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PROCALCITONIN MEASUREMENT USING PROCALCITONIN EASY DIAGNOSIS IMMUNOCHROMATOGRAPHIC ASSAY AND BRAHMS KRYPTOR SENSTIVE

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ABSTRACT

Aim: Procalcitonin is a calcitonin prohormone, its elevation used as one of many sepsis parameters. Procalcitonin Easy Diagnosis is an immunochromatographic cassette, its color intensity shift measured with imaging technology to determine procalcitonin level. Brahms Kryptor is an immunoassay that *uses* TRACE (Time Resolved Amplified Cryptate Emission) method. This study aims to determine agreement between the two method of measurement. **Method:** Test for comparison of two method is used. Precision test performed using control cassette with fixed level (25 ng/mL), run 10 times consecutively. Precision test using patient samples performed at low level (< 0.5 ng/mL), high (≥ 0.5 ng/mL), and very high (> 2 ng/mL), each runs 5 times consecutively. Subject of the study settled at 40, based on minimum sample requirement for comparison test. Correlation test and Bland-Altman plot used to analyze data set. P value < 0.05 is considered significant with 95% confidence interval. **Results:** Coefficient of variant (CV) obtained from control cassette is 2.528 %. Result for CV in low level (0.13 ng/mL), high 0.67 ng/mL), and very high (2.34 ng/mL) are 13.07%, 21.81%, and 13.77% respectively. Spearman correlation resulted r = 0.898 (p < 0.05). Bland-Altman analysis performed in four groups of data set, < 0.5 ng/mL, ≥ 0.5 ng/mL, ≥ 2 ng/mL, and ≥ 10 ng/mL.

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In the first group, there are two result outside limits of agreement. Agreement between the two methods is 95 %. **Discussion:** There is a significant difference between precision test using control cassette and patient samples. Control materials that fit standard requirement is needed. There is a good correlation between two methods. The two value outside limits of agreement in the first group are higher than > 0.2 ng/mL mean, showing a decreased agreement in higher procalcitonin level. **Conclusion:** There are good correlation and agreement between the two methods. It is advised to provide control material that fit control material requirements.

INTRODUCTION

Procalcitonin is calcitonin prohormone widely used as sepsis marker. Procalcitonin's ability to differentiate infectious inflammation from sterile inflammation is an advantage from other sepsis marker. In addition, procalcitonin correlates with bacterial load and decreased when host immune system and/or antibitotic usage succeed to overcome infections. Procalcitonin's elevation over two standard deviation from normal individual range is one of sepsis diagnostic criteria if there is documented or suspected sepsis infection. Therefore, a fast, reliable, and user friendly measurement method needed for this parameter. [1,2]

Procalcitonin Easy Diagnosis is a cassette strip that uses immunochromatographic (ICT) assay principle. Whole blood, serum, or heparinized plasma may be used for different type of cassette. Color intensity formed quantified with an imaging technology used in immune quantitative analyzer. The Immune quantitative analyzer has 30x40x20 cm dimension, easy to use, and give fast result. Procalcitonin cut-offs for ICT assay are < 0.5 ng/mL (systemic infection unlikely), $\geq$ 0.5 – 2 ng/mL (systemic infection possible), $\geq$ 2 – 10 ng/mL (systemic infection very likely), and $\geq$ 10 ng/mL (severe sepsis, sepsis shock likely).[3-5]

Brahms Kryptor is an immunoassay with TRACE (Time Resolved Amplified Cryptate Emission) principle. This assay measures changes in signal strength when there is an energy shift between europium labelled antibody and XL665 labelled antibody. The shift will occur when antibody-procalcitonin-antibody complex formed. Longer decrease in signal strength proportional with higher procalcitonin concentration. Procalcitonin cut-offs for this measurement method are < 0.05 ng/mL (healthy individual), < 0.5 ng/mL (local bacterial infection), $\geq$ 0.5 ng/mL - < 2 ng/mL (sepsis), $\geq$ 2 ng/mL - < 10 ng/mL (severe sepsis), dan $\geq$ 10 ng/mL (very severe sepsis leading to septic shock).[6]

This study aimed to compare and determine agreement in procalcitonin measurement between Procalcitonin Easy Diagnosis and Brahms Kryptor Compact Procalcitonin.

