



DOI:

10.22301/IJHMCR.2528-3189.54

Article can be accessed online on:

<http://www.ijhmcr.com>

ORIGINAL ARTICLE

**INTERNATIONAL JOURNAL
OF HEALTH MEDICINE AND
CURRENT RESEARCH**

COMPARISON OF PROTHROMBINE TIME MEASUREMENT BETWEEN ACL TOP 350 CTS AND CS-2100i

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ARTICLE INFO

Article History:

Received 15th June, 2016

Received in revised form

13th July, 2016

Accepted 17th August, 2016

Published online 30th September,
2016

Key words:

PT, coagulation, comparison test,
reference value.

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ABSTRACT

Background: There are many coagulometer that can be used for prothrombin time (PT) measurement. This study was designed to compare the results of PT measured by ACL TOP 350 CTS that uses RecombiPlasTin 2G reagents and by CS-2100i that uses Dade Innovin reagents. **Materials and methods:** We use 40 citrate plasma samples with PT that have been measured by CS-2100i and we compare them with ACL TOP 350 CTS. **Results:** We found good agreement and strong correlation for comparison test. Bland Altman analysis show that 95% of the data is in conformity limits. The result of Spearman correlation test was $r = 0.975$, $p = 0.0001$. **Conclusion:** There were an agreement with strong and significant correlation between PT measurement by means of the CS-2100i and ACL TOP 350 CTS. PT test results of both these coagulometers can be used interchangeably.

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Citation: Connie Frances Kumaat¹, Astuti Giantini¹, 2016 “Comparison Of Prothrombine Time Measurement Between Acl Top 350 Cts And Cs-2100i”, *International Journal of Health Medicine and Current Research*, 1, (01), 54-62.

INTRODUCTION

Prothrombin time (PT) measurement is a screening test for hemostasis disorders involving the extrinsic and common pathway. Additionally, PT was also used to monitor oral anticoagulant therapy that works by inhibiting the formation of vitamin K-dependent factor, namely prothrombin, F. VII, and F. X. PT test results can be reported in seconds, ratio, prothrombin activity, and index.

Prolonged PT can be found in extrinsic and deficiency, or it could be caused by inhibitor. Short PT found in the hypercoagulable state that caused by the increased of clotting factors and tissue factor.¹

There are many coagulometer that have been known to be used for PT measurement. In Dr. Cipto Mangunkusumo General Hospital Jakarta (RSUPNCM), we used coagulometer from Sysmex CS-2100i. Currently ACL TOP Family from Instrumentation Laboratory that been used abroad previously began to be marketed in Indonesia. This study aimed to compare the results of the PT measurement by ACL TOP 350 CTS (member of ACL TOP Family) which uses RecombiPlasTin 2G reagents and by CS-2100i which uses Dade Innovin reagents.

METHODS

Research subject

Sample size for this study was 40 citrate plasma which came to laboratory of RSUPNCM that request for PT measurement. Fourty citrate plasma samples consist of 20 samples with normal PT value, 10 samples with short PT value, and 10 samples with prolonged PT value based on measurement by CS-2100i.²

Materials, reagents, and controls

Material for comparison test used 2.7 mL of venous blood that were placed into tubes that had contained 0.3 mL of 0.109 M sodium citrate. Tubes should be inverted immediately, as much as 3-6 times. Tubes should be centrifuged 1500 g for 15 minute at room temperature to obtain platelet poor plasma (PPP). PT measurement carried out maximal 24 hours after materials obtained, measurement materials are placed in a room temperature.³ PT materials for measurement, shall not be refrigerated because the cold temperatures can activate F.VII.¹

