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Comparison Analysis of Total Cholesterol Level Examination Between Photometry and 3 Parameters Point of Care Testing Device

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Abstract: Total cholesterol is the composition of many substances including cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol. Cholesterol examination is one of the most frequent tests required in the laboratory to monitor vascular and cardiovascular diseases. Most clinical pathology laboratories use photometer to perform clinical chemistry checks. Cholesterol testing can also be done with Point of Care Testing (POCT) which has a working principle of biosensor technology. This research method is experimental, using 40 samples that can represent normal and pathological levels. All samples will be checked for total cholesterol with a photometer of CHOD-PAP method and 3 POCT Lipid Pro. The results showed linear regression y = 0.955x + 1.8325 with R^2 of 0.9955. The linear regression value is calculated by Total Error (TE), while the Total Error Allowable (TEa) cholesterol is 10%. The bias value is 0.31%, TE for normal level = 5.92% and TE for high pathological level = 3.00%, it can be stated the result of examination can be compared or accepted. The% TE value obtained is less than the TEa value of cholesterol. It can be concluded that the total cholesterol results examined by the photometer and LipidPro are comparable. For further research it is advisable to use a total cholesterol sample that has a value of more than 400 mg/dL.
Keywords: 3 Point of Care Testing (POCT); Cholesterol; Photometer

INTRODUCTION

Cholesterol, triglycerides and lipoproteins are important component of the composition of fat fractions in the human body (Dwizella, et al., 2018).

Cholesterol is unsaturated alcohol with steroid compounds. Cholesterol is essential for the function of animal cells and the basic constituent of cell membranes. Cholesterol is a precursor to various important compounds such as bile salts, adrenal steroid hormones and gonads. (Apro, 2015; Miller, 2013). Triglycerides are esters of glycerol fatty acids and it represent the main lipid components of fat from food and animal fat deposits (Tsoupras, et al., 2018).

Cholesterol and triglycerides are insoluble non-polar compounds transported by plasma lipoproteins (Welty, 2013). Plasma lipoproteins are divided according to density, electrophoretic mobility, size, and content of cholesterol, triglycerides, and proteins (Pan & Segrest, 2016). Lipoproteins are divided into five main classes, chylomicrons, very low density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) (Sacks & Brewer, 2014).

Preliminary studies of cholesterol are a key component of arterial plaque which raises the cholesterol hypothesis in the pathogenesis of atherosclerosis (Buja, 2014; Steinberg, 2013). Population studies have shown that elevated LDL cholesterol levels (Zmysłowski & Szterk, 2017) and apolipoprotein B (apoB) 100, The main structural protein LDL, is directly related to the risk of atherosclerotic cardiovascular events (ASCVE) (Ference et al., 2017). High triglyceride levels in the blood are one of the causes of atherosclerosis (Peng, Luo 2017). LDL peroxidation is the main key in the initiation and progression of atherosclerosis (Matsuura et al., 2014). Atherosclerosis is a systemic disease (Hoshino et al., 2018). About 60% of patients with peripheral artery disease will have ischemic heart disease, and 30% have cerebrovascular disease.

Within five years of diagnosis, 10-15% patients with intermittent claudication will die caused by cardiovascular disease. Therefore, treatment begins with identification and modification of common risk factors for peripheral artery disease, heart disease, and stroke (Frostegård, 2013; Morley et al., 2018).

Cholesterol examination is the most frequently requested tests in the laboratory to monitor vascular disease which includes coronary heart disease, cerebral vascular disease, and peripheral blood vessels (Sniderman et al., 2016). Most clinical pathology laboratories use photometer devices to conduct clinical chemistry examinations. This tool can determine the level of an ingredient in body fluids such as serum or plasma. Photometer is a standard method of clinical chemistry examination but has several disadvantages, such as expensive prices, invasive blood sampling and relatively longer examination times (Frostegård, 2013; Morley et al., 2018). The length of the examination can cause prolonged results and a delay in diagnosis. This will lead to services that are not suitable even fatal consequences can occur death. Continuously developed inspection technology is made to measure the inspection process, namely the Point of Care Testing (POCT) (Kost et al., 1999). Some countries like China have used POCT to reduce the number of people with dyslipidemia (Zhang et al., 2015).

Point of Care Testing (POCT) is a digital tool that uses cell measurements where certain reactions can take place. This cell can be a porous matrix, chamber or surface. Measuring devices can be visual, optical or monitoring electrochemical reactions that occur. Generally POCT mechanism use biosensor technology. Biosensor technology generated the electrical charge by chemical interactions between certain substances in blood and chemicals in dry reagents (strips) and will be measured then converted into numbers that correspond to the amount of electric charge. The resulting number is considered equal to the level of substance measured in blood. The advantage of the POCT is that the results are fast so that the diagnosis can be immediately enforced and the action / treatment can be given immediately. In addition, this tool is easy to use, the sample volume used is less, the device is smaller so it does not need a special room and can be carried or mobile. The disadvantage of this tool is that precision and accuracy are not good if compared to the reference method. In addition measurement capability is limited because it is mediated by temperature, humidity, and hematocrit value (Kemenkes, 2010).