METHODS AND MATERIALS

The study methods are cross-sectional two methods comparison performed at Clinical Pathology Laboratory Dr.Cipto Mangunkusumo National General Hospital between July 2014 – September 2014. Whole blood EDTA samples are used in this study. All measurement are performed using same lot number kit. Precision test for Procalcitonin Easy Diagnosis performed with a control cassette with an established value of 25 ng/mL and with patient's sample. Precision test using control cassette runs 10 times consecutively. Precision test using sample patients conducted in three concentration level obtained from Brahms that is low (> 0.1 ng/mL, < 0.5 ng/mL), high ($\geq$ 0.5 ng/mL), and very high (> 2 ng/mL). These level determination is based on its clinical significance. Each sample runs 5 times consecutively. All precision test performed by one competent laboratory analyst.

Sample number determined at 40 based on minimum sample requirement for two methods comparison.[7] Study subjects are Dr.Cipto Hospitals's patients that request procalcitonin examination and other laboratory parameter that use EDTA blood sample. Blood withdrawn at the same time. After all requested parameter finished and results are released, remaining EDTA samples taken for this study. The remaining samples runs within 6 hours after blood withdrawal. Subject with discordance in EDTA requirements volume, clotted, and/or hemolysis are excluded.

Procalcitonin Easy Diagnosis cassette kit uses GICA (*gold immunochromatographic assay*) principle that use double antibody sandwich method. Cassette divided by three areas: wells area, test area, and control area. (Figure 1).

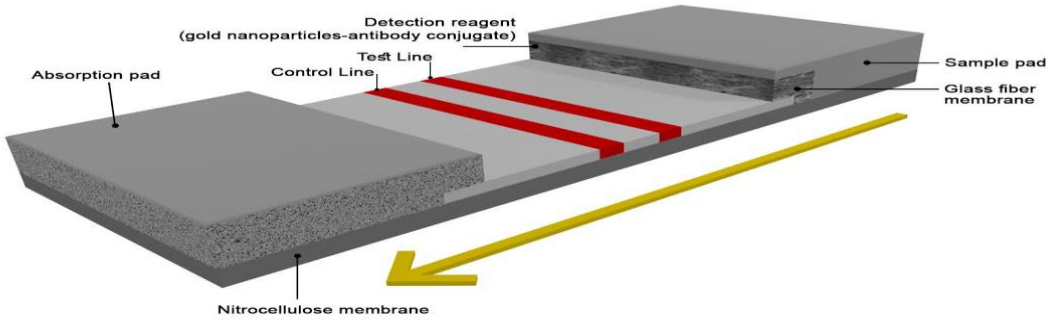

Figure 1. Procalcitonin Easy Diagnosis Cassette Kit

Eighty micro litre (80 uL) whole blood sample dropped on sample pad in sample well area. The sample filtered and captured with gold-labelled mouse monoclonal antiprocalcitonin antibody. The complex diffused along the kit to the test area. In this area, the complex captured with second monoclonal antiprocalcitonin antibody that fixed on cellulose membrane and form a red test line. The line intensity increased when there is a higher level of procalcitonin. Excess of gold-labelled antiprocalcitonin antibody continues to diffuse to control area, captured by anti mouse IgG antibody that is fixed on cellulose membrane and form a red control line. This line function as an internal control of the cassette.[3,8]

After 15 minutes, the cassette placed in immune quantitative reader. This reader uses imaging technology to analyze the line intensity. Software in the device plot the intensity level to a standard curve made for each kit lot number. Results obtained are the quantitative level of sample's procalcitonin. [9,10]

