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# Analytical evaluation of Diazyme procalcitonin (PCT) latex-enhanced immunoturbidimetric assay on Beckman Coulter AU5800

DOI 10.1515/cclm-2014-1118

Received November 14, 2014; accepted December 2, 2014

## Abstract

**Background:** This study was aimed to evaluate the analytical performance of the novel Diazyme procalcitonin (PCT) immunoturbidimetric assay on Beckman Coulter AU5800.

**Methods:** Diazyme PCT is a latex-enhanced immunoturbidimetric assay, developed for use on laboratory instruments with capability of reading absorbance at 600 nm. This analytical evaluation included the assessment of limit of blank (LOB), limit of detection (LOD), functional sensitivity, imprecision, linearity, carryover, and method comparison between Diazyme PCT and Kryptor PCT on 129 routine serum inpatient samples.

**Results:** The LOB, LOD, and functional sensitivity of Diazyme PCT were 0.16, 0.26, and 0.28 ng/mL, respectively. The intra- and inter-assay imprecision of Diazyme PCT was between 2.9% and 7.8%. The linearity was excellent in the range of PCT values between 0.16 and 56 ng/mL, and the carryover was negligible (0.02%). A highly significant agreement was found between Kryptor PCT and Diazyme PCT in a range of concentrations between 0.16 and 111 ng/mL (Diazyme PCT=1.10×Kryptor PCT–0.89;  $r=0.960$ ;  $p<0.001$ ). The mean bias was 0.48 ng/mL (95% CI, –0.58 to 1.54 ng/mL). The strength of agreement between Kryptor PCT and Diazyme PCT was between 85% and 96% at 0.50, 2.0, and 10 ng/mL cutoffs.

**Conclusions:** Diazyme PCT appears to be a reliable assay for diagnosis and management of critical care patients susceptible to severe bacterial infections.

**Keywords:** bacterial infections; immunoassay; measurement; procalcitonin; sepsis.

## Introduction

Sepsis is currently defined as a severe systemic reaction to frequently ordinary infections, which is mainly sustained by response of the immune system to injury [1]. The frequency of this life-threatening condition is relatively high, with as many as 751,000 cases of severe sepsis each year in the USA. The overall hospital mortality for sepsis is impressively high (i.e., approx. 30%), even greater than that for acute myocardial infarction and lung or breast cancer [2]. Because an early goal-directed therapy has a strong impact on the prognosis of sepsis, a timely diagnosis is necessary to reduce overall hospital stay and mortality [2, 3].

Procalcitonin (PCT) is the 116 amino acid precursor of the hormone calcitonin. Under physiological conditions, calcitonin is synthesized and released by the C cells of the thyroid gland, so that the concentration of PCT in the blood of healthy subjects is very low, typically  $\leq 0.05$  ng/mL [4]. It has been shown, however, that bacterial infections are capable to trigger ubiquitous expression of calcitonin gene (*CALC-1*), along with constitutive release of PCT from a number of tissues and differentiated cell types, so that a significant increase of PCT levels can be observed in patients with severe bacterial infection and/or sepsis [4]. A recent meta-analysis totalling 30 studies and 3244 patients concluded that PCT is a reliable biomarker for early diagnosis of sepsis [5]. More specifically, the area under the curve for early diagnosis of sepsis was 0.85, with 0.77 sensitivity and 0.79 specificity. According to this evidence, a discrete number of clinical cutoffs has been proposed.

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Specifically, PCT values between  $\geq 0.5$  and  $2.0 \text{ ng/mL}$  are suggestive of moderate risk for progression to systemic inflammation,  $\geq 2.0\text{--}10 \text{ ng/mL}$  are indicative of high risk for progression to systemic inflammation, and values  $\geq 10 \text{ ng/mL}$  reflect the high likelihood of developing severe sepsis and septic shock [4].

In a recent systematic review and meta-analysis based on 7 studies and 1075 patients with severe sepsis or septic shock, Prkno et al. [6] concluded that the duration of antimicrobial therapy could be significantly reduced when PCT-guided therapy is established (hazard ratio, 1.27; 95% CI, 1.01–1.53). This evidence was confirmed in another systematic review and meta-analysis of controlled trials [7], in which PCT-guided discontinuation of antibiotics was found effective to reduce the duration of antibiotic treatment by 2.05 days, with no effect on morbidity or mortality. In adult patients with respiratory tract infections, PCT guidance also reduced antibiotic duration by 2.35 days, antibiotic prescription rate by 22%, and total antibiotic exposure with no impact on morbidity or mortality. Interestingly, another meta-analysis of PCT-guided therapy in respiratory tract infections concluded that this approach appears effective to reduce antibiotic use without affecting overall mortality or length of stay in the hospital [8]. According to the current recommendations, antibiotic therapy may hence be encouraged when the concentration of PCT is in the range  $0.5\text{--}1.0 \text{ ng/mL}$ , whereas it should be “strongly” encouraged when PCT value is  $\geq 1 \text{ ng/mL}$  [9].

