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Comparative Study of Two Different Methodologies For Procalcitonin (PCT) Estimation

Ritu sharma[1], Rupal Dubey[1#]
[1]Department of Medical Laboratory Sciences, Lovely Professional University, Phagwara, Punjab- 144411, India
[#]email of corresponding author- rupal.25007@lpu.co.in
Phone no. - +91-9463592474

ABSTRACT

Background: Procalcitonin (PCT) is a biomarker for the diagnosis of bacterial infection and sepsis. The patients with bacterial infection are increasing day by day. Higher PCT values in serum sample shows that patient is suffering from bacterial infection. The first assays used to estimate PCT were based on the methods of manual immunochemistry (Brahms PCT LIA). These manual assays have been replaced by automated immunochemistry methods (BioMerieux Vidas, Brahms Kryptor). The first automated assay cleared by FDA in 2008 was BRAHMS Kryptor PCT assay.

Objective: Comparison of PCT with Enzyme Linked Fluorescent Assay (ELFA) viz. Biomerieux Vidas PCT assay and Time Resolved Amplified Cryptate Emission (TRACE) viz. BRAHMS Kryptor PCT assay.

Methods: Two different methods are used for the procalcitonin estimation. In this study 10 Values of each low and high control results were collected and compare the obtained mean with assigned mean. SD and CV were calculated for TRACE and ELFA.

Results: The results from our study demonstrated that both the methods offers improved sensitivity, precision and equivalent clinical performance, which serves as a valuable diagnostic tool for the early detection of sepsis.

Conclusion: We conclude that the two PCT methods investigated have a precision correlation i.e. **BIOMERIEUX VIDAS** with **BRAHMS PCT Kryptor ®** assay**.** But the two assays vary with respect to volume of sample required, measuring range, dilution capacity and analysis time.

Keywords: Procalcitonin, Sepsis, FDA, BioMerieux Vidas PCT assay, BRAHMS Kryptor PCT assay.

INTRODUCTION

Sepsis is a very common cause of illness and mortality even in this advance era of critical

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care management. [1] The number of patients with sepsis or septic shock is increasing gradually. PCT levels may increase in bacterial infection or sepsis patients. PCT assays can be used to support various patient classes in clinical management. PCT value aids in guiding the antibiotic use for bacterial infections. [2] The elimination of PCT through normal renal function indicates that the approximate half life of PCT is 30 hours. Patients with renal dysfunction may have a longer PCT half life because the kidneys do not appear to be the major pathway of PCT elimination from the blood. These results suggest that PCT can be used to detect systemic inflammation caused by bacteria. [3-5]

Procalcitonin (PCT) is the precusrsor to peptide superfamily of calcitonin hormone. PCT have 116 amino acids with the molecular weight of 14.5 KDa. [6] It undergoes three consecutive cleavages in the thyroid, lung and pancreas neuroendocrine cells, including PCT region's amino terminus, the immature calcitonin, and calcitonin carboxyl-terminus peptide-1 (CCP-1, also known as katacalcin). PCT belongs to group of closely related proteins including the calcitonin gene related peptides (CGRP) I and II, amylin, and adrenomedullin. [7] PCT is decoded by *CALC-1* gene present on chromosome 11 and is produced by alternative splicing of *CALC-1* mRNA transcript. The cleavage at the one site of *CALC-1* gene of primary transcript results in pre-PCT. The concentration of PCT usually remains below 0.1 ng/mL. [8,9] PCT values can vary with an infection's presence and severity. Except for the viral infections, if the cause of inflammation is bacterial or fungal the high serum level of PCT has been identified. [10] PCT is released in response to the microbial toxins and certain pro-inflammatory mediator like IL-1β, TNF-α, and IL-6 in bacterial infection. In the immune response to viral infections, cytokines are produced that block the release of PCT. Serum PCT levels rise within 2–6 hours following the onset of systemic infection and decrease by 50% everyday when infection is controlled by the host immune system or antibiotic therapy. Increased level of PCT (>2.0 ng/mL) have a high positive predictive value for the risk of progression to sepsis or septic shock. Low or normal levels of PCT (<0.50 ng/mL) have high negative predictive value and are useful to rule out sepsis. [11,12]

Sepsis is characterized as a systemic inflammation infection consisting of increased or decreased temperature or count of leukocytes, tachycardia, and rapid breathing. Sepsis

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affects organ systems from the source of infection. [13] Sepsis is caused by bacterial infection. It is a life-threatening syndrome. It is one of the world's major causes of sickness and death. The increased blood PCT concentration is caused by sepsis and septic shock. [14,15] Early PCT estimation was based on methods of manual immunochemistry. Many PCT immunoassays are currently available on automated laboratory analyzers. BRAHMS Kryptor PCT antibodies are used in most PCT immunoassays. It was developed by Thermo Fisher. The first PCT assay for measurement of the PCT level was put in place by BRAHMS. The BRAHMS Kryptor and VIDAS assay granted by US FDA, is based on ELFA (Enzyme-Linked Fluorescent Assay) while Kryptor is based on TRACE (Time Resolved Amplified Cryptate Emission) technology. [16-18]

