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VARIANCE INDEX SCORE (VIS) EVALUATION OF HOMEMADE AND COMMERCIAL CONTROL SERA ON AST AND ALT ASSAYS

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Abstract

Control materials are used as indicators of monitoring the performance of laboratory examinations so that the results issued are valid. Method of assessing the implementation of External Quality Assurance (EQAS) used Variance Index Score (VIS). This study aims to determine difference between pooled sera, homemade and commercial lyophilizates on AST and ALT parameters based on VIS. This research used comparative descriptive with quantitative approach in December 2023-May 2024. Homemade lyophilizates performed at UBAYA technobiology laboratory, determination of true value performed at 2 reference laboratories and clinical chemistry laboratory Poltekkes Surabaya, and determination of VIS performed at 10 primary level clinical laboratories in Surabaya Raya which determined by purposive sampling. The statistical analysis results showed no difference between homemade and commercial control materials based on VIS to the true value and all participating laboratories, so it was concluded that homemade control materials could be used as a substitute in implementing EQAS.

Keywords: *Pooled sera; Homemade Lyophilizate; Commercial Lyophilizate; VIS*

1. Introduction

Laboratory examination significantly influences patient safety, so that is necessary to guarantee the quality of the examination results to be issued, as the laboratory is a health facility that measures, determines, and tests human samples to determine the type and cause of disease (Sultana et al. 2024). Quality assurance is essential because poor-quality services resulting in low-quality results will impact increasing costs and increasing costs are only sometimes in line with the quality of the examination.

Efforts to optimize quality stabilization can be made by implementing quality. Quality implementation is divided into internal and external. Internal quality assurance is the regular implementation of quality assurance carried out by internal laboratories to reduce deviation factors and obtain accurate inspection results using the reliability of laboratory tests as a benchmark for its assessment. External Quality Assurance is the periodic implementation of quality assurance by external parties by evaluating the quality of a laboratory's examination results. External Quality Assessment Services (EQAS) in clinical chemistry uses the Variance Index Score (VIS) benchmark (Siregar et al. 2018).

Laboratory quality assurance must consider certain technical aspects in its implementation, one of which is precision and accuracy (Raden et al. 2022). Precision and accuracy are related to the use of control materials in monitoring the performance of laboratory examinations (Ramdhani 2023). Based on their form, control materials can be liquid (liquid control), solid powder, or freeze-dried (lyophilized). The control material used can be obtained commercially or homemade from a collection of patient serum residues (Pooled sera).

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Commercial control materials like lyophilizates are often used in clinical laboratories. However, their continuous use may not be economically feasible for developing countries due to their relatively high price and limited availability (Jamtsho 2013; Kulkarni, Pierre, and Kaliaperumal 2020). This study aims to provide a cost-effective alternative by demonstrating the comparable effectiveness of homemade control materials. By doing so, we hope to reassure laboratory professionals about the financial implications of their work and encourage the adoption of homemade control materials in quality implementation.

Research conducted by Salma et al., (2019) and Kulkarni et al., (2020) suggests that homemade control materials, particularly pooled sera, could be a cost-effective alternative to commercial control materials. The use of homemade control material as an alternative control material has several advantages, including easy to obtain, the manufacturing process requiring a relatively low cost, and the material comes from humans, which means the constituents are in accordance with the state of the specimen and the laboratory knows the origin of the control material (Sari 2021). Some studies also mention that homemade control serum has a reasonably long shelf life, Arlinda's research (2020) states that Pooled sera control material is stable for up to 2 months with a storage temperature of 0˚C to -10˚C on AST and ALT parameters. Another study states that homemade Pooled sera are stable for up to 3 months with a storage temperature in the freezer (- 20˚C) on fat parameters (Maulidiyanti et al. 2021). Research conducted by Jamtsho (2013) showed that homemade control material in the form of lyophilized without the addition of preservatives with a storage temperature of 2˚C-8˚C is still stable within 7 months, and freezer storage (-20˚C) is stable within 9 months. Homemade lyophilizate control material after dissolution (reconstitution) is stable for 2 months with storage temperatures of -2˚C to -4˚C and -20˚C on AST and ALT parameters (Hartani and Handayati 2023). The disadvantages of using homemade control is a complex manufacturing process and requires a long time, short stability time, and must perform statistical analysis every 3-4 months (Sari 2021). Whereas commercial control materials have advantages such as being ready to use, statistical analysis is only needs once, and can be used as a control of accuracy and precision because it has a reference value (Novalina and Shafrani 2020). However, commercial control materials also have disadvantages such as the possibility of errors during reconstitution, sometimes there are variations from vial to vial, and have a relatively high cost (Siregar et al. 2018).

