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Evaluation of Homemade Lyophilized Serum as External Quality Control Material for Clinical Chemistry Tests based on Variance Index Score

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ABSTRACT

Background: External Quality Assurance (EQAS) is crucial for maintaining accuracy in clinical laboratory results. However, the high cost of commercial serum controls often limits participation, particularly in developing countries. This study aims to compare the performance of homemade lyophilized serum with commercial control serum as a cost-effective alternative for EQAS, using the Variance Index Score (VIS) for evaluation. **Methods**: A comparative observational study was conducted in 15 general medical laboratories from January to September 2024. Homemade lyophilized serum was prepared from pooled serum of healthy volunteers. Both homemade and commercial serum were tested for eight clinical chemistry parameters: glucose, uric acid, cholesterol, triglyceride, SGOT, SGPT, urea, and creatinine. VIS was calculated based on the difference between laboratory results and target values, and statistical analysis compared the two types of serum. **Results**: Commercial control serum produced results with higher mean values and lower variation compared to homemade serum. Despite higher variability in the homemade serum, no significant difference in VIS was found between the two groups ($p > 0.05$) for all tested parameters. **Conclusion**: Homemade lyophilized serum, though more variable, demonstrated comparable performance to commercial controls, making it a viable cost-effective alternative for EQAS. Further studies are recommended to assess long-term stability and quality.

Keywords: External Quality Assurance (EQAS), homemade lyophilized serum, commercial control serum, Variance Index Score (VIS)

BACKGROUND

External Quality Assurance (EQAS) is a key element in ensuring the accuracy and consistency of clinical laboratory results. Through EQAS, laboratories can compare their test results to externally established standards, ultimately helping to identify and reduce potential analytical errors. One of the essential components of EQAS is the use of stable and reliable external control materials(Sciacovelli *et al.*, 2018)(Yagoot *et al.*, 2023). These control materials are generally commercial control sera used to monitor laboratory performance in testing clinical chemistry parameters, including glucose, cholesterol, triglycerides, SGOT, SGPT, urea, creatinine, and uric acid. However, many laboratories, especially in developing countries, face challenges in terms of the cost of procuring commercial control sera, which are often expensive and require import from abroad. This can limit laboratory participation in EQAS programs, especially in areas with limited resources. Therefore, research into more economical alternatives, such as home-made lyophilized sera, is highly relevant. Homemade lyophilized serum has the potential to be used as a more affordable external control material, with long-term stability if stored at the right temperature (Hartani and Handayati, 2023)(Rahayu,

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Handayati and Rahayuningsih, 2022)(Haile *et al.*, 2020) (Haile et al., 2020)(Kachhawa *et al.*, 2017).

The stability of home-made lyophilized serum and its ability as a control material have been tested in several studies, with results showing that this serum can be used to monitor laboratory performance with various clinical chemistry parameters(Jamtsho, 2013)(Kulkarni, Pierre and Kaliaperumal, 2020)(Handayati, 2022). One of the evaluation methods often used to assess laboratory performance in EQAS is by using the Variance Index Score (VIS), which measures the bias between laboratory results and the set target values. VIS is an important indicator in assessing laboratory accuracy in conducting clinical chemistry tests (Yerram, Sripad and Prabodh, 2018) (Jamtsho and Nuchpramool, 2012).

Previous research conducted by Ilma et al showed that there was no significant difference in the Variance Index Score (VIS) between homemade lyophilized serum and commercial control serum in several clinical chemistry parameters such as uric acid, AST, ALT, cholesterol, triglycerides, urea, and creatinine in the Puskesmas laboratory. However, there was a significant difference in the glucose parameter. These results indicate that homemade lyophilized serum has the potential to be used as an external control material, although further evaluation is still needed on certain parameters (Ilma Ainun Nisa et al., 2023) (Yagoot et al., 2023)

This study aims to expand on this study by evaluating the performance of homemade lyophilized serum and commercial control serum in an independent general medical laboratory at the primary level. The main focus of this study is to assess laboratory performance in clinical chemistry testing using the Variance Index Score (VIS) as an evaluation method, with the hope of confirming the potential use of homemade lyophilized serum as an economical alternative in external quality assurance of clinical laboratories.

RESEARCH METHODS

The type of research used is observational research using a comparative research design by analyzing the differences between homemade lyophilized serum and commercial control serum in the external quality assessment (EQA) parameters of glucose, uric acid, cholesterol, triglycerides, BUN, creatinine, SGOT and SGPT. This research was conducted in January 2024 - September 2024 at the Clinical Chemistry Laboratory of the Medical Laboratory Technology Department of the Surabaya Ministry of Health Polytechnic and in 15 general medical laboratories as participants or respondents.