Biomerieux VIDAS

The PCT value in serum or plasma is measured by VIDAS BRAHMS PCT assay. This is based on the automatic Enzyme-Linked Fluorescent Immunoassay (ELFA) technique. VIDAS BRAHMS PCT reagents kit offers the materials required for testing on the automated VIDAS® instruments, which takes approximately 20 minutes. The assay's test range is between 0.05-200 ng/mL. The assay has been providing the accuracy and precision at the diagnostic level. [19]

The VIDAS® analyzer contains two compartments called "sections". Each section (labeled A and B) can process six single reagent strips for a maximum capacity of twelve single or six dual tests. These sections operate independently allowing a variety of assays to be run on the VIDAS® analyzer at the same time. The reagent strip tray consists of six channels. The six positions of the block correspond to the six positions of the reagent strip tray. Each of the six channels constitutes a position in the section and reagent strips can be inserted per section for a total combined capacity of up to 12 tests. The reagent strip tray is pulled into the instrument during processing. The SPR® (Solid Phase Receptacles) block has six slots used to hold the SPR®. During processing, the SPR® block and SPRs® form a pipetting device that is used throughout the assay. PCT controls are available with each kit. Two levels controls are analysed before sample. [20]

BRAHMS KRYPTOR compact plus

Thermo Fisher Scientific BRAHMS KRYPTOR assay was the first automatic PCT

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estimation method. In March 2008, Kryptor assay was given FDA approval for use in US. This assay uses the technology of Time Resolved Amplified Cryptate Emission (TRACE) with the non radiative transfer energy. Kryptor method is based on polyclonal and monoclonal antibodies against calcitonin and katacalcin respectively. Calcitonin and katacalcin sequences bind with calcitonin pro-hormone. Serum or plasma (EDTA and heparin) samples are used for the quantitative measurement of PCT. The detection limit of the assay ranges from 0.02-50 ng/ml while automatic dilution expands with upper range upto 1000 ng/ml. [21]

MATERIALS AND METHODS

Serum sample was collected in the normal way from vein. For PCT estimation 200 µl serum sample is required using VIDAS and 50 µl for KRYPTOR. [22]

Reagents: VIDAS PCT Kit, KRYPTOR PCT Kit

The steps of the assay are followed according to the standard protocol. [23-25]

RESULTS

Estimation of procalcitonin with VIDAS:

There are two controls in VIDAS for PCT estimation to ensure that the results for the samples are accurate. QC1 and QC2 values were taken of 10 days and then their mean, standard deviation and coefficient of variance was calculated (Table 1 and 2).

For each control value Variance % and Coefficient of Variation (CV) is calculated as:

Variance % = ((B-A)/A) × 100

CV = (Standard Deviation (SD)/Mean) × 100

Table 1: Calculation of Mean, SD and CV for QC1 in Biomerieux VIDAS.

	Assigned Mean (A)	Achieved Result (B)	Variance %
		16.24	9.37
		18.41	2.73
		16.39	8.53
		16.91	5.63

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	17.92	16.39	8.53
		19.06	6.36
		18.79	4.85
		19.19	7.08
		20.48	14.28
		18.62	3.90
Mean		**18.04**	
Standard Deviation		**1.46**	
CV		**8.09**	

Table 2: Calculation of Mean, SD and CV for QC2 in BioMerieux VIDAS.

	Assigned Mean	**Achieved Result**	**Variance %**
	1.87	1.69	9.62
		1.56	16.57
		1.96	4.81
		1.82	2.67
		2.08	6.95
		1.99	6.41
		1.96	4.81
		1.82	2.67
		2.01	7.48
		1.55	17.11
Mean		**1.844**	
Standard Deviation		**0.189**	
CV		**10.24**	

Estimation of procalcitonin with KRYPTOR:

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There are two controls viz. low and high levels in Kryptor to ensure that the results of PCT samples are accurate. Variance % and CV for each control is calculated as shown in Table 3 and 4.

Table 3: Calculation of Mean, SD and CV for Control1 in KRYPTOR.