Brahms-Kryptor Compact used TRACE (Time Resolved Amplified Cryptate Emission) principle. In TRACE, there is an energy shift between first europium labelled anti-procalcitonin antibody and second XL665 labelled anti-procalcitonin antibody. The energy shift occurs if antibody-procalcitonin-antibody complex formed. The energy emission signals reduction per time unit associated with procalcitonin level. Longer signal reduction proportional with higher procalcitonin level.[11]

Data normality tests with Kolmogorov-Smirnoff. Data analysis continued with correlation test and two methods agreement test (Bland-Altman). Statistical Product and Service Solution (SPSS) ver 20 and MedCalc ver 16.4.3 used to process the data. P value < 0.05 considered significant with 95% confidence interval.

RESULTS

Usage of whole blood EDTA samples has advantages in lesser blood volume withdrawal, no need for plain serum tube, and no sentrifugation step. Therefore shorter turn around time, more convenient for patients, and lesser cost. Procalcitonin Easy Diagnosis total turn around time from sample receival to result obtained is 20 minutes, faster than Brahms Kryptor Compact (30 – 40 minutes).

Precision Test

Precision test using control cassette gives mean of 25 ng/mL, standard deviation (SD) of 0.632 ng/mL, and coefficient of variance (CV) 2.528 %. Precision test results from patient's sample can be viewed at Table 1.

Table 1. Precission test results using patient's samples on Procalcitonin Easy Diagnosis

Level	Mean (ng/mL)	Standard Deviation (ng/mL)	Coefficient of Variance (%)
Low (0.13 ng/mL)	0.114	0.015	13.07
High (0.67 ng/mL)	0.628	0.137	21.81
Very High (2.34 ng/mL)	1.222	0.168	13.77

Correlation and comparison of two methods

Data normality from both methods shows abrnomal distributions. Therefore, Spearman correlation test used with results r = 0.898 (p < 0.05). Bland-Altman analysis performed on four data groups based on Brahms result, there are < 0.5 ng/mL, ≥ 0.5 - < 2 ng/mL, ≥ 2 ng/mL, and ≥ 10 ng/mL. This grouping aimed to reach better agreement evaluation for each cut-off. The results may be viewed in Figure 2-5.

