ASIAN JOURNAL OF HEALTH AND APPLIED SCIENCES
2022, VOL. 1, NO. 2, 7–14
https://doi.org/10.53402/ajhas.v1i2.15

Validity test of POCT (Point of Care Testing) method on blood glucose examination using whole blood samples, serum, and EDTA plasma

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ABSTRACT
Medical laboratory services can support disease diagnosis or monitor patient recovery. The reality of the POCT method has been widely used by clinical laboratories. The tool is not only used for screening but is also used to check the diagnosis of diabetes mellitus. This research aims to determine the validity of the POCT method on whole blood, serum, and plasma EDTA samples on blood glucose tests. The research method of this study used the descriptive-analytical method using the POCT method on blood glucose tests using three different types of samples, whole blood, serum, and plasma EDTA. Precision tests were accepted on normal and pathological serum samples with CV% of 2.02% and 2.27%. The accuracy test was accepted on a normal and pathological serum with TE% values of 8.54% and 6.03%. The linearity test is accepted on serum–plasma EDTA samples with an r2 value of 0.998. The sigma values are in the unacceptable area. The use of the POCT tool for blood glucose examination has a valid performance value. The deviation of the examination results is influenced by pre-analytical errors such as sampling and processing samples so that the total error obtained is higher than the total allowed error. The POCT tool can be used for all types of samples.

KEYWORDS
Validity; POCT; Blood glucose; Whole blood; Serum; EDTA plasma

Received: March 2, 2022
Accepted: June 3, 2022
Published: June 27, 2022

Introduction

Health laboratory services are services that can support disease diagnosis or monitor patient recovery. One of the parameters of laboratory service quality is to overcome several error factors. In the laboratory, errors in service are divided into three categories: errors in the pre-analytical or pre-analytical process such as sample identification and sampling errors, request errors, sampling technique errors, and selection of tools and materials (Apriani, et al, 2018).

Based on the research, representative sampling of capillary blood vessels has a large enough failure factor. it is known that from a total of 40,490 analytical sample analyses in the laboratory there are 4.5% errors. The sample error accounts for the largest error in the pre-analytic stage (60-70%), analytic (10-15%), and post-analytic (15-18%) (Apriani, et al 2018).

According to the National Standard ISO 15189 and ISO 17025, each laboratory must use a standard method that has been validated, and any modified standard method needs to be validated to be applied in the laboratory. It is necessary to use analytical methods to ensure that certain methods have been used according to the assessment, the behaviour meets the intended use requirements, and experiments with certain parameters (Nurhayati, et al, 2019).

There are several reasons for the need for method validation: method validation is an important element of quality control and helps ensure the measurement will be reliable. One of the method validation stages is the selective and sensitive ability of an examination method for various disturbances and various types of samples against target compounds. In some areas, method validation is a regulatory requirement (Riyanto, 2012)

Glucose is the most important carbohydrate which is mostly absorbed into the bloodstream as glucose and other sugars are converted to glucose in the liver (Wungouw, et al. 2015). Examination of blood glucose levels has been proposed by many clinicians for screening or monitoring diabetes mellitus. Diabetes Mellitus is a disorder of carbohydrate metabolism characterized by an increase in blood sugar levels (hyperglycemia) of 200 mg/dL. WHO predicts an increase in the number of people with DM in Indonesia from 8.4 million in 2000 to around 21.3 million in 2030. This shows an increase of 2-3 times the number of people with DM in 2030 in Indonesia (Garini, et al, 2019). According to Riskesdas (2013), the highest number of people with diabetes mellitus in Indonesia is in West Java. In West Java itself, the incidence of diabetes reached 4.2% with the number of prediabetes at 7.8% (West Java Provincial Government, 2017).

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The results of laboratory examinations that are fast and precise are one of the problems that are often encountered in providing satisfaction for patients or users of clinical laboratory services. However, laboratories need to always maintain the quality of examination results, in the sense of having a proven level of accuracy and precision. Accuracy (accuracy) is the closeness of the result to the true value determined by the standard method. While precision (accuracy) is how close an examination result is when repeated with the same sample.