Control materials are often used to monitor the quality of a laboratory examination, often on parameters commonly used for Medical Checkups (MCU). One of the MCU examinations used for liver function tests is AST and ALT. These parameters were chosen as the research object because they are commonly used in monitoring liver function and are expected to represent enzymatic examinations. It is necessary to know whether homemade control materials can be an alternative for implementing laboratory quality assurance by comparing deviations in the Variance Index Score (VIS) of homemade (Pooled sera, lyophilization) and commercial (lyophilization) control materials against Aspartate Transaminase (AST) and Alanine Transaminase (ALT) parameters.

2. Method

This type of research uses a comparative descriptive with a quantitative approach. The research was conducted in December 2023-May 2024. The manufacture of homemade lyophilizates was carried out in the UBAYA technobiology laboratory, the determination of true value was carried out in 2 reference laboratories and the Poltekkes Kemenkes Surabaya clinical chemistry laboratory and the determination of VIS was carried out in 10 primary level clinical laboratories in the Surabaya Raya area which were determined by purposive sampling. The test materials used included pooled sera, homemade lyophilizates, and "Human" brand commercial lyophilizates.

The implementation was carried out by preparing the test materials. Pooled sera were obtained from the pooled sera of respondents who had met the criteria as test materials, namely free from HIV and hepatitis, while homemade lyophilizates were obtained from pooled sera that were freeze-dried through the freeze-drying process. Lyophilized serum was reconstituted using distilled water, then the three test materials were examined for AST and ALT parameters in the laboratory where the study was conducted.

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The results of the three test materials are tabulated and then the average or target value is calculated, after which the Variance Index (VI) and Variance Index Score (VIS) are calculated using the following formula (Siregar et al. 2018):

$$\text{target value} = \frac{\text{sum of inspection results}}{\text{number of laboratory participants}}$$

$$\text{Standar Deviation (SD)} = \sqrt{\frac{\Sigma\,(examination\ results\ of\ each\ laboratory - target\ value)}{number\ of\ laboratory\ participants - 1}}$$

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

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

The Chosen Coefficient of Variation (CCV) for the AST parameter is set at 12.5%, while for the ALT parameter, it is 17.3%. To assess laboratory quality, the Variance Index (VI) results are further converted into Variance Index Scores (VIS). These scores are evaluated based on predefined quality criteria. The VIS criteria categorize laboratory performance into four levels: scores ranging from 0 to 100 indicate "good" quality, 101 to 200 signify "sufficient" quality, 201 to 300 are categorized as "less" quality, and scores exceeding 300 reflect "bad" quality. This classification system provides a standardized benchmark for assessing and improving laboratory assay performance.

The VIS results obtained were then subjected to comparative statistical tests using Kruskal-Wallis to determine the differences between test materials, including pooled sera, homemade, and commercial lyophilizates on AST and ALT parameters. The significance value decision was based on the test result obtained. If the significance value is > 0.05 there is no difference from the VIS results, otherwise if the significance value is < 0.05 it means that there is a difference in VIS results.

3. Result and Discussion

Target Value Determination

To determine the VIS value, it is necessary to calculate the target value obtained from the examination of the control materials on the AST and ALT parameters by the laboratories. The target value is the average value of the examination result.