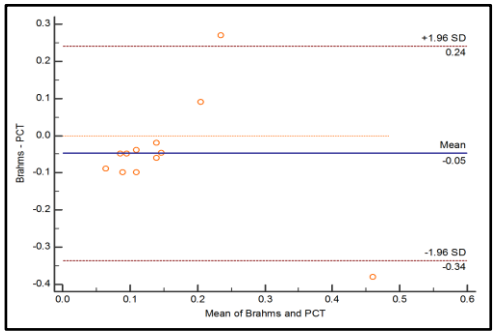

Figure 2. Bland Altman plot for the levels < 0.5 ng/mL

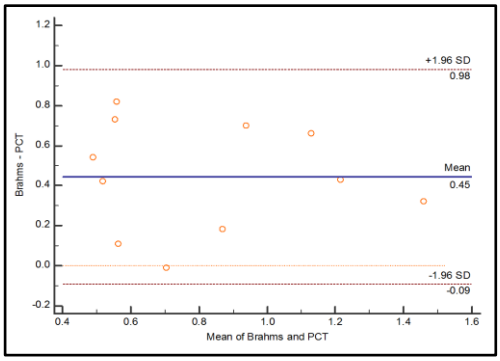

Figure 3. Bland Altman plot for the levels ≥ 0.5 ng/mL - < 2 ng/mL

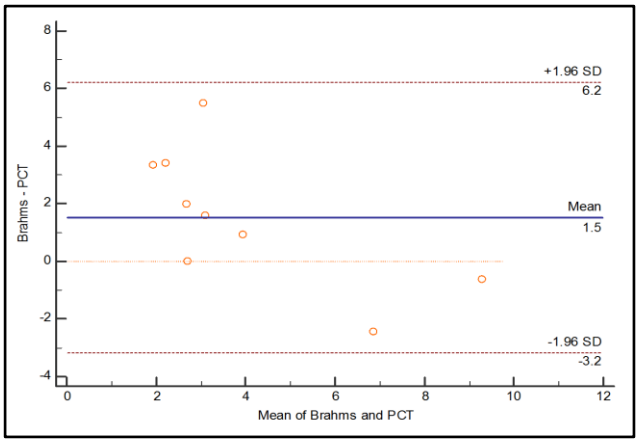

Figure 4. Bland Altman plot for the levels ≥ 2 ng/mL - < 10 ng/mL

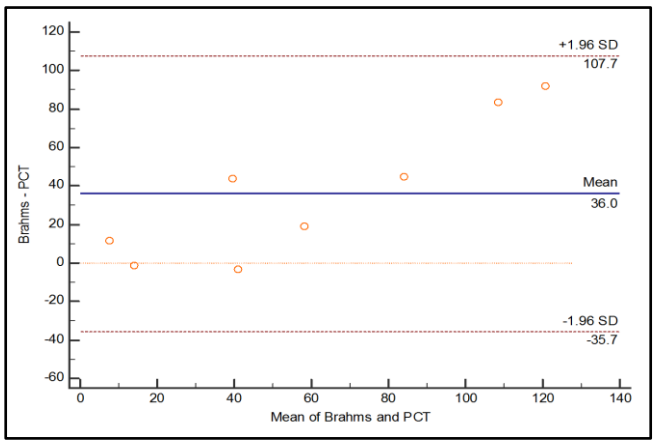

Figure 5. Bland Altman plot for the levels ≥ 10 ng/mL

Table 2. Data results from Brahms Kryptor Compact and Procalcitonin Easy Diagnosis

Sample	Sex	Age (year)	Brahms (ng/mL)	PCT Easy Diagnosis (ng/mL)
1	Male	63	3.6	0.26
2	Female	33	5.64	8.1
3	Male	80	5.80	0.32
4	Male	46	0.07	0.12
5	Female	11	13.53	2.2
6	Female	66	0.09	0.13
7	Female	3	0.1227	0.17
8	Female	26	0.0611	0.11
9	Male	35	106.6	62
10	Male	56	39.31	43
11	Male	42	67.7	49
12	Male	52	0.96	0.78
13	Male	64	150.2	67
14	Male	58	2.71	2.7
15	Female	23	166.8	75
16	Male	70	3.89	2.3
17	Male	67	1.43	1
18	Male	59	0.11	0.17
19	Male	55	1.62	1.3
20	Male	44	1.46	0.8
21	Male	59	4.41	3.5
22	Male	19	61.45	18
23	Female	21	8.98	9.6
Sample	Sex	Age	Brahms	PCT

		(year)	(ng/mL)	Easy Diagnosis (ng/mL)
24	Female	9	3.93	0.52
25	Male	46	0.97	0.15
26	Male	34	0.73	0.31
27	Male	30	0.13	0.15
28	Male	45	0.25	0.16
29	Female	83	0.04	0.14
30	Female	31	0.27	0.65
31	Female	59	0.7	0.71
32	Female	43	13.36	15
33	Female	57	0.06	0.16
34	Female	9	0.02	0.11
35	Male	69	0.62	0.51
36	Male	52	1.29	0.59
37	Female	57	0.92	0.19
38	Female	67	0.76	0.22
39	Male	62	0.37	0.1
40	Female	47	3.67	1.7

Table 3. Precision test result with patient's samples on Procalcitonin Easy Diagnosis