The reality of POCT has been widely used by clinical laboratories. The tool is not only used for screening tests but is also used to diagnose diabetes mellitus. POCT is patient besides testing where the meaning of the examination is for emergency conditions or monitoring during the treatment process. POCT should be used by trained health personnel. However, at this time, POCT can be used independently by anyone (not health workers), at any time, and does not pay attention to the rules of correct blood sampling (Anggraeni, et al, 2017).

Determination of the validity of a sample is determined based on the results of the precision (CV%) and accuracy (d%) of the performance of the method. The performance calculation uses the Westgard multi-rule with medical decision concentration by comparing the total error (TE) to the total error allowed (TEa) by Clinical Laboratory Standard International. Errors that occur in the sample can cause a decrease in the performance of a method, even rejection of the test results.

So based on this background, researchers are interested in conducting research entitled "Test the Validity of the POCT method (Point of Care Testing) on Blood Glucose Examination Using Whole Blood Serum and EDTA Plasma Samples". The author considers this title important considering that in West Java blood glucose checks are often carried out to support diabetes mellitus, therefore the methods and stages of blood glucose examination must be properly validated so that public confidence in the laboratory increases.

Methods

The research used in this thesis uses the descriptive-analytic method, which is a study that aims to provide an overview of the reality of the object under study. (Nurhayati, et al., 2019). The parameters measured to determine the validity of the method are precision (CV%), accuracy (d%), standard deviation (SD), linear regression equation, systematic error (SE), random error (RE), total error (TE), determination of sigma category with Medical Decision Concentration (MDC) for normal glucose and hyperglycemia. The Calculation is followed in Table 1.

Table 1. Parameter formula of Validation methode

No.	Parameters	Formula
1	CV%	$Bias\ (\%) = \sqrt{\frac{1}{n} \sum (x_1^2 + x_2^2 + \ldots\ldots x_n^2)}$,
2	d%	$Accuracy = \frac{N - E - R}{N} \times 100$
3	SD	$s = \sqrt{\frac{\sum(x_n - \bar{x})^2}{n - 1}}$
4	SE	$SE = SD + 3*CV$
5	RE	$RE = Bias + Imprecision$
6	TE	$TE = SE + RE$

Reference: Westgard Multi Rule (2015)

The description of the acceptability of the performance of the POCT method on blood glucose examination using 3 different types of samples compared with control are whole blood, serum, and plasma EDTA was obtained by collecting and analyzing data from the test results for the 3 validation parameters with 35 for each of sample. The sample used is blood taken by not a health worker but by the patient or the patient's family who are at Pindad General Hospital According to POCT function, widely used for self-monitoring without special skills.

In this study, two conditions of glucose levels were used, normal glucose and hyperglycemia. Grouping was done after blood glucose levels were obtained from patients using whole blood. Subsequently, additional blood was drawn from the capillaries to obtain sufficient blood for processing blood samples using EDTA anticoagulant and without anticoagulant (serum). The additional collection is in accordance with the instructions for using POCT, that for glucose examination, various types of blood samples can be used. In every inspection, control material is checked every day to show that the inspection results can be published and have good accuracy and precision followed by Westgard multi-rule.

Results

Before the inspection, the control material was inspected. Control materials are materials used to monitor the accuracy of an examination in a laboratory or to monitor the quality of daily inspection results (Apriliana, 2019) for the material graph can be seen in Figure 1.