According to recent studies, the clinical utility of PCT assessment is rapidly expanding beyond the boundaries of sepsis, to embrace the diagnosis and therapeutic guidance of a number of clinically relevant bacterial infections such as community-acquired pneumonia [10], peritonitis [11], endocarditis, meningitis, arthritis, and urinary or abdominal tract infections [12].

The availability of commercial PCT immunoassays has consistently increased over the past decade, with automated immunochemical methods replacing the former manual immunoassay. These recent techniques are now mostly based on time-resolved amplified cryptate emission, enzyme-linked fluorescent assay, chemiluminescent immunoassay, and electro-chemiluminescent immunoassay technologies [4, 13], and are hence only available on immunochemistry instrumentation [4]. Therefore, the aim of this study was to evaluate the analytical performance of the novel Diazyme PCT immunoturbidimetric assay, which has been developed for use on a large number of conventional laboratory analyzers.

## Materials and methods

### Method description

Diazyme PCT (Diazyme Laboratories, Poway, CA, USA) is a latex-enhanced immunoturbidimetric assay, developed for use on laboratory instrumentations with capability of reading absorbance at 600 nm. In this test, the PCT present in the patient sample binds to specific anti-PCT antibodies coated on latex particles, thus triggering their agglutination. The relative degree of turbidity generated by agglutination is then optically measured at 600 nm and is proportional to the concentration of PCT in the test sample. The PCT value is finally calculated by interpolation of optical signal against a six-point calibration curve. The amount of sample required for testing is  $20 \mu\text{L}$ , and the first test result is available in 10 min. In this study, the analytical evaluation of Diazyme PCT was performed using a Beckman Coulter AU5800 clinical chemistry analyzer (Beckman Coulter, Brea, CA, USA) [14].

### Analytical characteristics

The analytical characteristics of Diazyme PCT were defined by calculation of the limit of blank (LOB), limit of detection (LOD), and the functional sensitivity. Specifically, the LOB was calculated as the value corresponding to the sum of the mean and the  $1.645 \times \text{standard deviation (SD)}$  of 20 consecutive replicates of saline, as suggested by Armbruster and Pry [15]. The LOD was calculated as the sum of the LOB and  $1.645 \times \text{SD}$  of 20 replicates of an inpatient serum pool with the lowest measurable PCT value (i.e.,  $0.16 \text{ ng/mL}$ ) [15]. The functional sensitivity was defined as the lowest PCT concentration that could be analyzed with a coefficient of variation (CV)  $\leq 20\%$ , as suggested by Steinbach et al. [16]. This value was calculated by preparing serial dilutions (i.e., 1:2, 1:4, 1:8, and 1:16) from an inpatient serum pool with PCT concentration of  $1.26 \text{ ng/mL}$  and sample buffer. After measuring each dilution in ten replicates, the CV was calculated for each dilution. A model fit was then developed to identify the PCT value with 20% CV.

### Imprecision studies

The imprecision studies were performed using three serum pools obtained from routine inpatient samples, exhibiting low, intermediate, and high PCT values. The intra- and inter-assay imprecision of Diazyme PCT were assessed in 20 and 10 sequential runs (i.e., 10 consecutive working days for the inter-assay imprecision), using identical lot of reagents and the same calibration curve. The results were finally expressed as CV.

### Linearity

A pool obtained from routine inpatient serum samples and exhibiting a high PCT value (i.e.,  $56 \text{ ng/mL}$ ) was serially diluted at fixed ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) with a pool also

obtained from routine inpatient serum samples and displaying very low PCT concentration (i.e., 0.16 ng/mL), to cover the clinically significant range of concentration of this biomarker. The serial dilutions were then assessed in duplicate with Diazyme PCT and the theoretical values were calculated from measured values of undiluted serum pool. Linearity was tested by linear regression analysis and Spearman correlation coefficient ( $r$ ).