Level	Run	Result
Low (0.13 ng/mL)	1	0.1
	2	0.1
	3	0.11
	4	0.14
	5	0.12
Mean (ng/mL)		0.11
SD		0.015
CV (%)		13.07
Level	**Run**	**Result**
High (0.67 ng/mL)	1	0.55
	2	0.73
	3	0.8
	4	0.65
	5	0.41
Mean (ng/mL)		0.63
SD		0.137
CV (%)		21.81

Level	Run	Result
Very high (2.34 ng/mL)	1	0.91
	2	1.3
	3	1.2
	4	1.4
	5	1.3
Mean (ng/mL)		1.22
SD		0.168
CV (%)		13.77

Mean difference of the first group result is -0.05 ng/mL with (-0.34) – 0.24 ng/mL limits of agreement. There are two mean value outside the limits of agreement, those are 0.23 ng/mL and 0.46 ng/mL. Mean difference of the second group result is 0.45 ng/mL with (-0.09) – 0.98 ng/mL limits of agreement, all mean value are within limits of agreement. Mean difference of the third group result is 1.5 ng/mL with 3.2 – 6.2 ng/mL limits of agreement, all mean value are within limits of agreement.

Table 4. Precision test result with Procalcitonin Easy Diagnosis cassette control

Run	Result
1	25
2	25
3	24
4	25
5	25
6	26
7	24
8	25
9	26
10	25
Mean (ng/mL)	25
Target value (ng/mL)	25
SD	0.632
CV (%)	2.528
D	0

the fourth group result is 36 ng/mL with (-35.7) – 107.7 ng/mL limits of agreement, all mean value are within limits of agreement.

DISCUSSION

Results from precision tests shows that CV measurement using control cassette is satisfactory. Neverthless, usage of control cassette as an external control does not meet quality control material requirements (stable, packed in individual vial or aliquote, minimum variation between vial/aliquote, may withstand long period of time, and has same matrix as samples evaluated). However, the data gives information that the imaging technology used in the immune quantitative analyzer is satisfactory.[12]

There is a significant discrepancy between CV result from cassette control and with patient's samples. Significant difference also observed between results from this study and from the kit leaflet. On the leaflet, kit's CV obtained from 20 times measurement on procalcitonin concentration of 0.25 ng/mL and 40 ng/mL are 9.53% and 7.18%. Difference in procalcitonin levels measured, number of repetition, and type of control material used might cause this discrepancy. Difference also observed between CV from patient's sample and Brahms leaflet. Based on Brahms leaflet, CV for 0.1 ng/mL, 0.2 ng/mL, and 0.3 ng/mL are 15%, 10 %, and less than 6% respectively.[3,13]

Based on researcher's knowledge, no studies have reported quantitative CV from procalcitonin ICT assay, determination using imaging technology, and whole blood without sample preparation. Similar measurement method performed by Chuang et al using serum and alpha-fetoprotein (AFP) as analyte. Coefficient of variance results from this study are 1.5% for mild elevation and 1.1% for high elevation of AFP.[14] Serum sample used might cause this difference, but further study needed for confirmation.

Correlation test shows a strong correlation between Procalcitonin Easy Diagnosis and Brahms Kryptor Compact (r=0.898). Jiang et al and Chuang et al also obtained similar results with AFP as analyte. Jiang et al carried out correlation test between chemiluminescent method and ICT method with r = 0.961 ($p < 0.005$) as a result. Chuang et al compares radioactive immunoassay method and ICT , a correlation coefficient of r = 0.997 ($p < 0.0001$) obtained.[8,14]

In the first group, majority of agreement values are within the limits of agreement. There are two mean values outside the limits of agreement. The first mean value outlier measured 0.37 ng/mL on Brahms Kryptor and 0.1 ng/mL on Procalcitonin Easy Diagnosis. The second mean value outlier measured 0.27 ng/mL on Brahms Kryptor and 0.65 ng/mL on Procalcitonin Easy Diagnosis. These results shows there is a good agreement at mean value less than 0.2 ng/mL, but decreases on higher mean value.