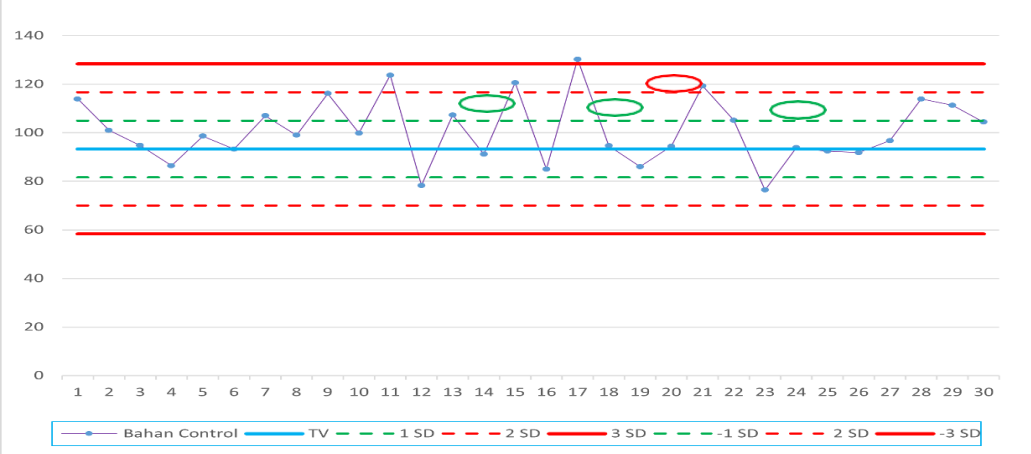


Figure 1. Control Material Chart

This control chart is analysed using the Westgard rule, the Westgard rule presents a series of rules that help the evaluation process of the control chart examination (Apriliana, 2019). Based on the graph, there are criteria for the Westgard 1.2s rule on data numbers 11, 15 and 21, so it can be categorised as a warning because the control has passed +2 SD. Criterion 1.3s is found in data number 17, where the data exceeds the +3 SD limit, so it is a rejection criterion that indicates a random error.

The validity of blood glucose levels in 35 samples for normal glucose and hyperglycemia at Pindad General Hospital with three different types of samples namely whole blood, serum, and plasma EDTA, obtained the following results in Table 2.

Table 2. Precision & Accuracy Test Results Data

Characteristics Data	Normal (Level 1)			Hyperglycemia (Level 2)		
	Whole blood	Plasma	Serum	Whole blood	Plasma	Serum
Average (mg/dL)	91	116	112	168	195	193
Standard Deviation (SD)	15,13	15.27	2.34	26.23	24.49	4.43
Bias (d%)	23.68	27.89	3.40	15.33	16.24	0.78
Coefficient of Variation (CV%)	13.50	13.18	2.02	13.52	12.53	2.27
Tea %	10	10	10	10	10	10
TE %	15.75	21.73	8.54	31.51	33.10	6.03

Precision test

The acceptance criteria for the Precision Test is that the CV% value is less than 3.3. The value of 3.3 was obtained from the calculation of 0.33x TEa where the TEa value for glucose based on the CLIA was 10%, so 0.33x TEa was 3.3 (Nurhayati, 2019).

Based on the data in Table 2, it was obtained that CV% met the acceptance criteria for the Precision Test on normal glucose serum data (Level 1) and hyperglycemia serum (Level 2) where the CV% value was 2.02% for Level 1 and 2.27% for Level 2. Then the Precision Test is accepted because of the CV% value < 3.3. While the whole blood data has a CV% value of 13.50% for Level 1 and 13.52% for Level 2 and EDTA plasma has a CV% value of 13.18% for Level 1 and 12.53% for Level 2, so the Precision Test the four data are not accepted because of the value of CV%> 3.3.

Total Error combined with accuracy Test

Based on the data in Table 2, the TE (Total Error) values that meet the criteria are normal glucose serum data (Level 1) and hyperglycemia serum (Level 2) serum samples with TE values of 8.54% for level 1 and 6.03% for level 2, then the Accuracy Test can be accepted because of the value of TE<TEa. Meanwhile, whole blood and plasma TE values

did not meet the criteria with whole blood TE values of 15.75% at Level 1 and 31.51% at Level 2, and plasma TE values of 21.73% at Level 1 and 33.10% at Level 1. 2, so the Accuracy Test cannot be accepted because of the value of TE>Tea.

On the results of the Accuracy Test, a Method Decisions Chart is made. This graph is used to combine random error and systematic error in assessing the performance of a method.

Figure 2. Decision Chart Method with 10% TEa

Figure 2 shows a method's performance and the combination of random error and a systematic error of a method. In MDC, whole blood with a concentration of 91 mg/dL is in the unacceptable outer region with a sigma value of 1.01 and in MDC serum with a concentration of 112 mg/dL, it is in the unacceptable outer region with a sigma value of 1.80. And the MDC plasma EDTA with a concentration of 116 mg/dl is outside the Unacceptable region with a sigma value of 1.35. So from the graph data, the POCT method cannot be accepted.

