

Technical Note

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Comparison of enzyme-linked fluorescent assay and electrochemiluminescence immune assay in procalcitonin measurement

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Abstract

Background: Procalcitonin (PCT) measurement is required for intensive care patients with systemic inflammation symptoms, early diagnosis of possible infections, and evaluation of sepsis severity and prognosis.

Objectives: We aimed to determine the analytical performance of PCT measurement in a Roche Modular E170 (ECLIA) analyzer and compare the performance with VIDAS (BRAHMS/ELFA) analyzer findings.

Material and methods: Within-day and between-day precision value, linearity was determined, and two methods were compared with regression and Bland–Altman analysis.

Results: Both ECLIA and ELFA assays indicated excellent precision, where within-day precision varied between 1.18% and 3.97% CV, and between-day precision varied between 1.77% and 3.93% CV. The ECLIA method was linear up to 62.15 ng/mL. The arithmetic mean was 6.02 ng/mL with the ECLIA method and 8.02 ng/mL with the ELFA method. The correlation coefficient was $r=0.996$ and $p=0.001$. The correlation was linear between the two methods. Regression equation was found $y=0.78x - 0.23$.

The Bland–Altman figure was revealed the difference between the methods was specifically in lower concentrations (<0.15 ng/mL).

Conclusions: Both methods show good precision and correlation. It was determined that the difference between methods was significant, especially at <0.15 ng/mL concentration.

Keywords: comparison; electrochemiluminescence; fluorescent assay; procalcitonin; sepsis.

Introduction

There is an arising demand for PCT tests from all hospital departments, especially emergency and intensive care units, for early diagnosis and monitoring of sepsis cases. The utilization of antibiotics following PCT results in sepsis and the other infections led to a shorter treatment period and antibiotic use [1–3].

Despite there isn't recently approved reference method for PCT measurement, the BRAHMS PCT Kryptor method fairly improved from the original BRAHMS luminometric immunoassay [4]. A few diagnostic companies such as BioMerieux, Roche, DiaSorin, and Siemens have since collaborated with BRAHMS for establishing automated PCT assays on their registered devices utilizing other methods. Additionally, a novel amplified luminescent proximity homogeneous assay (Alpha LISA) was established [5].

New methods have been investigated for various reasons, such as increasing total laboratory and hospital quality, providing further medical benefits, increasing precision, accuracy, and specificity in diagnosis, and reducing costs.

The present study aimed to determine the analytical performance of PCT measurement with ECLIA method and compare the performance of the ECLIA method with the ELFA method.

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Materials and methods

The 62 serum samples of the patients admitted to Vakıf Gureba Training and Research Hospital Intensive Care Unit and PCT requests were included in the study. All study procedures were approved by Bezmialem Valide Sultan Vakıf Gureba Education and Research Hospital Ethics Committee (25.02.2009-B.02.1.VGM.2.03.01). Thirty minutes after collecting patient blood samples to determine PCT, the samples were centrifuged in a Becton Dickinson vacutainer tube for 10 min at 4,000 rpm on Nuve 1200 centrifuge.

After PCT levels were measured with the ELFA method on the VIDAS autoanalyzer, samples that varied between 0.05 ng/mL and 68.51 ng/mL were stored at -20°C until the measurements were conducted with the ECLIA method on Roche Modular E 170 autoanalyzer. Original reagents were used for the measurement on both auto analyzers. We divided samples <0.15 ng/mL ($n=8$), 0.15 – 2 ng/mL ($n=28$) and >2 ng/mL ($n=26$).

Icteric and hemolyzed samples were excluded from the study. The instrument measurement performances were compared with the modified CLSI EP09-c (Clinical Laboratory Standards Institute) protocol [6].

Serum samples were used to develop low and high serum pools. The low serum pool was negative for the levels of procalcitonin, such as blank. CLSI EP10-A3 AMD protocol was applied for within-day and between-day precisions [7] with low and high serum pools. Each one was divided into 20 portions and stored at -20°C . The serums with low PCT concentration (0.377 ng/mL) and those with high PCT concentration (9.932 ng/mL) were studied 20 times consecutively for within-day precision (Table 1). Low and high concentrations of PCT pool samples were studied for 20 consecutive days for between-day precision. All samples were analyzed in duplicate for linearity and precision assays.