CS-2100i used Innovin Dade reagents with lot numbers 539 370, with expired date of August 13, 2018. The composition of that reagent consists of recombinant human tissue factor, synthetic phospholipids, calcium ions, heparin neutralizing components, buffers, and stabilizers (bovine serum albumin). Recombinant human tissue factor serves as tissue thromboplastin. Synthetic phospholipid serves as platelet factor 3 (PF3). Calcium ions serve to replace for calcium ions from blood of the subject that has been bonded to citrate anticoagulant. Heparin neutralizing component serves to neutralize heparin if the sample contains heparin. Heparin cooperate with antithrombin (AT) to inhibit the activity of thrombin and F.Xa in the common pathway. Thus,

common pathway coagulation factor heparin will also prolong the PT. Because PT devoted as a test for monitoring oral anticoagulant therapy, heparin neutralizing reagent were given to eliminate the effects of heparin on the PT measurement. So if the results of the PT was prolonged, it can be ascertained was due to oral anticoagulant therapy or deficiency of coagulation factors in the extrinsic and common pathway, not because of heparin therapy. Control materials used are the Sysmex normal plasma control (CPN) with lot number 507 712 expired date July 8, 2017 and Sysmex pathological plasma control (CPP) with lot number 509 984 expired date May 10, 2017.

ACL TOP 350 CTS tool used reagent RecombiPlasTin 254 715 2G with the lot number, the expired date February 2017. The composition of that reagent consists of recombinant human tissue factor, synthetic phospholipids with stabilizers, preservatives, and buffers. diluent containing calcium chloride, polybrene, and preservatives used to dissolve reagents. Polybrene is a trade name of hexadimethrine bromide which is a heparin neutralizer. Control materials used are Instrumentation Laboratory normal controls with lot number 254 540 expired date February 2018, Instrumentation Laboratory abnormal low control with lot number 657 090 expired date June 2018, and Instrumentation Laboratory abnormal high control with lot number 556 262 expired date May 2018.

The principle of measurement

CS-2100i coagulometer used optical method with multiwavelength technology (340 nm, 405 nm, 575 nm, 660 nm and 800 nm). The wavelength of 405 nm, 660 nm and 800 nm are used for parameters with coagulation methods, including PT. This coagulometer also introduced a system of detection of hemolytic, icteric, and lipemic conditions on the sample, which is used as a reference wavelength displacement if it is found to be potential interference on readings at the wavelength that was used in the beginning. Beam was directed to the mixture of plasma and reagents. When there was a change in refractive index due to the formation of fibrin from fibrinogen, there will be a change of light transmission. The change of the transmitted light is proportional to the amount of fibrin is formed. As soon as the reagent is added, prior to coagulation, the degree of intensity of light that is absorbed is considered 0%. Then immediately after clot formed, the degree of intensity is considered 100%. Coagulation time is the time required to change the intensity of absorbed light by 50%.⁴

ACL TOP 350 CTS using coagulation methods with a wavelength of 671 nm for measurement PT. The detection system of hemolytic, icteric, and lipemic conditions of the sample is also available on this instrument. For turbidity detection, wavelength of 671 nm is used. For the detection of turbidity and hemolytic, wavelength of 535 nm is used. As for the detection of turbidity, hemolytic, and icteric, wavelength of 405 nm is used. Clot detection is based on the principle that when the reagents is added to the sample, fibrinogen is converted to fibrin. Light passes through a medium will be absorbed by the threads of fibrin, then transmitted over the sample. The light transmission will be captured by a photodetector which is positioned at 180° to the source. The light absorption will increase during fibrin formation takes place. As a result, the transmission of light through the sample will continue to decline. Photodetector will generate an electrical signal in proportion to the transmission of light captured. The electrical signals are then processed by computer software through a series of algorithms to determine the point of bekuan.⁵

Working method

Test of within run accuracy and precision is done both on the CS-2100i and the ACL TOP 350 CTS. Test of within run accuracy was carried out by using citrate plasma control for the normal, short, and prolonged PT. Test of accuracy is simply done by using a control material. Within run precision and accuracy test used control material performed 10 times in a row, while the citrate plasma material is only performed 5 times in a row. In addition, the accuracy of the test was also done between day during 10 days using control materials.