Linearity test

The results for the Linearity Test are plotted using a scatter plot diagram so that a regression line equation is obtained which shows the relationship between glucose control with variant glucose samples.

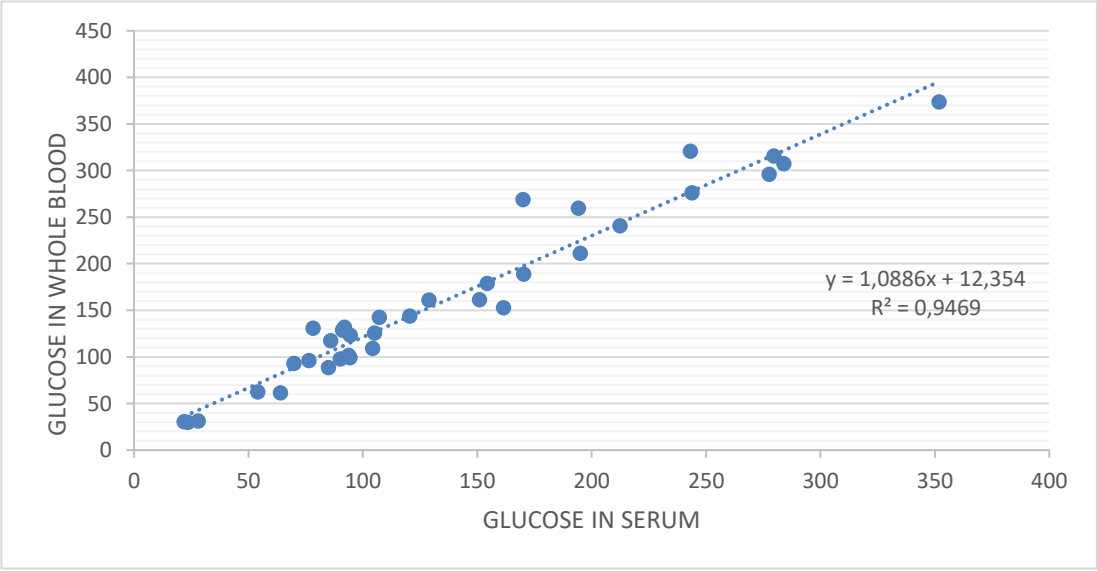


Figure 3. Scatter Plot Graph linearity between Glucose in Whole Blood and Glucose in serum

Based on figure 3, the results of the regression line equation are Y = 1.0886x + 12.354 with a correlation coefficient (r) or r^2 which is 0.9469, so the Linearity Test is not accepted because the value (r^2) < 0.98. In the scatter, plot graph, a significant difference test or statistical T-Test test is also made using MS. Excel, for the results of the T-test on whole blood-serum samples. The calculation results show the T-Test's probability value (P), which is 0.00. So

it can be concluded that there are differences in the accuracy of the POCT method of blood glucose examination on serum samples.