CLSI EP06 protocol was applied for linearity analysis [8]. Serum sample with 62.15 ng/mL PCT concentration was used to determine linearity by mixing 100%, 75%, 50%, 30%, 20%, 10% and 0% serum samples at 0.041 ng/mL PCT concentration in Roche Modular E 170.

SPSS version 12.0 (Statistical Package for Social Sciences) for Windows software was used for the analysis of the study findings. The findings were analyzed within the 95% confidence interval and $p<0.05$ significance level. The comparison of the quantitative data was based on between-day and within-day precisions and the two methods (regression analysis, correlation coefficient, Deming regression analysis, Bland–Altman graphic).

Results

ECLIA PCT assay was linear between 0.041 and 62.15 ng/mL ($r^2=0.99$).

The arithmetic mean $\pm$ SD (min–max) was 6.02 ± 11.8 (0.02–51.6) ng/mL with the ECLIA method and 8.02 ± 15.1 (0.05–68.1) ng/mL with the ELFA method.

95% confidence interval of the difference was 3.00–9.03 ng/mL for ECLIA method and 4.17–11.87 ng/mL for ELFA method.

The correlations between the findings were analyzed with regression analysis and expressed as the correlation coefficient. Correlation coefficient was found 0.996 and $p=0.001$. The regression equation was $y=0.78x - 0.23$ ($n=62$).

The correlation coefficient was high between the two instruments. Curve fit analysis was conducted to investigate the form of the correlation, and it was determined that the correlation was linear (Figure 1).

Deming regression analysis was also conducted (Figure 2). Deming regression equation was $y=0.78x - 0.26$; $Sy/x=1.14$.

Bland–Altman figure (the graph of differences between the means) was plotted with the differences of the measurements from the means for the two devices (Figure 3) for three groups.

Discussion

Steinbach et al. [9] conducted a PCT study on a Cryptor analyzer, and they determined that within-day and between-day assay precisions were $<5\%$ and $<10\%$, respectively. Another study was carried out by Wolf and et al. on the Roche Modular E170 analyzer. It was determined that within-day and between-day precisions were 3% and 2.8% for low concentration and 1.3% and 6.3% for

Table 1: Within-day and between-day assay precision at low and high concentration for Roche Modular E170 and VIDAS.

Roche modular E170		Within-day		Between-day	
Concentration		Low concentration (0.377 ng/mL)	High concentration (9.932 ng/mL)	Low concentration (0.411 ng/mL)	High concentration (10.169 ng/mL)
%CV		3.97	1.18	3.16	1.77
SD		0.015	0.117	0.013	0.180
VIDAS		Within-day		Between-day	
Concentration		Low concentration (0.151 ng/mL)	High concentration (10.590 ng/mL)	Low concentration (1.698 ng/mL)	High concentration (17.048 ng/mL)
%CV		3.30	3.18	2.65	3.93
SD		0.005	0.337	0.045	0.67

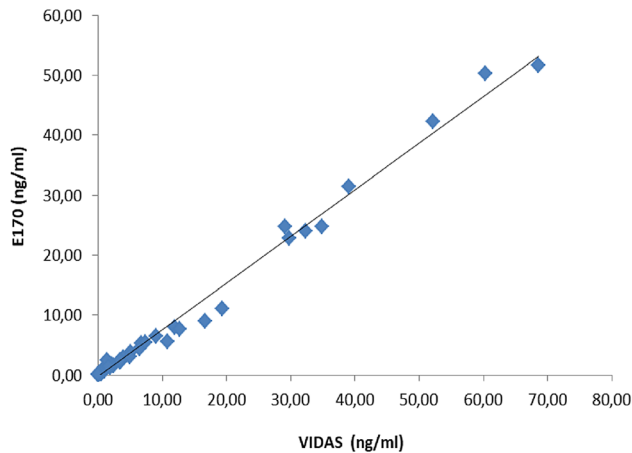


Figure 1: Linear relation graphic of E170 and VIDAS measurement results. Regression equation was found $y=0.78x - 0.23$.

high concentration, respectively [10]. These results were consistent with our results.

PCT does not have an allowed CV value determined by the CLIA. In such situations, the CV value obtained in studies that investigated the biological variations of analyte could be used. In the present study, the value determined by Barassi et al. [11] on biological variations of PCT in a healthy population was used as the acceptable CV value. The target CV% cited in that study was 8.1%, the present study CV% was quite good.