Based on Table 2 the results of the true value examination obtained the largest Standard Deviation (SD) located in the pooled sera ALT parameter. Based on Table 3, the participant's laboratory examination results obtained the largest Standard Deviation (SD) located in the commercial lyophilization ALT parameter.

The examination results obtained were calculated for the target value and Standard Deviation (SD) using the formula in the method section. Based on the calculation, it was found that on average, all participants had a Standard Deviation (SD) value greater than the target value based on laboratory reference This can occur because some participant laboratories use semi-automatic tools that allow random errors originating from humans. This statement is the following Fauziah et al. (2019), which explains that random errors can be obtained from inconsistencies in the volume of reagents or control materials examined due to the tilt of the micropipette and the way of homogenization.

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Table 1. Reference Laboratory test results

Parameter	Control Materials	Target Value	Standard Deviation (SD)	Range (Mean ± 2SD)
AST (U/L)	Pooled sera	20.63	1.07	18.49 – 22.27
	Homemade Lyophilizate	17.94	1.1	15.74 – 20.14
	Commercial Lyophilizate	31.91	0.81	30.29 – 33.53
ALT (U/L)	Pooled sera	15.34	1.69	11.96 – 18.72
	Homemade Lyophilizate	12.02	1.01	10 – 14.04
	Commercial Lyophilizate	28.3	0.71	26.88 – 29.72

Table 3. Participant's Laboratory Test Results

Parameter	Control Materials	Target Value	Standard Deviation (SD)	Range (Mean ± 2SD)
AST (U/L)	Pooled sera	20.7	1.57	17.56 – 23.84
	Homemade Lyophilizate	18.21	2.08	14.05 – 22.37
	Commercial Lyophilizate	28.94	2.56	23.82 – 34.06
ALT (U/L)	Pooled sera	17.95	1.99	13.97 – 21.93
	Homemade Lyophilizate	12.85	2.14	8.57 – 17.13
	Commercial Lyophilizate	28.75	3.81	21.13 – 36.37

Calculation of Variance Index Score

The Variance Index Score (VIS) is calculated for each participating laboratory based on the target value obtained. If a negative VIS is obtained, then the result needs to be converted to positive by rounding. Then the results of the VIS are converted into the criteria in Table 1 to determine the quality of the control material in the laboratory examination. VIS results on pooled sera, homemade and commercial lyophilizates on AST and ALT parameters are presented in the diagrams in Figures 1 and 2.

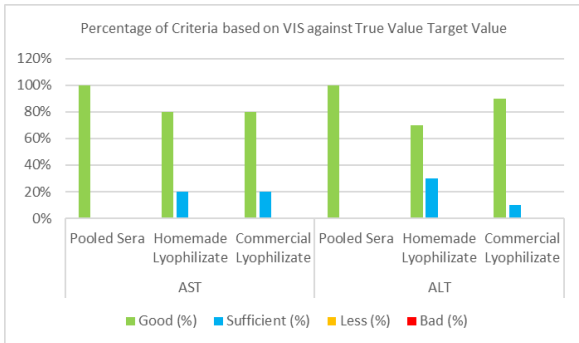

Figure 1. *Graph of the percentage of criteria based on VIS against the true value target value*

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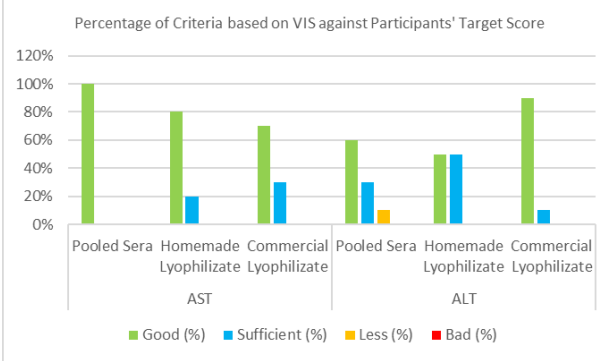

Figure 2. *Graph of the percentage of criteria based on VIS against the target score of all participants.*