All value lies within the limits of agreement in the remaining groups. This shows excellent agreement in procalcitonin level more than $\geq$ 0.5 ng/mL. Overall agreement is 95 % values lie within the limits of agreement.[15]

CONCLUSION

Comparison between procalcitonin results using Procalcitonin Easy Diagnosis Kit and Brahms Kryptor Compact has conducted. The use of EDTA whole blood samples favor Procalcitonin Easy Diagnosis as more convenient for patients, more economical, and gives faster results than Brahms Kryptor Sensitive. Precision test using control cassette provide good results, however, the use of control cassette does not comply with control materials requirements. Precision test using patient samples results <22%. Appropriate control materials required to provide better precision test evaluation and kit stability monitoring. It is advisable to seek a reference value of normal individuals using Procalcitonin Easy Diagnosis kit so cutoff point of procalcitonin elevation in accordance with the severity of the infection can be determined. The correlation between the two methods results r = 0898 ($P < 0.05$) with Bland-Altman analysis result a good agreement (95%).

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REFERENCES

1. Reinhart K, Bauer M, Riedeman NC, Hartog CS. New approaches to sepsis: molecular diagnostics and biomarkers. Clin Microbiol Rev 2012;25:609-27
2. Dellinger RP, Levy M, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, et al. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock : 2012. Crit Care Med J 2012;41:580-4
3. Wuhan Easy Diagnosis Biomedicine. Procalcitonin Rapid Test Kit Instruction for Use. Wuhan: Wuhan Corporation; 2013
4. Clayton J. Procalcitonin Analyte Monographs. National Laboratory Medicine Catalogue. Association for Clinical Biochemistry and Laboratory Medicine. United Kingdom; 2013
5. Brahms PCT-Q Measuring Principle. BRAHMS GmbH, 2013 (Accessed at 18 July, 2014, http://www.procalcitonin.com/default.aspx?tree=4_7_0&key=pctq2.)
6. Brahms Kryptor Procalcitonin Assay Measuring Principle. BRAHMS GmbH, 2013 (Accessed at 18 July, 2014, at http://www.procalcitonin.com/default.aspx?tree=4_7_0&key=pctq2.)
7. Linnet K, Boyd JC. Selection and Analytical Evaluation of Methods-With Statistical Techniques. In: Burtis CA, Burns DE. Tietz Fundamentals of Clinical Chemistry. 7th ed. Missouri: Elsevier-Saunders; 2015:16-21
8. Jiang H, Du M, Ke D. A rapid quantitative determination method of AFP concentration with gold immunochromatographic strip. J of Comp 2012;7:2868-75
9. Wuhan Easy Diagnosis Biomedicine. The immune quantitative analyzer manual. Wuhan: Wuhan Corporation; 2013
10. Dezhi Z, Wu Z, Shuai W. Detecting method of quantitative colloidal gold test strip concentration based on the DSP image processing. Intl J of Electr and Electro Engr J 2010;10:1-4.
11. Brahms GmbH. Brahms Kryptor Compact Manual Ver 4.00. Hennigsdorf: BRAHMS GmbH; 2010.
12. Klee GG, Westgard JO. Quality Management. In: Burtis CA, Burns DE. Tietz Fundamentals of Clinical Chemistry. 7th ed. Missouri: Elsevier-Saunders; 2015:97-9

13. Brahms GmbH. Brahms PCT Sensitive Kryptor Instruction for Use Ver R.16en. Hennigsdorf: BRAHMS GmbH; 2011.
14. Chuang L, Hwang JY, Chang HC, Chang FM, Jong SB. Rapid and simple quantitative measurement of alpha-fetoprotein by combining immunochromatographic strip test and artificial neural network image analysis system. Clin C Acta 2004;348:87-93
15. Bland JM, Altman D. Measuring agreement in methdod comparison studies. Stat Methods Res 1999;8:135-60

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