### Comparison study

The comparison study included 129 consecutive inpatient serum samples (68 from the intensive care unit, 22 from the emergency department, 12 from the infective disease unit, and the remaining 27 from other medical and surgical wards) referred to the laboratory in one working day for routine PCT measurement and with values covering the most clinically significant range of PCT concentrations (i.e., 0.16–111 ng/mL). All samples were tested within 3 h from collection. The results of Diazyme PCT were compared with those obtained on the same serum samples with a well-validated automated immunoassay (i.e., BRAHMS Kryptor system, BRAHMS, Hennigsdorf, Germany). The functional sensitivity of Kryptor PCT is 0.04 ng/mL, and the imprecision is between 0.7% and 8.3% [16]. The correlation between methods was assessed with Deming fit and Spearman correlation coefficient, whereas the mean bias and the 95% CI were calculated with Bland-Altman difference plot. The concordance between Diazyme PCT and Kryptor PCT at the three clinically relevant cutoffs for severe bacterial infections (i.e., 0.50, 2.0, and 10 ng/mL) was estimated by  $\kappa$  coefficient. The statistical evaluation was performed with Analyse-it (Analyse-it Software, Leeds, UK).

### Carryover

The potential carryover of Diazyme PCT was assessed by measuring a specific sequence “A1-A2-B1-B2-B3” in two sequential runs, where “A” was an inpatient serum sample with a very high PCT value (286 ng/mL; final value obtained after 1:10 external dilution with sample diluent), and “B” was an inpatient serum sample with a PCT value corresponding to the LOD of the immunoturbidimetric assay (i.e., 0.26 ng/mL). The carryover was finally calculated according to the formula proposed by Broughton, as follows: carryover, % =  $100 \times (B1 - B3) / (A2 - B3)$  [17].

### Ethical approval

This analytical evaluation was entirely based on pre-existing inpatient serum samples referred for routine PCT testing, and the material was obtained after analysis was completed. Therefore, no patient informed consent was necessary. However, the study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation.

## Results

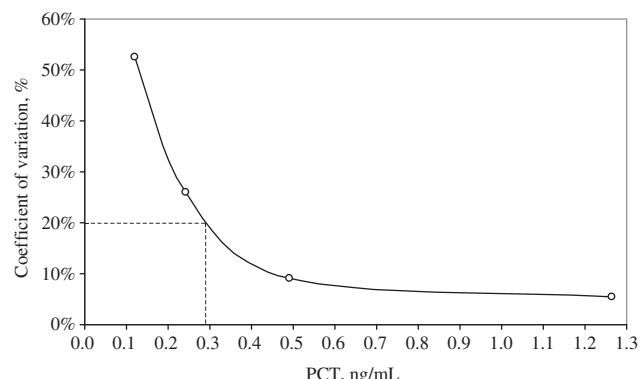
The LOB, LOD, and functional sensitivity of Diazyme PCT, calculated as previously described, were 0.16, 0.26, and

0.28 ng/mL, respectively (Figure 1). The imprecision of Diazyme PCT assay is shown in Table 1. In brief, the intra- and inter-assay imprecision was between 2.9% and 7.7% and between 3.5% and 7.8%, respectively. The linearity of the test was excellent in the range of PCT values between 0.16 and 56 ng/mL, as mirrored by the linear regression analysis obtained by plotting measured and theoretical concentrations ( $r=0.997$ ;  $p<0.001$ ). The carryover calculated according to the formula of Broughton was 0.02%, and hence negligible.

The results of the method comparison study are shown in Figure 2. A highly significant agreement was found between Kryptor PCT and Diazyme PCT in a range of concentrations between 0.16 and 111 ng/mL ( $r=0.960$ ;  $p<0.001$ ). The resulting equation of the Deming fit was Diazyme PCT =  $1.10 \times$  Kryptor PCT – 0.89. The mean bias calculated with Bland-Altman plot analysis was 0.48 ng/mL (95% CI, –0.58 to 1.54 ng/mL). The strength of agreement between Kryptor PCT and Diazyme PCT was 85% at the 0.50-ng/mL cutoff ( $\kappa$ , 0.24; 95% CI, 0.02–0.46;  $p<0.001$ ), 91% at the 2.0-ng/mL cutoff ( $\kappa$ , 0.80; 95% CI, 0.670–0.91;  $p<0.001$ ), and 96% at the 10-ng/mL cutoff ( $\kappa$ , 0.91; 95% CI, 0.84–0.99;  $p<0.001$ ), respectively. The strength of agreement between Kryptor PCT and Diazyme PCT at the 0.25-ng/mL threshold, which is used in emergency care for antibiotic stewardship [18], was 92%, and hence satisfactory ( $\kappa$ , 0.14; 95% CI, –0.13 to 0.42;  $p=0.024$ ).