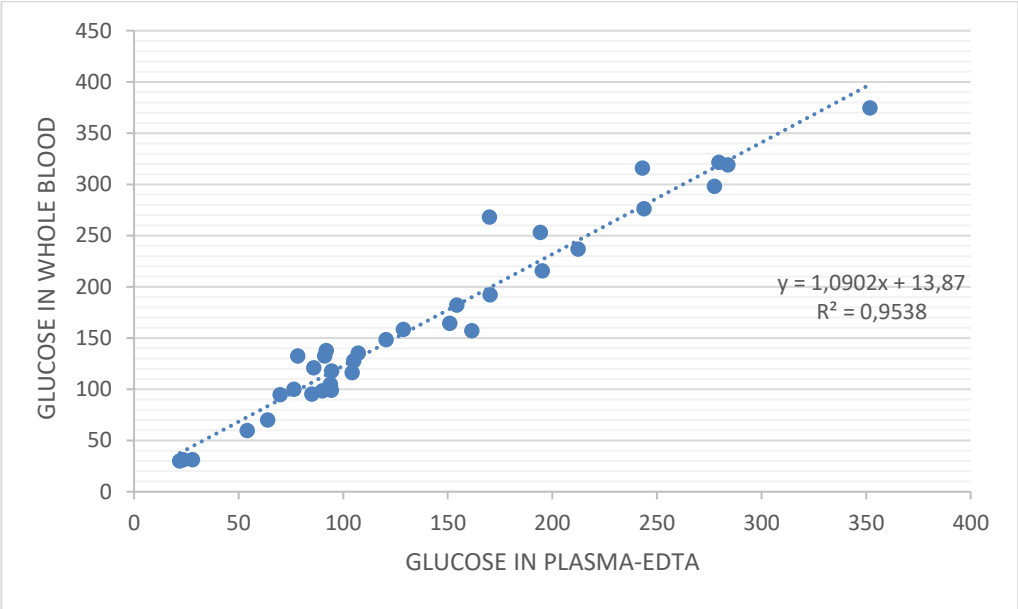


Figure 4. Scatter Plot Graph linearity between Glucose in Whole Blood and Glucose in whole blood

The scatter plot graph of blood glucose examination on whole blood samples can be seen in Figure 4. The results of this test obtained the following linear equation y = 1.0902x + 13.87 with a correlation coefficient (r^2) of 0.9538, so the Linearity Test was not accepted because of the value (r^2) < 0.98. For the results of the significant difference test on whole blood and plasma samples, the probability value (P) is 0.00. So it can be concluded that there are differences in accuracy in plasma samples.

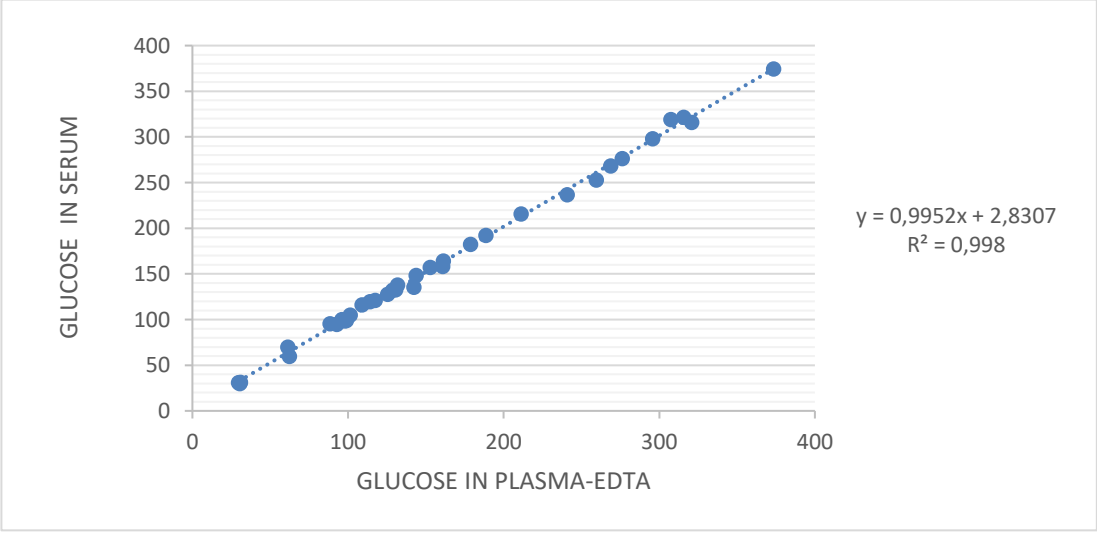


Figure 5. Scatter Plot Graph linearity between Glucose in serum and Glucose in plasma-EDTA