The use of POCT only takes less than 2 minutes to perform cholesterol and triglyceride tests (Xavier et al., 2016). This makes POCT suitable for disease screening tests (Ferreira et al., 2015). POCT can be used as a screening filter and diagnosis of hypercholesterolemia (Peverelle et al., 2018), and CVD risk assessment (OAM, 2016), and long-term monitoring of patients who have undergone treatment (Plüddemann et al., 2012).

POCT tools can be used by someone who does not have the basis of laboratory knowledge, they do not understand quality control over the results of POCT examination. Based on Food and Drug Administration (FDA) data from the United States between 1984 and 1992, there were 24 deaths and 984 morbidity due to POCT use because of inappropriate testing. Errors in dealing with patients are usually 50% due to incorrect instructions (indications), 32% fail to act because they are not in accordance with the test results (Kost et al., 1999).

POCT that is often used in hospital and clinic laboratories is LipidPro. The advantage of using this tool is faster inspection time and can reduce medical waste because 1 strip can directly check 3 parameters. Lipid Pro is a tool to check blood lipid levels in vitro which helps with practical and easy measurement of total cholesterol, HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein), and triglycerides. The principle of this tool is to read the reflection of light based on changes in the color of the results of the enzymatic reaction between the substrate (total cholesterol, HDL cholesterol and triglycerides) and the enzymes in the strip. When the sample is dropped on the test strip, the sample will react and produce the color that will be read by the tool. The intensity of the measured color is proportional to its concentration. The instrument component changes the resulting color to a numerical value and displays the value on the screen (Osang Healthcare, 2017).

MATERIAL AND METHOD

This research method is experimental. The sample of the study was 40 patients who were examined for cholesterol levels in the Hospital in Bandung in the period February-March 2018. This research is approved by Jenderal Achmad Yani School of Sciences ethical committee declared by the ethical clearance number 013/KEPK/VIII/2018.

Before the examination, the patient had to fast beforehand to avoid the influence of the examination from the food. Specimens used are serum that is not hemolysis. The number of samples is 40 serum in accordance with Westgard provisions for comparability testing.

Cholesterol level examination using ELITech Selectra Pro-M and POCT Lipid Pro Photometers. The photometer used is capable of checking 266 tests / hour, can detect barcodes, with a Quartz-Iodine light source 12V-20W, absorbance photometric range of -0.1 to 3. ELITech Selectra Pro-M photometers using reagents have specifications can check cholesterol by range cholesterol levels of 20-600 mg / dL, measurements with the end point method, and 505nm wavelength. Cholesterol testing with POCT Lipidpro with the specifications of one tool can check total cholesterol, triglycerides, and HDL-cholesterol, measurement time of 2 minutes, and have 200 data memory. LipidPro uses a kit with 5 unit cholesterol esterase enzyme specifications, 3.3 units of cholesterol oxidase, 3.3 units of peroxidase, 4-9 µg of Aminoanthipirin, and 81 µg of aniline derivate.

The material used in this study was normal control serum (CTN17L04 LOT) and pathological (No 01-1030 LOT), patient serum, Elitech Group cholesterol reagent, POCT LipidPro total cholesterol strip and tools. Cholesterol examination with an ELItech Selectra Pro-M photometer performed an internal calibration before conducting the examination, followed by inserting 0.5-50 µL of serum into the cuvette, choosing a cholesterol check then clicking start (Elitech Group, 2018). Cholesterol examination with LipidPro by inserting the strip into the tool, entering the strip code on the tool, using the lancing device to take a blood sample and drop the sample on the strip. The results will be read after two minutes (Osang Healthcare, 2017).

The examination begins with internal quality stabilization with serum control checks. After the data is on the target value and range of examination, then it is followed by samples check. For normal control serum, the target value is 131 mg/dL with a range of 105-157 mg / dL. Pathological control series, the target value is 221 mg / dL with a range of 177-265 mg / dL. Data obtained from measurements of samples in the form of absorbance values for both Photometers and Lipids Pro. Data analysis technique uses linearity regression test calculation. Linear regression equation from Y to X is formulated shown by equation 1;

$$Y = a + b\,X$$

$$b = \frac{\Sigma[(x1 - \bar{x})(y1 - \bar{y})]}{\Sigma(x1 - \bar{x})^2}$$

$$a = \bar{y} - (b.\bar{x})$$

Description:
Y = dependent variable
X = independent variable
a = intercept
b = regression coefficient / slope