	Assigned Mean	**Achieved Result**	**Variance %**
	0.25	0.21	16.0
		0.27	8.0
		0.30	20.0
		0.23	8.0
		0.29	16.0
		0.30	20.0
		0.28	12.0
		0.29	16.0
		0.28	12.0
		0.24	4.0
Mean		**0.26**	
Standard Deviation		**0.03**	
CV		**11.53**	

Table 4: Calculation of Mean, SD and CV for Control2 in KRYPTOR

	Assigned Mean	**Achieved Result**	**Variance %**
	10.1	9.991	1.07
		9.456	6.37
		9.947	1.51
		10.18	0.79
		10.46	3.56
		9.915	1.83
		9.856	2.41

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		10.07	0.29
		10.19	0.89
		10.22	1.18
Mean		**10.04**	

Method	Control	Mean	SD	CV
A (VIDAS)	**QC1**	**18.04**	**1.46**	**8.09**
	QC2	**1.884**	**0.189**	**10.24**
B (KRYPTOR)	**QC1**	**0.26**	**0.03**	**11.53**
	QC2	**10.04**	**0.268**	**2.66**

Standard Deviation		**0.268**	
CV		**2.66**	

Table 5: Summarization of Mean, SD and CV for both the methods

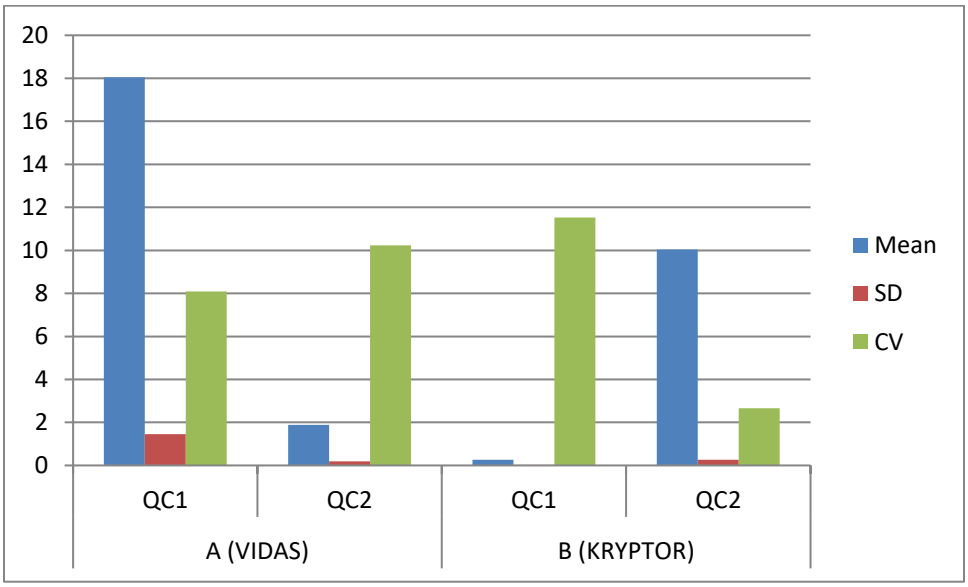

Figure 1: Comparison of VIDAS and KRYPTOR PCT Assays

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DISCUSSION

Aspects like sample volume, analysis time, dilutions of higher range samples, measurement range and also the cost and quality or traceability of a method can effect the decision of a laboratory to achieve new method. PCT assay comparison has highlighted the difference between the sample volumes of the two assays: BRAHMS Kryptor may use lower volume samples (50µL), while Biomerieux may use higher volume samples (200µL). The BRAHMS Kryptor PCT method is best for specimens with low volume and this shows its importance in a clinical setting where mostly neonatal specimens are used. PCT assays measurement ranges also differs, Biomerieux Vidas having range of 0.05 to 200 ng/mL and BRAHMS Kryptor with range of 0.02 - 50.0 ng/mL. Specimen crossing the upper limit of assay may need to be diluted as a serial quantitative values are needed to control bacterial infection or sepsis. The BRAHMS Kryptor does dilutions on board with its own diluents; while the Biomerieux Vidas requires manual dilution. The mean value of all 10 QC1 of the BioMerieux Vidas is 18.04 nearest around the assigned mean 17.95, whereas the BRAHMS Kryptor mean value is 0.26 nearest around the assigned mean 0.25. [26, 27]

CONCLUSION

The Biomerieux VIDAS procalcitonin controls (QC1 and QC2) were assayed per day for 10 days (10 days for QC1 and QC2). Results are summarized in the Table 5. Both the assays demonstrated excellent precision with: VIDAS assay ranging from 2.73-14.28 % CV for QC1 and 2.67-17.11 % CV for QC2. KRYPTOR assay ranging from 4.0-20.0 % CV for low Control and 0.29-6.37 % CV for high control. The results from our study demonstrated that both the methods offers improved sensitivity, precision and equivalent clinical performance, that serves as a valuable diagnostic tool for the detection of sepsis. It has been found that two tested PCT methods correlate well i.e. BIOMERIEUX VIDAS with BRAHMS PCT Kryptor ® assay. PCT assay shows better accuracy near diagnostic level of 0.5 ng/mL. BRAHMS Kryptor ® method required specimen of low volume as compared to Biomerieux Vidas. The BRAHMS Kryptor provides automated dilution of higher ranges of PCT specimens. The Biomerieux Vidas require serial dilution from outside range of PCT specimen. [28]

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CONFLICT OF AUTHOR

Author declare no conflict of interest

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