Comparison test conducted by measuring the remaining sample material that has been measured on CS-2100i PT to the ACL TOP 350 CTS.

Statistic analysis

Statistical analysis in this study used Statistical Product and Service Solution (SPSS) ver.20 and MedCalc®ver.16.4.3. SPSS is used to find the correlation test. MedCalc® program is used for Bland Altman analysis.

To test the correlation, initially test data distribution with for each groups of the PT data (CS-2100i and ACL TOP 350 CTS). Shapiro-Wilk test was chosen for the test of data distribution for only 40 data. If the data were normally distributed ($p > 0.05$), Pearson correlation test will be used. If the data distribution is not normal ($p \leq 0.05$), a logarithmic transformation will be performed to normalize the data. If transformation was not successful, then the Spearman correlation test will be used. For Bland Altman analysis, the results of the measurement of PT by the CS-2100i and ACL TOP 350 CTS were incorporated into the program. The program will display the Bland Altman plot when we choose a menu Bland Altman automatically.

This research was conducted at the laboratory RSUPNCM in March 2016 and has received a permission letter by the Research Ethics Committee of the Faculty of Medicine, University of Indonesia number 936/UN2.F1/ETIK/2015.

RESULTS

Test of accuracy and precision

Within run precision test result of CS-2100i with CPN obtained an average 10.9 seconds, standard deviation (SD) 0.05, and a coefficient of variation (CV) 0.46%. With CPP, we obtained a mean of 18.2 seconds, SD 0.06, and CV 0.25%. In the accuracy test results by using CPN, we obtained deviation (d) ranges from 1.9 to 2.8% and total error (TE) 2.93%. By using CPP we obtained deviation (d) ranged between -1.6 - (-0.5)% and TE 1.21%. (Table 1, and Table 2).

Table 1. Within run precision and accuration test using control material on CS-2100i

Test number	PT (second)	
	CPN	CPP
1	10.8	18.2
2	10.8	18.2
3	10.9	18.1
4	10.9	18.2
5	10.9	18.3
6	10.9	18.3
7	10.9	18.2
8	10.9	18.2
9	10.8	18.2
10	10.9	18.2

Mean (second)	10.9	18.2
Target (second)	10.6	18.4
SD	0.05	0.06
CV (%)	0.46	0.33
d (%)	1.9 – 2.8	-1.6 – (-0.5)
Bias (%)	2.83	1.09
TE (%)	2.93	1.21

Table 2. Within run precision and accuracy test using control material on ACL TOP 350 CTS

Test number	PT (second)		
	Normal	Abnormal Low	Abnormal High
1	11.1	19.2	30.5
2	11.2	19.6	31.3
3	10.9	19.0	31.4
4	11.2	19.2	31.1
5	11.0	18.8	31.1
6	11.2	19.4	31.9
7	11.2	19.4	31.0
8	11.2	19.3	30.8
9	11.1	18.8	31.7
10	11.1	19.3	31.0
Mean (second)	11.1	19.2	31.2
Target (second)	11.1	22.3	37.8
SD	0.10	0.26	0.41
CV (%)	0.93	1.37	1.32
d (%)	-1.8 – 0.9	-15.7 – (-12.1)	-19.3 – (-15.6)
Bias (%)	0	13.9	17.46
TE (%)	0.2	14.42	18.28

By using normal PT value sample for within run precision test of the samples, we obtained a mean 9.7 seconds, SD 0.09, and CV 0.93%. From the short PT value samples, we obtained mean 9.3 seconds, SD 0.00, and CV 0%. From the prolonged PT value samples, we obtained mean 50.9 seconds, SD 0.31, and CV 0.61%. (Table 3 and Table 4).