Then the bias calculation is performed. Bias is the difference between the results of the serum control level and the actual value. The bias value is processed into the total error value and the allowable (Tea) cholesterol total error value. Cholesterol TEa is 10% (Westgard, 1992). Tea eaquation shown by equation 2;

$$\%Bias = \frac{mean - TV}{TV} \times 100\%$$

$$CV = \frac{SD}{mean} \times 100\%$$

$$TE = |Bias| + (2.SD)$$

RESULT AND DISCUSSION

This research was conducted in one hospital in the Bandung with the parameter total cholesterol levels. The number of samples used were 40 samples and were examined for 7 days, it measured by using POCT and Photometer. Before measuring the patient sample, quality control (QC) performed by observing control serum during the examination period. The results of the control serum examination show that a sample check can be performed. The results of the examination of the normal control serum on the photometer are in table 1.

Table 1 Quality control result for Photometer

Description	Average mg/dL	SD mg/dL	CV %	Target range mg/dL	Intepretation
Normal control	108,75	1,78	1,89	105-157	Accepted

Table 2 Quality control result for POCT

Description	Average mg/dL	SD mg/dL	CV %	Target range mg/dL	Intepretation
Normal control	126,4	1,43	1,13	105-157	Accepted
Pathological control	206,8	1,39	0,7	177-265	Accepted

Description:
SD = Standard deviation
CV = Coefficient of Variation

Based on the measurement data of control values that performed everyday, the data is accepted because it is in the value range of the control material of photometer. After being calculated, it was obtained the value of SD = 1.78, CV = 1.89% and the value of TEa 10%. SD & CV values obtained <½ TEa so that the QC results are acceptable. Based on the results of this QC, the photometer can be used as a reference in measuring cholesterol levels.

Furthermore, also performed control quality for POCT. The monitoring was carried out using normal and high pathological control serum. POCT control material test results can be seen in table 2. The POCT is used to compare, therefore the impression test is done first. The control material was examined for 5 consecutive days and carried out in duplicate. This impression test aims to estimate random errors in a method and also the initial step in determining the method validation (Biswas et al., 2015). POCT control serum measurement results for normal levels found SD = 1.43 mg / dl, CV = 1.8% while high pathological values obtained SD = 1.39 mg / dl, CV = 0.7%, and TEa at this examination is 10%. After the calculation is obtained SD and CV values <½ TEa value, then the tool has a good impression, so it can be used to compare and measure samples.

From the results of the control serum measurements carried out for 5 days showing the value of the control received either using a Photometer or POCT, the test can be done using a photometer and also POCT. The results of measurements and linear regression calculations can be seen in table 3.

Table 3 shows the average results obtained from photometer is 205.1 mg/dL while the average obtained from POCT is 206 mg/dL. Slope value (b) = 0.9955x, while intercept value (a) = 1.83 and linear regression value (R^2) = 0.9915. After obtaining a linear regression value calculated TE (Total Error), while the total allowable error value (TEa) cholesterol is 10% (Westgard, 1992). The results obtained bias value is 0.31%, TE for the normal level is 5.92% and TE for the high pathological level is 3.00%. It can be stated that the results of the examination can be compared or accepted. The data is plotted into the graph to find out the equation of the line and the regression value can be seen in figure 1

Figure 1 shows the linearity of sample absorption measured using a photometer compared to POCT obtained R2 = 0.995. Then the correlation and regression tests were carried out. The results of the correlation and regression test can be seen in table 4.

The R2 value is 0.995 then proceed with calculating linear regression, and the test used was T Pearson. T test showed p-value is 0.000, it can be stated that there is a correlation between the result performed by photometer and POCT. For more details, correlation and regression test is conducted. Correlation test results obtained 0.995, the results is more than 0.99 then the T test used is T person.

Table 3 Cholesterol total result

No.	POCT mg/dL	Photometer mg/dL	y-x
1.	127	125	2
2.	130	127	3
3.	136	133	3
4.	141	145	-4
5.	160	155	5
6.	167	160	7
7.	170	166	4
8.	170	168	2
9.	173	174	-1
10.	173	177	-4
11.	180	179	1
12.	184	180	4
13.	180	181	-1
14.	179	184	-5
15.	192	188	4
16.	192	189	3
17.	188	190	-2
18	191	194	-3
19.	193	198	-5
20.	193	199	-6
21.	207	203	4
22.	224	221	3
23.	233	230	3
24.	221	225	-4
24.	230	233	-3
26.	211	206	5
27.	258	251	7
28.	230	226	4
29	251	246	5
30	218	221	-3
31.	227	224	3
32.	235	230	5
33.	224	219	5
34.	201	204	-3
35.	273	269	4
36.	278	275	3
37.	266	269	-3
38.	265	270	-5
39.	276	281	-5
40.	293	289	4
$\bar{x}$	206 mg/dl	205,1 mg/dl	

