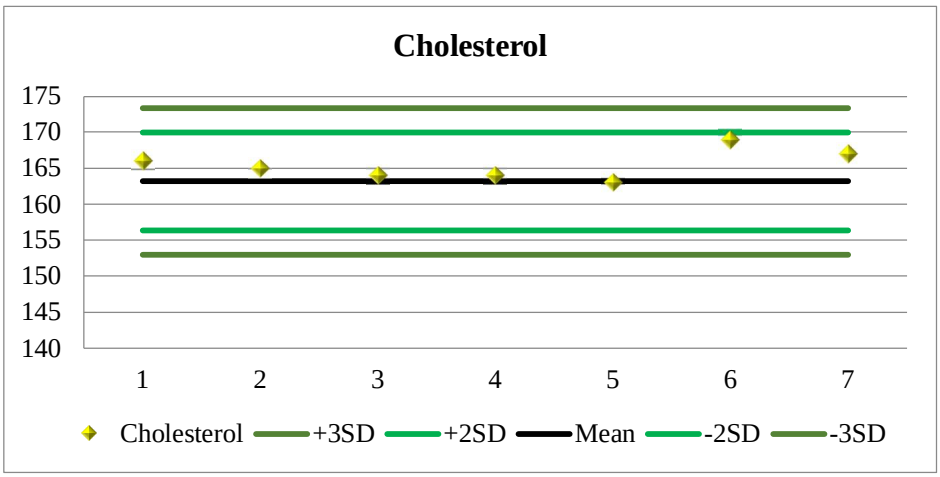

Figure 3. Levey jenning graph of Cholesterol levels at refrigerator temperature 2-8ºC