Specimen Collection

The test material used in this study was a collection of normal serum (pooled sera) that was free from HIV, HBsAg, non-lipemic and non-icteric. The pooled sera procedure was carried out by taking blood from 10 healthy volunteers, 10 ml each, and centrifuging to obtain serum. Pooled sera screening is done by testing pooled sera with HbsAg and anti-HIV examinations. All serum samples were declared negative for both parameters. Serum that met the criteria was collected and homogenized using a vortex. The collected serum was divided into 30 vials, each containing 3 mL of serum, then freeze-dried using the freeze dry technique.

Reconstitution and distribution process of control serum

Homemade control serum and commercial serum that were already in lyophilized form were dissolved/reconstituted with aquabides with 3 ml for homemade lyophilized serum and 5 ml for commercial control serum using a volume pipette. After the homogenization process, the serum was divided into 500 microliters of microcups

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per microtube to be distributed to each participating laboratory, 1 microcup of homemade serum and 1 microtube of commercial control serum. The distribution of control serum used a coolbox that was kept at a temperature of 2-8^{o}C.

Performance Assessment Process

The results of the examination of homemade lyophilized serum and commercial control serum obtained from each laboratory were then tabulated and the average was calculated. Furthermore, the Variance Index (VI) and Variance Index Score (VIS) were calculated using the following formula

$$\%V = \frac{\text{x} - \text{target value}}{\text{target value}} \text{ x } 100\% \text{ ;}$$

$$Variance\ Index\ (\text{VI}) = \frac{\%V}{\text{CCV}} \text{ x } 100$$

% Variation (V) is the difference between the participant's examination results and the target value expressed as a percentage of the target value. Variance Index (VI) is % Variation divided by CCV and multiplied by a factor of 100. CCV is a scale or unit used as a benchmark to determine the extent to which the examination results deviate from the expected results. Chosen Coefficient of Variation (CCV) on blood glucose parameters 7.7%, uric acid 7.7%, cholesterol 7.6%, triglycerides 7.6%, AST 12.5%, ALT 17.3%, urea 5.7% and creatinine 8.9%. The VI results are then converted into VIS to determine the quality criteria for each laboratory's examination results as listed in the table below:

Table 1. Variance Index Score Criteria

Value	Criteria
0 – 100	good
100 – 200	enough
201 – 300	less
> 300	bad

The VIS data obtained was then subjected to comparative statistical tests to determine the differences between the homemade lyophilized serum and the commercial control serum

RESULTS AND DISCUSSION

Results

The results of the examination of the test materials of homemade lyophilized serum and commercial lyophilized serum in 15 participating laboratories for the parameters of blood glucose, uric acid, cholesterol, triglycerides, AST, ALT, urea and creatinine are tabulated in Table 2 below:

Table 2. Average results of examination of homemade lyophilized serum and commercial lyophilized serum for blood glucose, uric acid, cholesterol, triglycerides, AST, ALT, urea and creatinine parameters in 15 participating laboratories

Parameter	Type of serum	Mean (target value)	SD	CV	CV Max
Glucose	homemade lyophilized	83.53	3.85	4.61	5
	Commercial	105.4	4.21	3.99	5
Uric acid	homemade lyophilized	4.24	0.24	5.59	6
	Commercial	4.71	0.18	3.75	6
AST	homemade lyophilized	24.53	1.96	7.98	7
	Commercial	32.13	1.55	4.83	7
ALT	homemade lyophilized	20.2	1.4	6.92	7
	Commercial	27.87	1.77	6.34	7
Cholesterol	homemade lyophilized	148.13	7.53	5.09	6
	Commercial	155.47	5.5	3.54	6
TG	homemade lyophilized	85.47	4.64	5.43	7
	Commercial	92.2	3.57	3.87	7

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Ureum	homemade lyophilized	22.67	1.16	5.12	8
	Commercial	27.8	1.21	4.34	8
Cretinine	homemade lyophilized	0.865	0.04	4.41	6
	Commercial	0.959	0.03	2.89	6

Table 2. shows that commercial lyophilized serum tends to give higher mean values compared to homemade lyophilized serum in all parameters tested. The lowest coefficient of variation (CV) value of homemade lyophilized serum is 4.41% in creatinine and the highest is 7.98% in AST parameters. The CV value of commercial serum is the lowest 2.89% in creatinine and 4.83% in AST. Thus, homemade lyophilized serum has higher variation compared to commercial serum in all parameters tested.