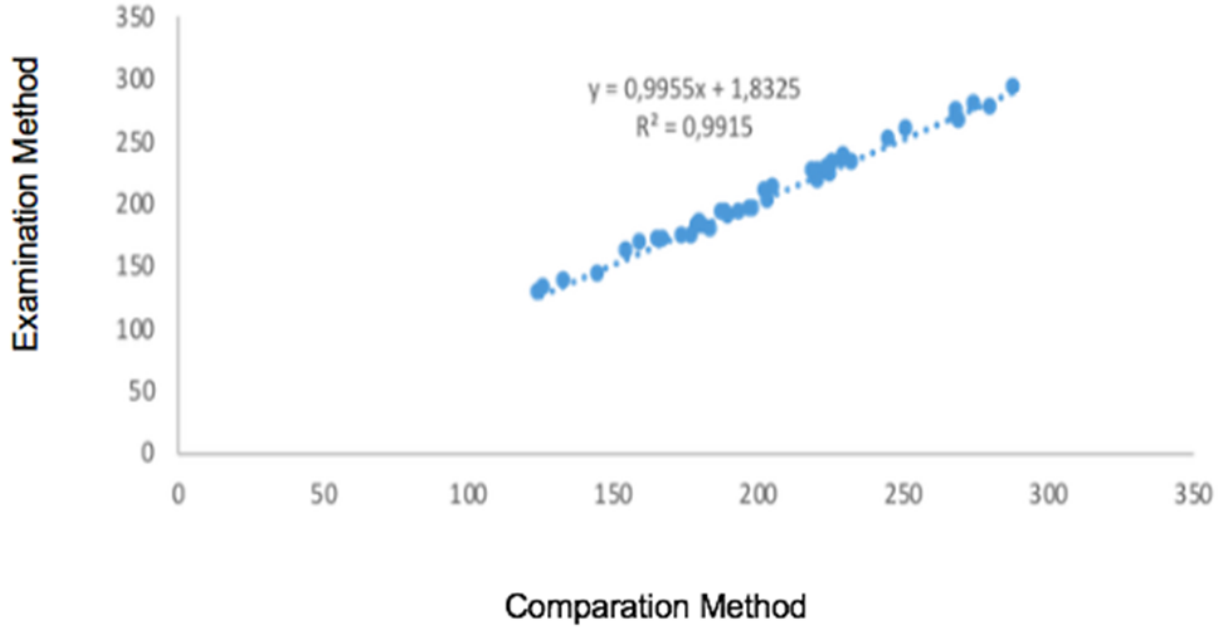

Fig. 1. Graphic Photometer and POCT comparison test

Table 4 Correlation and Regression for Total Cholesterol Examination using POCT with Photometer

	$\bar{x}$	SD	r	t	p-value
POCT	208,02	42,48	0,995	66,593	0,000
Fotometer	207,15	42,86			

The T person test is obtained p-value 0.000, which means the value less than 0.005, it can be stated Ha: there is correlation. In this correlation test has a limit of 0-1.0, in this test the correlation value obtained is 0.995, so it shows a very strong correlation because the value is close to 1.0.

In this study, all subjects had total cholesterol levels in accordance with the limit of ability (range) of detection devices that is 100-400 mg/dL. This is related to the cut-off value of the tool and also the limited number of enzymes contained in the test strip. The minimum value on the measurement results obtained is 127 mg / dl and the maximum value is 293 mg / dl, then the sample results obtained into the range also represent normal and pathological values. Thus it can be stated that there is correlation between the POCT and the Photometer, but only at this limit. For a value of 300-400 it still needs to be tested furthermore. The availability of POCT for monitoring lipid levels has increased in recent years (Abel, 2015). Each POCT must be validated for biases and inaccuracies to ensure that appropriate screening decisions and disease tests are carried out (Park et al., 2016).

Bias (inaccuracy) is defined as the difference between laboratory results and standards. The National Cholesterol Education Program (NCEP) in the United States recommends a bias value of 3% for cholesterol and 5% for triglycerides (McPherson & Pincus, 2017). In this study, the bias value is not more than 3%, which is 0.31%. Impression refers to the reproducibility of test results. Impression allowed is 3% for cholesterol and 5% for triglycerides (McPherson & Pincus, 2017)

Overall, the analytical results of POCT must be the same as the equipment used in the laboratory such as a photometer, so as to ensure that POCT does not endanger patient examination standards and clinical decision making. In addition, knowledge is needed especially for lay users, that POCT is different from laboratory settings (Onovughakpo-Sakpa et al., 2015).

CONCLUSSION

The results of examination of total cholesterol levels using a photometer and POCT showed results that were almost the same as the tolerance limit or TE <Tea and a bias (inaccuracy) of 0.31%. It can be concluded that total cholesterol results using comparable photometer and POCT Lipid Pro.

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