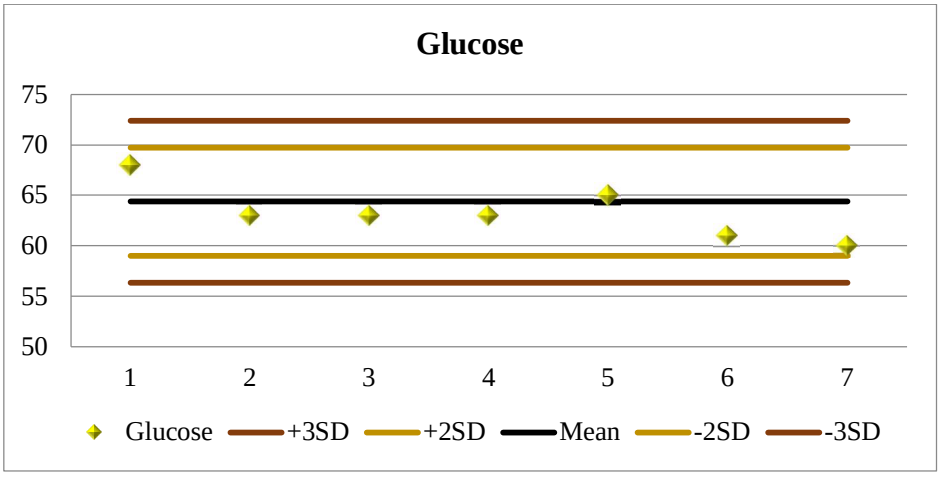

Figure 4. Levey jenning graph of glucose levels at freezer temperature 0ºC

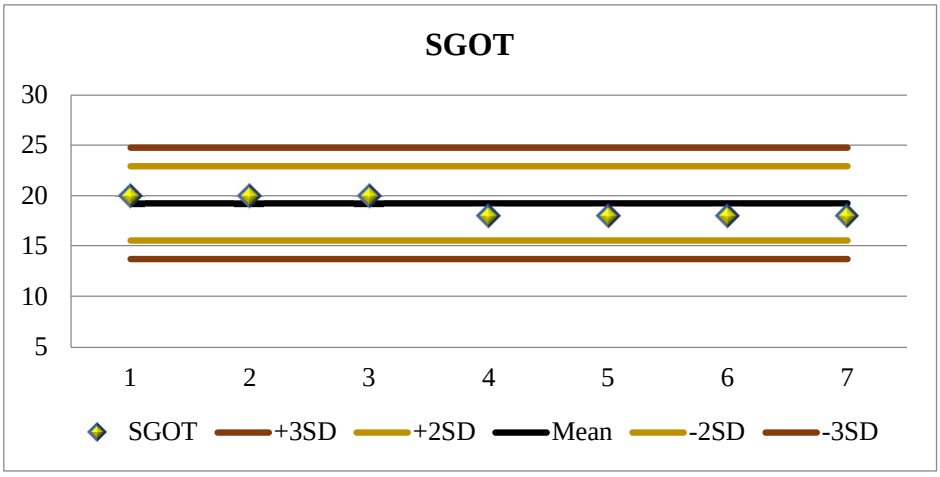

Figure 5. Levey jenning graph of SGOT levels at freezer temperature 0ºC

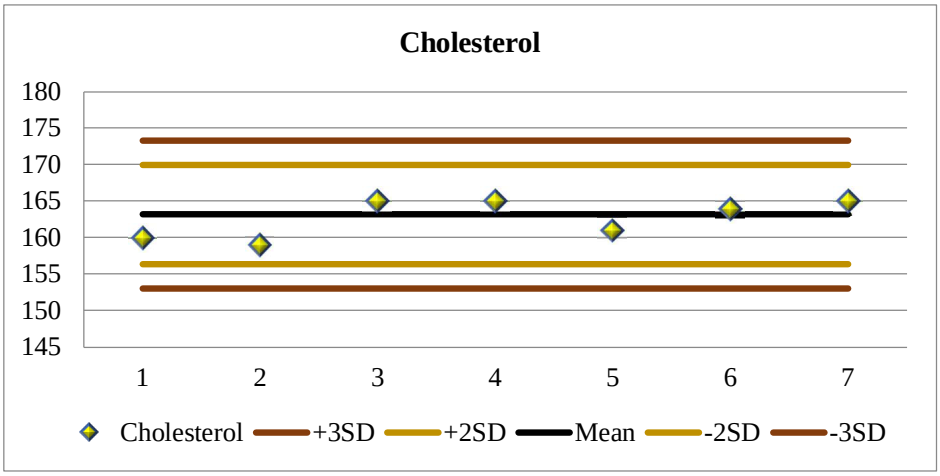

Figure 6. Levey jenning graph of cholesterol levels at freezer temperature 0ºC

DISCUSSION

Based on the results of the stability test of homemade lyophilized serum for 7 months, the Coefficient of Variation (CV) of glucose, uric acid, SGOT, SGPT, creatinine, cholesterol, and triglyceride levels did not exceed the limits of the CV (CCV). Meanwhile, the results of the examination of BUN levels have a CV value that exceeds the normal limit, the results of the examination of BUN levels in the control serum have a CV value of

6,31%, the CCV value of BUN levels of 5,7%. Thus, the results of examination of glucose, uric acid, SGOT, SGPT, creatinine, BUN, cholesterol, and triglyceride levels in homemade lyophilized serum during 7 monthss of storage had variations in results that were not much different.

The Levey Jennings chart shows that the distribution of glucose, uric acid, SGOT, SGPT, creatinine, BUN, cholesterol, and triglyceride test results is in the mean ± 2 SD area for 7 months of storage and does not follow the Westgard law prohibition, meaning that the results of homemade lyophilized serum are well controlled (in control) for 7 months 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 parameters in homemade lyophilized serum stored at for 7 months showed varying results. Examination of parameters in homemade lyophilized serum increased and decreased levels during the 7 months storage period, this was concluded due to variations in results because the distribution of data still showed in the mean±2SD area on the Levey-Jennings chart. This shows that homemade lyophilized serum is stable during a storage period of 7 months at refrigerator (2-8ºC) and freezer (0ºC) temperatures. [9]

In this study, glucose levels in homemade control serum decreased during storage. Glucose levels in homemade lyophilized serum slightly decreased during storage due to degradation by microbial agents during storage and as well as room temperature and ambient temperature where serum is stored before being examined which results in a decrease in blood glucose level [8,10]. Previous research stated that the decrease in SGPT levels could be caused by enzyme activity in long storage and interference by LDH in SGPT [11].

Homemade 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 [8,10]. The stability of lyophilized homemade serum 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 [1].

CONCLUSION

Based on the results of the research and data analysis conducted, it is known that the CV values for glucose, uric acid, SGOT, SGPT, creatinine, cholesterol, and triglyceride levels do not exceed the maximum CV limit (CCV). The Levey-Jennings chart shows that the parameters examined do not deviate from the mean ±2SD and are not rejection of the Westgard-Multirule law. Thus it can be concluded that homemade lyophilized serum using the freeze dry technique is stable, and can be used as an alternative to commercial control serum for examining glucose, uric acid, SGOT, SGPT, creatinine, BUN, cholesterol, and triglycerides because it has good accuracy and precision for 7 month.

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