Table 3. Within run precision test using citrate plasma sample on CS-2100i

Test number	PT (second)		
	Short PT	Normal PT	Prolonged PT
1	9.3	9.7	51.3
2	9.3	9.6	50.8
3	9.3	9.6	51.1
4	9.3	9.6	50.5
5	9.3	9.7	50.8
Mean (second)	9.3	9.7	50.9
SD	0.00	0.09	0.31
CV (%)	0.00	0.93	0.61

Table 4. Within run precision test using citrate plasma sample on ACL TOP 350 CTS

Test number	PT (second)		
	Short PT	Normal PT	Prolonged PT
1	9.5	9.9	53.7
2	9.6	9.9	53.4
3	9.5	9.8	52.5
4	9.5	9.8	52.0
5	9.5	9.8	51.5
Mean (second)	9.52	9.8	52.6
SD	0.04	0.05	0.93
CV (%)	0.47	0.56	1.76

Test of ACL TOP 350 CTS within run accuracy with normal controls obtained an average of 11.1 seconds, SD 0.10, and CV 0.93%. With abnormal low control, we obtained mean 19.2 seconds, SD 0.26, and CV 1.37%. With high abnormal control, we obtained mean 31.2 seconds, SD 0.41, and CV 1.32%. In the accuracy test using normal control, we obtained deviation (d) ranged between -1.8 - 0.9% and TE 0.2%. By using abnormal low control, we obtained deviation (d) is ranged from -15.7 - (12.1)% and TE 14.42%. By using high abnormal control, we obtained deviation (d) ranged from -19.3 - (-15.6)% and TE 18.28%.

(Appendix 1). By using normal PT value sample for within run precision test, we obtained mean 9.8 seconds, SD 0.05, and CV 0.56%. By using short PT value sample, we obtained mean 9.5 seconds, SD 0.04, and CV 0.47%. By using prolonged PT value sample, we obtained mean 52.6 seconds, 0.93 SD, and CV 1.76%. (Appendix 2).

In between day precision test for CS-2100i with CPN, we obtained mean 11.0 seconds, SD 0.25, and CV 2.33%. By using CPP, we obtained mean of 17.8 seconds, SD 0.37, and CV of 2.06%. (Table 3).

Table 5. Between day precision test using control material on CS-2100i

Day	PT (second)	
	CPN	CPP
1	10.8	18.2
2	10.9	17.5
3	10.9	17.5
4	11.6	17.7
5	11.1	17.9
6	10.8	17.4
7	11.0	18.2
8	10.8	17.6
9	10.8	17.8
10	10.9	18.6
Mean (second)	11.0	17.8
Target (second)	10.6	18.4
SD	0.25	0.39
CV (%)	2.24	2.17

Table 6. Between day precision test using control material on ACL TOP 350 CTS

Day	PT (second)		
	Normal	Abnormal Low	Abnormal High
1	11.1	19.2	30.5
2	11.3	20.1	31.3
3	11.3	20.6	31.4
4	11.5	21.2	31.1
5	11.6	20.6	31.1
6	11.5	20.2	31.9
7	12.0	21.2	31.0
8	11.8	20.3	30.8
9	11.4	19.5	31.7
10	11.2	19.3	31.0
Mean (second)	11.5	20.2	31.2
Target (second)	11.1	22.3	37.8
SD	0.28	0.72	0.41
CV (%)	2.40	3.55	1.32

In between day precision test for ACL TOP 350 CTS with normal control, we obtained mean 11.5 seconds, SD 0.26, and CV 2.23%. By using abnormal low control, we obtained mean 20.3 seconds, SD 0.66, and CV 3.24%. By using high abnormal control, we obtained mean of 32.4 seconds, SD 0.94, and CV of 2.92%. (Table 7).