Calculation of the variance index score (VIS) of homemade lyophilized serum and commercial control serum from each participant laboratory against the target value (mean value of all participants), with parameters of glucose, uric acid, cholesterol, triglycerides, AST, ALT, urea and creatinine tabulated in table 3 below.

Table 3. Variance index score (VIS) of homemade lyophilized serum and commercial control serum against participant target values (average of all participants)

		Participant Laboratory														
Parameter	Types of serum	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Glucose	Homemade	55	55	120	69	55	85	122	69	39	55	137	55	39	23	101
	Commercial	7	67	57	120	81	17	17	135	32	42	30	57	81	101	67
Uric acid	Homemade	118	12	43	49	80	187	43	73	49	80	73	141	73	160	141
	Commercial	52	123	3	3	58	117	85	25	52	30	25	52	120	52	25
AST	Homemade	122	115	180	50	48	15	50	15	113	48	50	110	83	81	48
	Commercial	47	28	47	114	53	22	28	3	22	28	53	140	28	71	28
ALT	Homemade	121	52	34	110	23	52	63	34	23	34	23	34	34	132	52
	Commercial	3	23	18	60	18	3	60	39	44	23	65	44	146	18	3
Cholesterol	Homemade	8	90	34	70	126	105	19	28	81	90	162	90	34	70	70
	Commercial	47	4	55	46	81	38	55	140	55	13	29	55	30	64	4
TG	Homemade	23	70	80	54	84	85	69	38	166	216	84	85	167	70	110
	Commercial	26	132	74	54	54	46	155	106	40	54	88	40	60	54	31
Ureum	Homemade	52	26	210	52	26	103	52	129	52	180	103	214	129	52	26
	Commercial	50	13	50	76	13	50	118	50	139	76	50	76	205	76	102

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Cretinine	Homemade	58	116	71	45	32	71	110	71	19	19	105	97	58	32	64
	Commercial	17	110	41	32	41	102	41	29	87	16	32	77	47	29	36

The Variance Index Score (VIS) obtained is grouped based on the criteria as listed in Table 1 to determine the quality criteria for the examination results of each laboratory as listed in Figure 1 below:

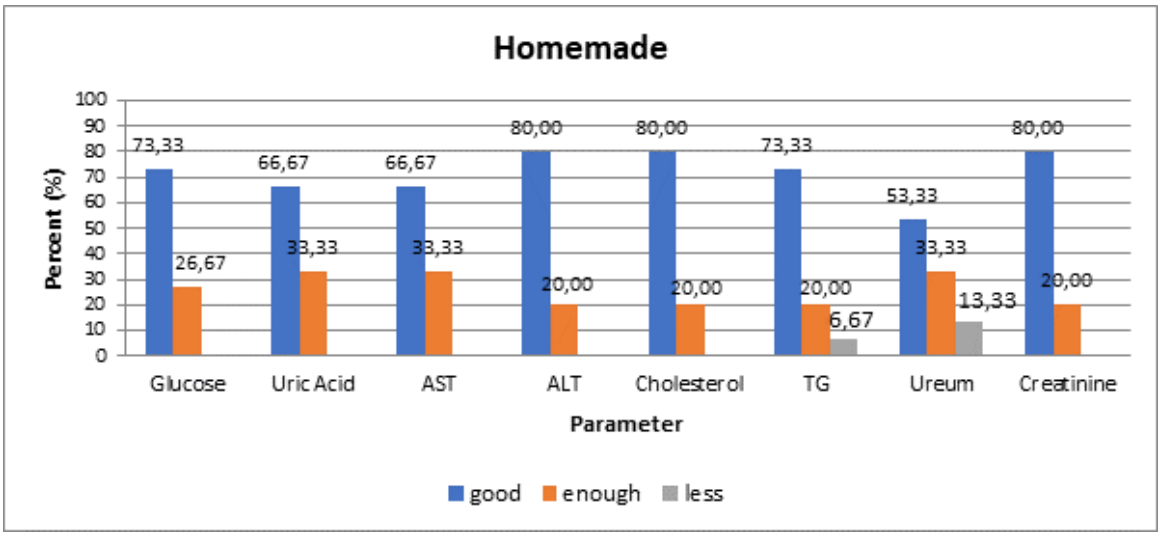

Figure 1. Percentage graph of homemade lyophilisate serum result criteria based on VIS against participant target values

Based on the graph above, the results of the homemade lyophilisate serum examination that had the highest good results were ALT, Cholesterol, and Creatinine, which reached 80% in the good category. The Urea parameter showed a more varied quality with a significant proportion in the sufficient category of 33.33% and less than 13.33%. Glucose, Uric Acid, AST, and Triglycerides were also mostly good, but there was still a proportion of sufficient results. The percentage of the less category was only found in two parameters, namely Triglycerides (TG) of 6.67% and Urea 13.33%.