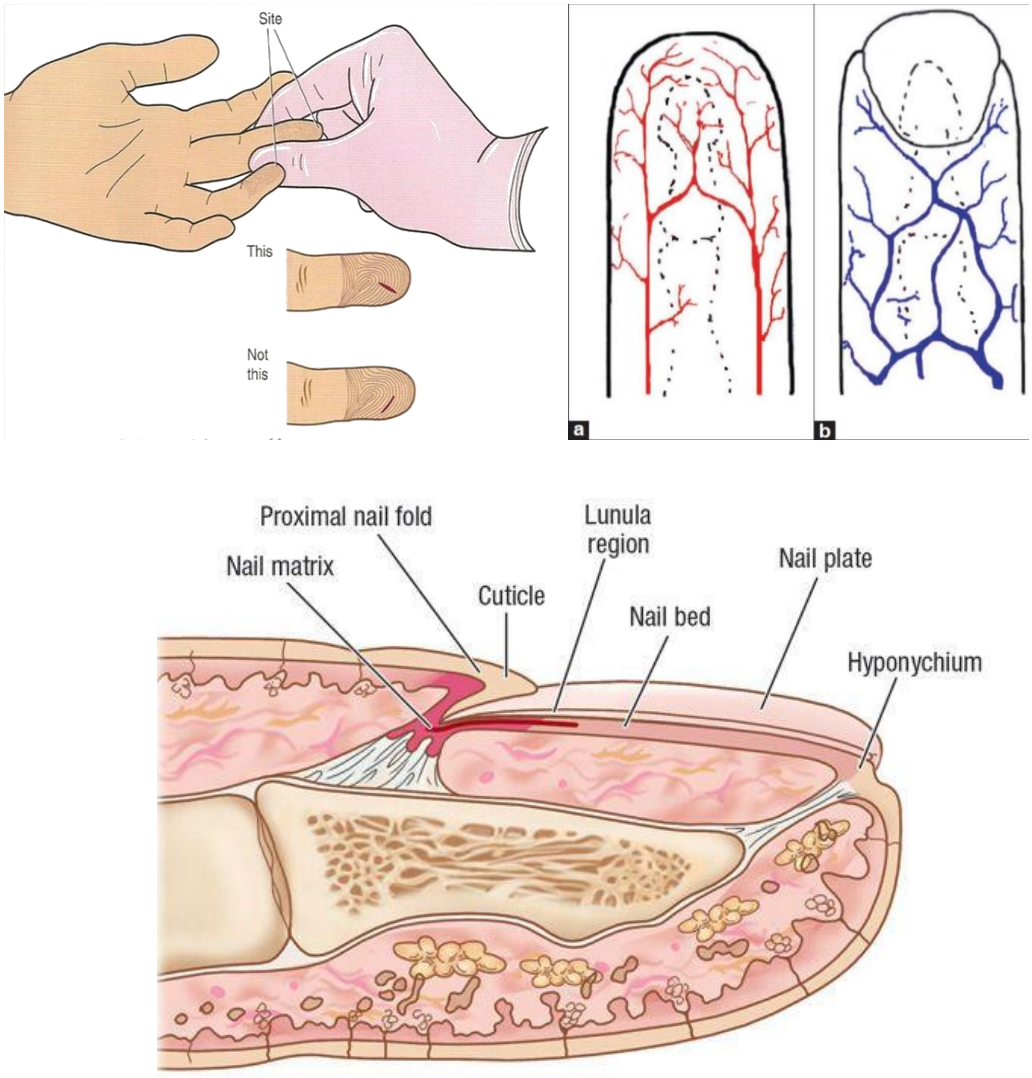
The scatter plot graph of blood glucose examination on plasma-EDTA samples can be seen in Figure 5. From the graph, the regression line equation is as follows Y= 0.9952x + 2.8307 and the correlation coefficient (r^2) is 0.998. So that the Linearity Test is accepted because the value (r^2) > 0.98. For the results of the Significant Difference Test or T-test on serum-plasma samples, the probability value (P) is 0.339. So it can be concluded that there is good accuracy and precision.

Discussion

This method of measuring blood glucose in POCT uses the principle of amperometry. The instructions for using the device state that the blood samples that can be used are whole blood, serum, and plasma-EDTA. The use of

POCT has good validity with various requirements, including blood sampling technique, procedures for using electrode strips, and measurement time. This error factor can be avoided for health workers who have been trained, but the general public who uses it directly can provide biased glucose test results.

Based on Figure 2. Illustrates that the limit of total error allowed for glucose examination using the POCT amperometry method is 10% according to CLSI. The total error includes systematic error and random error. The method's decision chart shows the POCT method's performance category with all factors that can be corrected by trained personnel. In Figure 2. The total error value of blood glucose examination results with the sample type variant is outside the allowable total error. The total error value obtained is caused by the magnitude of the random error made from the variance of the sample type. The pre-analytic error has a percentage of ± 72%, the analytical error is ±12%, and the post-analytic error is ±16% (Siren, et al. 2017). Random errors and systematic errors were obtained through linear regression equations of each variant against the control material.