Table 7. Data Subject of Research Test Koparasi

No	CS-2100i (detik)	ACL TOP 350 CTS (detik)	Selisih (detik) (CS – ACL)	Rerata (detik) (CS+ACL/2)
1	9,5	9,3	0,2	9,4
2	9,6	9,4	0,2	9,5
3	9,6	9,7	-0,1	9,65
4	9,5	9,2	0,3	9,35
5	9,6	9,7	-0,1	9,65
6	9,1	9,1	0	9,1
7	9,7	9,6	0,1	9,65
8	9,2	9,4	-0,2	9,3
9	9,0	9,1	-0,1	9,05
10	9,6	9,6	0	9,6
11	10,4	10,6	-0,2	10,5
12	10,1	10,2	-0,1	10,15
13	10,0	9,9	0,1	9,95
14	9,9	10,0	-0,1	9,95
15	9,8	10,1	-0,3	9,95
16	10,0	9,9	0,1	9,95
17	10,6	10,8	-0,2	10,7
18	9,8	9,8	0	9,8
19	10,0	10,0	0	10
20	10,1	10,4	-0,3	10,25
21	10,2	10,2	0	10,2
22	10,6	10,7	-0,1	10,65
23	9,8	10,0	-0,2	9,9
24	10,7	10,8	-0,1	10,75
25	10,8	10,8	0	10,8
26	10,0	10,3	-0,3	10,15
27	9,9	10,1	-0,2	10
28	10,1	10,4	-0,3	10,25
29	10,9	11,1	-0,2	11
30	10,1	9,9	0,2	10

31	16,1	16,0	0,1	16,05
32	14	14,5	-0,5	14,25
33	12,5	12,9	-0,4	12,7
34	16,5	17,1	-0,6	16,8
35	23,4	22,7	0,7	23,05
36	22,7	23,2	-0,5	22,95
37	57,6	58,8	-1,2	58,2
38	20,4	20,8	-0,4	20,6
39	14,3	14,8	-0,5	14,55
40	11,8	11,3	0,5	11,55

Analysis of the test data comparison

From the Shapiro-Wilk normality test was obtained $p < 0.001$ both in the group of data of the CS-2100i PT and ACL TOP 350 CTS, which means the data is not normally distributed. We done logarithmic transformation to normalize the data, but not successful. Therefore we selected nonparametric Spearman

correlation test. Spearman correlation test obtained a very strong correlation with $r=0.975$ and $p<0.0001$. Based on linear regression, the obtained equation: $ACL\ TOP\ 350\ CTS = (1.021 \times CS-2100i) - 0.152$. Results of correlation test between the value of PT which is measured by CS-2100i and ACL TOP 350 CTS can be seen in Figure 1.

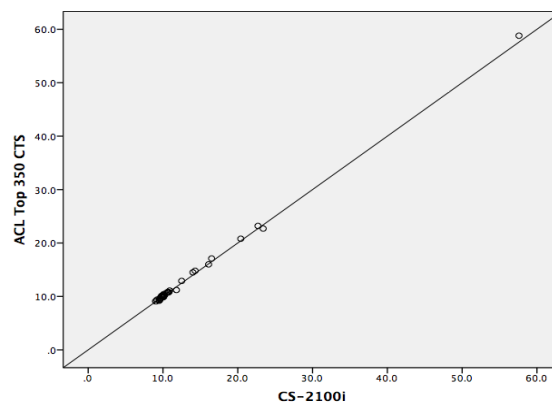


Figure 1. Spearman correlation of PT measurement between CS-2100i and ACL TOP 350 CTS

Bland-Altman analysis used to determine the compatibility between the value of the PT measured by CS-2100i and ACL TOP 350 CTS. The results were 38 samples (95%) have a good agreement, with a mean difference of -0.12 seconds. Limit of agreement between

the two results of the measurement ($\pm 1.96\ SD$) is between -0.74 seconds to 0.51 seconds. The agreement test results of PT value measured by CS-2100i and ACL TOP 350 CTS can be seen in Figure 2.