Criteria based on VIS against the target value of participants and true value against the AST parameter, the highest percentage is found in pooled sera, which has good criteria of 100%, while in the ALT parameter, the best percentage is found in commercial lyophilizates, which has good criteria of 90% and sufficient of 10%. Variations in the percentage of criteria based on various VIS are located in the ALT parameter, including the VIS value of pooled sera based on the participant's target value has good criteria of 100%, but when compared to the true value target value, pooled sera has good criteria of 60%, sufficient 30%, and less 10%, then the VIS value of homemade lyophilizate based on the participant's target value has good criteria of 70% and sufficient 30% while the VIS value based on the true value target value obtained good and sufficient criteria of 50% each. The variety of VIS results can be influenced by several factors, including pre-analytic, analytic, and post-analytic stages, in addition to differences in the tools and reagents used, which can also affect the results (Widyastuti et al. 2020).

The highest percentage of deviations came from the results of the pooled sera and homemade lyophilizate test materials on the ALT parameter, both against the true value target value and against the average of all participants. The deviation may be influenced by the use of inappropriate reagents, such as working reagent preparation, homogenization between reagent and sample, or reagent temperature during the examination process, as this is related to the enzymatic kinetic reaction method that measures the speed of enzymes to break down substrates. The substrate level and enzyme activity determine the reaction speed. If the enzyme level is excessive but the substrate is limited, it results in substrate depletion, while if the enzyme is limited. Still, the substrate is excessive, which can result in substrate inhibition, and both of these cause false low results. Inconsistency results in pooled sera test material according to Fauziah et al (2019) inconsistent results were influenced by the lack of homogenization and pipetting, which caused bias between the sample tubes. The inconsistency in the homemade lyophilizate results is due to the time lag between the start of pooled sera preparation and the lyophilization process. This is due to the preparation of the test material without additional stabilizers, which can affect ALT levels. This statement follows Arlinda (2020), who suggests a decrease in ALT levels due to loss of enzyme activity due to storage factors.

To determine the differences between pooled sera, homemade and commercial lyophilizates based on AST and ALT parameters, statistical analysis was used on the VIS results of the true value of target value and the target value of all participant laboratories using the Kruskal-Wallis test which can be seen in Table 4.

Table 4. Statistical Data Analysis Results

Parameter	Significance (P Value)	
	VIS to target true value	VIS against participants laboratory target values
AST	0.199	0.558
ALT	0.117	0.123

The results of the Kruskal-Wallis test showed that the significance obtained by the three test materials on AST and ALT parameters based on VIS against the true value target value and the target value of all participants was significance value > 0.05, meaning that there is no significant difference

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between pooled sera, homemade and commercial lyophilizates. The result is based on the decision of the Kruskal-Wallis test that if the significance value is > 0,05 there is no difference from the VIS results, otherwise if the significance value is < 0,05 it means that there is a difference in VIS results.

Results that have been obtained in line with Kulkarni et al., (2020) explained that there is a difference in effectiveness between pooled sera and commercial sera, but the difference is insignificant, so pooled sera can still be used as an alternative to commercial substitutes. Another similar study stated that there was no difference between homemade lyophilized serum and commercial lyophilized serum on the parameters of SGOT, SGPT, BUN, and Creatinine towards the implementation of laboratory quality assurance (Nisa et al., 2023). Other studies also mention that the implementation of External Quality Assurance using homemade lyophilizate control materials can improve the implementation of laboratory proficiency testing because it has good stability and minimizes costs incurred (Jamtsho and Nuchpramool 2012).

4. Conclusion and Suggestion

Based on the Variance Index Score (VIS) analysis between pooled sera, homemade, and commercial lyophilizate on AST and ALT parameters, accuracy deviations were obtained from several participating laboratories, this was due to differences in the tools and reagents used. Although there are deviations in the results of some participating laboratories, homemade control materials can be used as a substitute for the implementation of External Quality Assurance (EQAS). Further research can be conducted research with a larger number of participating laboratories and questionnaires for participating laboratories related to the tools and reagents used.

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