In our linearity study, PCT measurements were found to be linear between 0.041 and 62.15 ng/mL. In a linearity study conducted by Steinbach et al. [9], it was linear between 0.04 and 50 ng/mL.

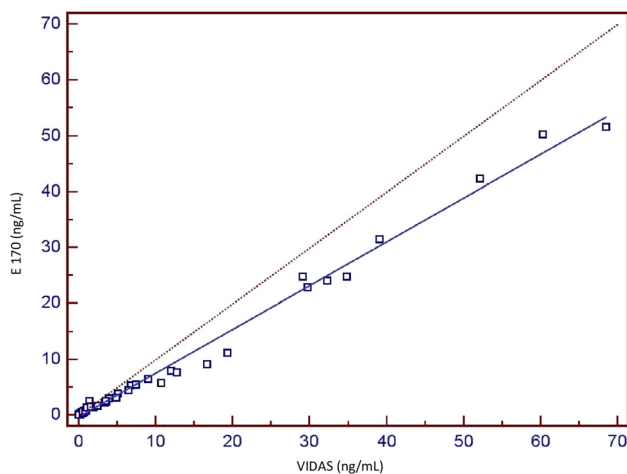


Figure 2: Deming regression analysis of E170 and VIDAS measurement results. Deming regression equation was found $y=0.78x - 0.26$ ($Sy/x=1.14$).

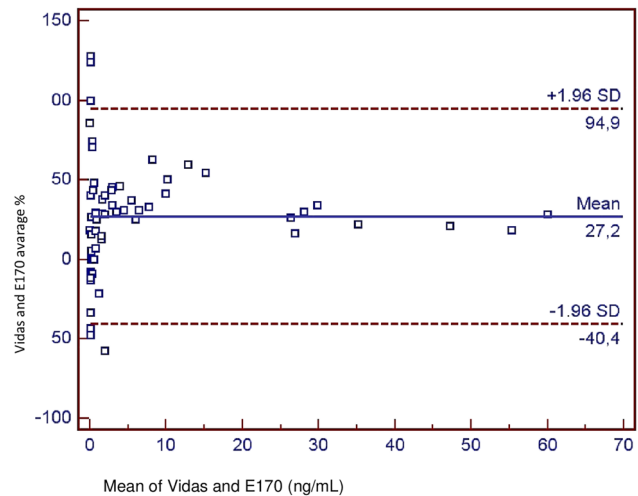


Figure 3: Bland-Altman graphic for measurement results of E170 and VIDAS.

The comparison of the two methods was conducted with regression analysis with the method comparison experiment data. The regression equation was $y=0.78x - 0.23$ and correlation coefficient was $r=0.996$ ($p=0.001$, $n=62$) (Figure 1). Our r value demonstrated an excellent correlation between the two methods.

Since the comparison method adopted in the ELFA method was not a reference method for PCT measurements, Deming regression analysis was also conducted [12]. The disadvantage of our study is that the ELFA method is not a reference method. In addition, our small sample size was a disadvantage of our work. Deming regression equation was $y=0.78x - 0.26$; $Sy/x=1.14$ (Figure 2). It was determined that there was a proportional difference and the difference decreased with the increase in concentration. A novel PCT immunoassay kit on HYBIOME AE-180 system compared to VIDAS BRAHMS PCT by Wang et al. They revealed that the new kit has excellent analytical performance, including analytical sensitivity and a wide linear range. But the new kit slightly overestimated the PCT values, which approves that harmonization of different measurement methods of PCT has not yet been achieved [13].

In conclusion, the present study demonstrated that the analytical performance of the ECLIA method was quite good based on the precision and linearity findings. However, in comparing the methods, the analysis of the Deming regression figure revealed the proportional difference between the two methods. We found that y values corresponded to x values in increasing concentrations starting from 0.15 ng/mL in the equation $Y=0.78x - 0.26$. It was determined that the difference decreased with the increase in concentration. Bland-Altman Figure 3 supported these findings. All

findings suggested that the proportional difference between the two instruments should be revised for the cut-off values specified in the ECLIA method. Also, it was determined in the plot that the difference between the methods was specifically in lower concentrations.

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Competing interests: Authors state no conflict of interest.

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