Source: Wolff, et al. (2008)

Figure 6. The random error that has the greatest potential to increase pre-analytic error is blood sampling through capillaries.

Peripheral blood sampling requires a special technique that complies with international standards. Errors in choosing the site location and the depth of the puncture caused the blood samples obtained to be unrepresentative. To initiate peripheral blood sampling, the patient sits or lies in a chair with the arms extended on the armrests. The skin on the tip of the ring finger is then cleaned with 70% alcohol, and let dry. Insert the sterile needle using an auto click with a depth of 5 mm, and attach it to the collection container. Once collected, the scar is pressed using dry gauze for 5 minutes to stop the bleeding. Random errors at this stage are choosing the wrong site location and non-standardized needle insertion depth. If this happens, it will be challenging to perform a search to correct invalid check results.

Based on Figures 3 and 4, blood glucose levels of serum and plasma-EDTA samples have less than optimal precision, accuracy, and correlation with whole blood samples. In serum and plasma-EDTA samples, processing of serum through peripheral blood has problems because the samples obtained are quite small. In addition, finger

massage provides additional tissue fluid that comes out of the epidermis and hypodermis layers. the total glycogen synthase activity increases approximately 4-fold and hence glycogen synthase I activity increases at the same rate during the process of injury and epidermal regeneration. Changes in enzyme activity and metabolite concentrations were very similar to those found in the epithelium and the proportion of phosphorylase in the A form was increased relative to the normal epithelium (Harmon, 2014).

Statistically, the correlation between glucose in plasma-EDTA and glucose in whole blood is better than glucose in serum. In plasma-EDTA samples, there was no effect on glucose because EDTA reacted with calcium ions. The amount of glucose detected in plasma-EDTA and serum samples was more than in whole blood samples. This is caused by the reaction of glucose oxidase with glucose in the sample that occurs on the sample electrode strip, not blocked by blood cells. Glucose substrate is converted by GOx FAD Oxidized to Gluconolactone and $FADH_2$. The very small and narrow surface of the electrode provides a limit to the amount of glucose that can be detected (Accucheck, 2013). The presence of a sufficient number of blood cells can potentially provide mechanical resistance to the working electrode.

Other factors that can cause random errors include unstable instrument variations, variations in examination procedures including sampling; mixing and incubation time, air bubbles in the sample, variations in temperature (temperature), variations in operators or officers in using tools in the laboratory, open strip storage and non-standard finger pricking (Anasari, 2020). Systematic errors can also occur in the POCT device. Calibration process, selection of strip lot code, and expiration date. Therefore, making decisions on the results of the inspection is not only based on quality control but also on corrections and conclusions from all errors that occur

Based on this study, if the decision to decide the validity of the glucose test results in 3 types of samples is using the standard deviation (SD) control rules, the corrections made are only to assess accuracy and precision so that the interval or range of categories to receive measurement results is wide enough. Therefore, Westgard controls the distribution of the data over this interval using Westgard's 10 rules. Westgard's rule does not count Random Error (RE) & Systematic Error (SE). (Fatturahman et al, 2018). This rule only identifies possible Random Errors (RE) and Systematic Errors (SE) during the examination so that the results can be issued to the patient.

If the decision is made using the six sigma value, then the feasibility of the examination results is determined by Medical Decision Concentration (MDC), and sigma takes into account Systematic Error (SE) and Random Error (RE) during the examination process so that the quality of the examination is guaranteed. Even though the results of in-control measurements on the SD scheme, the performance of the method is not necessarily included in the six sigma category. (Rahayu et al, 2020) In the six sigma rule, there are Random Errors and Systematic Errors that cannot be eliminated so that control values cannot be issued to patients. Therefore, the solution to this problem is that the deviation is minimized so that the performance of the method is acceptable and the results can be issued.

Conclusion

The use of the POCT tool for blood glucose examination has a valid performance value. The deviation of the examination results is influenced by pre-analytical errors such as sampling and processing samples so that the total error obtained is higher than the total allowed error. The POCT tool can be used for all types of samples.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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