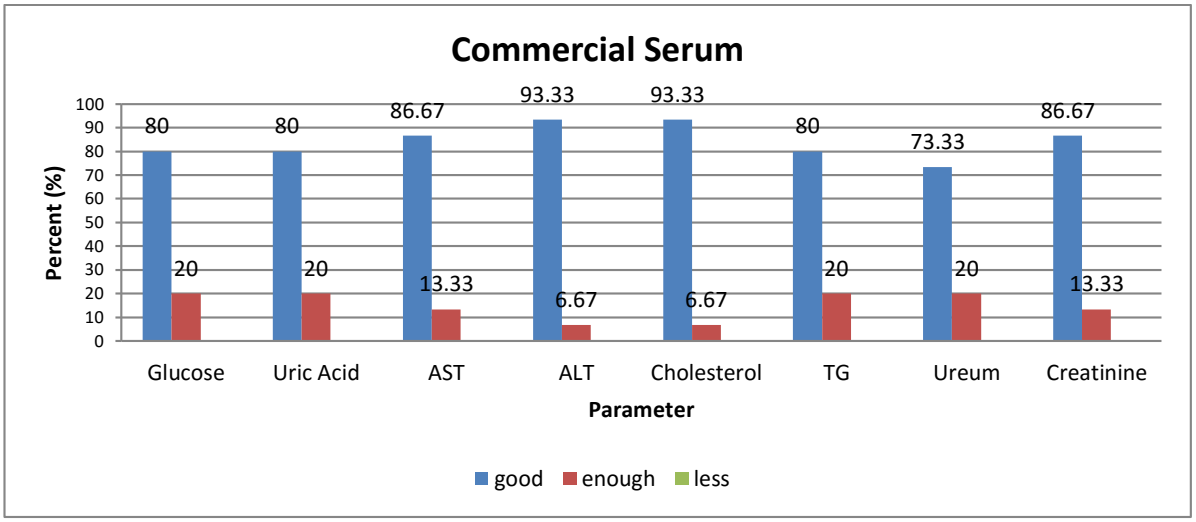

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Figure 2. Percentage graph of commercial lyophilized serum result criteria based on VIS against participant target values

Based on the graph above, the results of commercial serum control examination, ALT and Cholesterol parameters showed very good results, with more than 90% in the good category. Parameters such as Glucose, Uric Acid, Triglycerides, and Creatinine were dominated by the good category of 80%, but there were still around 20% sufficient results. Only Urea had results in the less category, but the number was very small (6.67%).

The difference between the two test materials can be determined through statistical analysis using the T test for normally distributed data and Mann Whitney for non-normally distributed data. Below are the results of the difference test of homemade and commercial lyophilisates on glucose, uric acid, cholesterol, triglycerides, AST, ALT, urea and creatinine parameters against the target values of participants/average of all participants:

Table 4. Results of independent T test VIS of homemade lyophilized serum and commercial control serum on glucose and uric acid parameters against participant target values

Parameter	Sig	Description
Glukocose	0.403	No difference observed
Asam urat	0.054	No difference observed

Table 5. Results of the Mann Whitney VIS test of homemade lyophilized serum and commercial control serum on glucose and uric acid parameters against the target values of participants

Parameter	Sig	Description
SGOT	0.067	No difference observed
SGPT	0.137	No difference observed
Cholesterol	0.074	No difference observed
Triglycerides (TG)	0.106	No difference observed
Ureum	0.367	No difference observed
Creatinine	0.161	No difference observed

The VIS difference test showed that the significance value obtained from both test materials on the parameters of glucose, uric acid, cholesterol, triglycerides, AST, ALT, urea and creatinine against the target values of all participants was > 0.05. These results indicate that there is no significant difference between homemade lyophilized serum and commercial control serum.

Discussion

In this study, a comparison was conducted between homemade lyophilized serum and commercial control serum based on the results of various biochemical parameters. The data obtained showed several significant differences in terms of the coefficient of variation (CV), Variance Index Score (VIS), and the results of statistical tests. Interpretation of the results of this study provides several new insights regarding the advantages and disadvantages of the two types of serum tested.