## Discussion

The concerning frequency of severe bacterial infections and sepsis combined with the high mortality rate supports the notion that diagnosis and treatment of this condition should be established as soon as possible [2, 3]. Because the measurement of inflammatory biomarkers

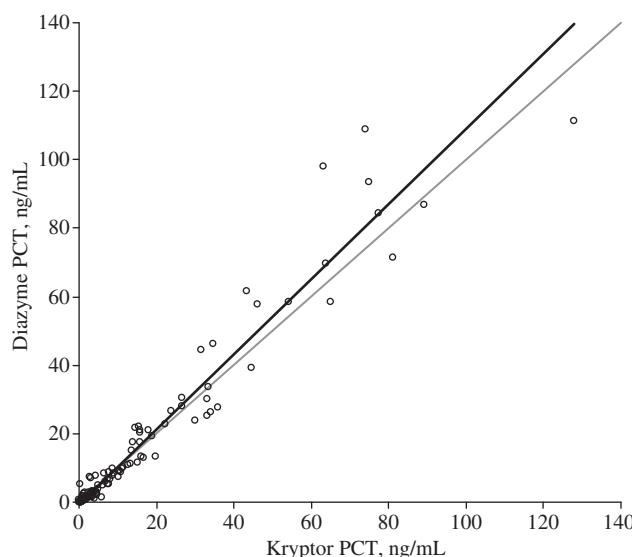


**Figure 1** Calculation of functional sensitivity of Diazyme PCT assay.

**Table 1** Intra- and inter-assay imprecision of Diazyme PCT assay.

Serum pool	Intra-assay, n=20		Inter-assay, n=10	
	Value, ng/mL	CV, %	Value, ng/mL	CV, %
Low	0.93±0.07	7.7	0.86±0.07	7.8
Medium	1.27±0.09	7.1	1.31±0.10	7.7
High	18.2±0.5	2.9	17.1±0.6	3.5

Results are expressed as coefficient of variation (CV).



**Figure 2** Deming fit of Diazyme PCT immunoturbidimetric assay compared with Kryptor PCT.

such as PCT has now become a cornerstone in the clinical management of severe bacterial infections or sepsis [19, 20], the widespread availability of methods that can be applied to a vast array of laboratory instrumentation such as the novel Diazyme immunoturbidimetric assay should be regarded as an attractive perspective for broadening the use of PCT in clinical practice.

The results of this analytical evaluation show that the LOB (0.16 ng/mL) and the LOD (0.26 ng/mL) of Diazyme PCT are approximately threefold and fivefold higher than the upper limit of the reference range of PCT in blood of healthy subjects (i.e.,  $\leq 0.05$  ng/mL). Although the functional sensitivity of Diazyme PCT (0.28 ng/mL) was closer to that quoted by the manufacturer (0.17 ng/mL), and sevenfold higher than that of Kryptor PCT (0.04 ng/mL) [16], this limit appears still suitable for diagnosis and management (i.e., PCT-guided therapy) of severe bacterial infections and sepsis, as for current recommendations [9].

The intra- and inter-assay imprecision of Diazyme PCT was between 2.9% and 7.8%, thus comparable to that previously reported for Kryptor PCT (i.e., 0.7%–8.3%) [16]. No problems of carryover were observed, even using a serum pool exhibiting a PCT value as high as 286 ng/mL. As regards the correlation with Kryptor PCT, Diazyme PCT exhibit a good agreement ( $r=0.960$ ) and a limited positive bias (0.48 ng/mL), thus displaying results comparable to those of other automated immunoassays [21]. Even more importantly, the strength of agreement at the clinically relevant cutoffs for severe bacterial infections (i.e., 0.5, 2.0, and 10 ng/mL) was even better than that observed in previous studies that compared VIDAS PCT with Kryptor PCT (i.e., 85%–96% vs. 83%–85%) [21, 22].

According to these results, Diazyme PCT appears to be a reliable method for diagnosis and management of critical care patients susceptible to severe bacterial infections. Compared with other existing automated immunoassays, Diazyme PCT has some technical and analytical advantages. These basically include the low volume of sample required (i.e., 20 vs. 50  $\mu$ L with Kryptor PCT), the fast turnaround time (the assay is completed within 10 vs. 19 min on Kryptor PCT), the lower cost, as well as the high throughput attributable to practicable implementation in a large number of fully automated and efficient laboratory analyzers, which would make it available to virtually all clinical laboratories. Indeed, the LOD and the functional sensitivity of Diazyme PCT is higher than that of other automated commercial immunoassays, which makes the use of this method unadvisable in clinical conditions characterized by very modest increase of PCT values (i.e., between 0.05 and 0.26 ng/mL). In a fully automated laboratory and in hospital daily practice, it may hence be feasible to use Diazyme PCT as a screening test not only in infectious diseases wards or internal medicine but also in emergency departments, then measuring PCT samples with concentration  $<0.26$  ng/mL with more sensitive immunoassays, as a “reflex test”.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Financial support:** None declared.

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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