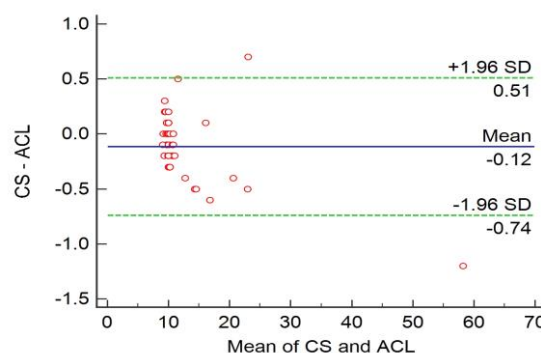


Figure 2. Bland-Altman plot of PT value between CS-2100i and ACL TOP 350 CTS

DISCUSSION

Within run and between day precision test using control material and citrate plasma were done in this study. The results obtained on both the CS-2100i and the ACL TOP 350 CTS are all fulfilled the requirement of the CV that is defined by NCCLS / CLSI for PT test, that is 5%.⁶ Prakoso study also testing the within run and between day precision on CS-2100i. For within run CV, they obtained mean 0.54% with CPN and 0.37% with CPP. For between day CV, they obtained mean 2.67% with CPN and 1.86% with CPP.⁷ Widiasih study testing within run and between day precision only by using CPN. Within run and between day CV obtained is 0.62% and 1.07%.⁸ The factory (Instrumentation Laboratory) testing testing the within run and between day precision on the ACL TOP 350 CTS. For within run, they obtained CV 0.7%, 0.9%, and 0.9% respectively for the normal, abnormal low, and abnormal high control. For between day, they obtained CV 1.2%, 1.7%, and 2.2% respectively for normal, abnormal low, and abnormal high control.⁹

Limits on the accuracy (d) of the factory to the CS-2100i and ACL TOP 350 CTS does not available. Other studies also do not include the accuracy of the test results so that the accuracy of test results in this study can not be compared. According to the Clinical Laboratory Improvement Amendments (CLIA), the total error allowable (TEA) for PT is 15%.¹⁰ In this study, TE

from CS-2100i using either CPN and CPP meets the recommendation of TEA limit set by CLIA. As for TE

from ACL TOP 350 CTS, meets TEA limit set by CLIA only by using the normal controls. In general, the within run precision and accuracy test results of CS-2100i is better than ACL TOP 350 CTS.

Correlation between the value of PT on CS-2100i with ACL TOP 350 CTS in this study show a strong positive correlation and this was statistically significant ($r = 0.975$; $p = 0.0001$). The positive correlation indicates that the higher the value of the PT of CS-2100i will be the higher PT value of the ACL TOP 350 CTS. The results of this study are similar to Milos et al study that compared the results of PT between ACL TOP and BCS of Dade Behring with the value of $r = 0.913$ and $p = 0,0247$.¹¹

In Bland Altman analysis in this study, the mean difference was -0.12 seconds with the limit of agreement between -0.74 seconds to 0.51 seconds and 95% of the data are within the limits of agreement. These results suggest that there is an agreement between the PT

measurement on CS-2100i and ACL TOP 350 CTS. Milos et al study that expressed the results of PT in the percentage also obtain similar results with this study. They obtained mean difference -0.8% with limit of agreement between -25.7% to 24.1% and 95.8% of the data were within the limit of agreement.¹⁴ Rathod et al compared the results of PT between ACL TOP and Compact Max from Stago. They distinguish the analysis of samples with normal and abnormal PT values. The analysis of normal PT value samples obtained mean difference 0.77 seconds with limit of agreement between -1.125 to -0.415 seconds and 95% of the data is within the limit of agreement. The analysis of samples with abnormal PT values obtained mean difference -1.234 seconds with limit of agreement between -3.340 seconds to 0.871 seconds and 100% of data are within the limit of agreement.¹²

CONCLUSION

There were an agreement with strong and significant correlation between PT measurement by means of the CS-2100i and ACL TOP 350 CTS. In Bland Altman analysis, we obtained 95% of the data has a good agreement. In Spearman correlation test, we obtained $r = 0.975$ and $p = 0.0001$. PT test results of both these coagulometers can be used interchangeably.

ACKNOWLEDGEMENTS

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