The results of the study showed that homemade lyophilized serum had a higher coefficient of variation value than commercial control serum in all parameters tested. The largest variation occurred in the AST parameter with a CV value of 7.98% for homemade serum, compared to commercial serum which only had a CV value of 4.83%. This indicates that homemade serum is more susceptible to variability between measurements, which can affect the accuracy of laboratory test results (Wilson *et al.*, 2011)(Prasad *et al.*, 2019)(Arabsolghar and Askari, 2014)

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Previous studies have also reported similar results,(Ahn *et al.*, 2017) in their study comparing the quality of homemade and commercial control serum, found that commercial serum tended to have lower variation due to a more standardized production process. This process produces a more homogeneous product between batches, compared to homemade serum which can be affected by external variables such as inconsistent drying processes, storage conditions, or lyophilization techniques (Selvakumar, Swaminathan and Fleming, 2004)(Kaur, Kumar Kare and Madaan, 2018)(Cuhadar *et al.*, 2013)

However, these results are also in line with the findings of (Jiskani *et al.*, 2021), who stated that although homemade serum has higher variation, this does not always cause significant differences in clinical test results, as long as the variation is still within an acceptable range.

In this study, the Variance Index Score (VIS) of both types of serum showed that the commercial control serum had a higher proportion of results in the good category, especially in parameters such as ALT and Cholesterol, where more than 90% of the results were in the good category. Homemade serum, although it had relatively good results in parameters such as ALT, Cholesterol, and Creatinine (80% good each), still showed higher variation, especially in the Urea and Triglyceride parameters, where there was a significant proportion in the sufficient and deficient categories.

Ilma et al, 2023 also reported that commercial serum tended to produce better results in VIS testing compared to homemade serum. They concluded that this was related to tighter quality control in commercial products, which were produced using more consistent materials and methods. This study strengthens the evidence that the use of commercial serum provides more reliable results, especially in laboratory testing that requires a high level of accuracy (Ilma Ainun Nisa *et al.*, 2023)(Yagoot *et al.*, 2023)

Although the data showed that commercial serum had advantages in terms of consistency and better VIS results, the results of the T-test and Mann-Whitney U statistical tests showed that there was no significant difference between homemade lyophilized serum and commercial control serum in the parameters of glucose, uric acid, cholesterol, triglycerides, AST, ALT, urea, and creatinine (significance value > 0.05).

These results indicate that although homemade serum has higher variation, overall, the performance of both can still be considered equivalent in the context of clinical use. This finding is consistent with the study Jamtsho, 2012 , who reported that differences in VIS between homemade and commercial serum did not significantly affect the final results of laboratory tests, especially when used for routine testing (Jamtsho and Nuchpramool, 2012)

This study offers novelty in several ways. First, this study introduces a comprehensive analysis between homemade lyophilized serum and commercial control serum using the VIS and CV approach which has not been widely performed in various laboratories. Second, although previous studies have emphasized the superiority of commercial serum in terms of consistency, this study shows that homemade serum can still provide clinically feasible results, albeit with a higher degree of variation.

These findings have the potential to provide an alternative solution for laboratories with limited budget or access to commercial serum. Thus, small laboratories or those in remote areas can consider the use of homemade serum as an economical option, as long as quality control procedures are implemented properly.

Although the results of this study provide useful insights, there are some limitations that need to be acknowledged,

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variability in homemade serum preparation, because homemade serum preparation involves more uncontrolled factors (e.g., storage conditions, lyophilization techniques), there may be greater variation between batches, and this could impact the results if not handled properly. Scale of the study, the number of participating laboratories may not be representative of the broader laboratory population. Therefore, further studies with a larger number of laboratories are needed to ensure that these results are generalizable. Limitations of the parameters tested, although this study covered common parameters in biochemical testing, there are many other parameters that could be tested to determine if similar results would be seen outside the scope of this study.

CONCLUSION

Based on the results of this study, it can be concluded that homemade lyophilized serum has significant potential as an alternative control material in the external quality assurance program of clinical laboratories. The absence of significant differences in the Variance Index Score (VIS) between homemade lyophilized serum and commercial control serum indicates that homemade serum can provide results equivalent to commercial control materials in testing clinical chemistry parameters, including glucose, cholesterol, triglycerides, SGOT, SGPT, urea, creatinine, and uric acid.

This study supports the use of homemade lyophilized serum as a more economical solution, especially for laboratories with limited budgets, and can help reduce dependence on more expensive commercial control materials. In addition, the stability of homemade serum also makes it a viable option for long-term external laboratory quality assurance.

This study provides a basis for further studies with several recommendations, studies with a wider laboratory sample and more test parameters to ascertain whether the trends found in this study also apply to other parameters, development of standard protocols for homemade serum preparation that can be used to reduce variability in results and improve consistency, more in-depth studies to evaluate the cost-benefit between the use of homemade and commercial serum, especially in laboratories facing budget constraints

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