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Analytical evaluation of the performances of a new procalcitonin immunoassay

Keywords: clinical concordance; correlation; interferences; precision; procalcitonin(PCT); stability.

To the Editor,

All the clinical cut-off values and algorithms have been defined according to the first available method for measuring procalcitonin (PCT), the BRAHMS PCT assay. It should also be noted that the KRYPTOR assay is considered as a surrogate reference method but it is not a higher order reference method listed in Joint Committee for Traceability in Laboratory Medicine database.

Recently, several manufacturers have implemented BRAHMS PCT assays on their instruments using common antibodies and/or calibrators from BRAHMS GmbH. By contrast, several companies have developed their own tests using monoclonal antibodies including Radiometer [1], Boditech [2], Diazyme [3], DiaSys [4], and recently Beckman Coulter [5].

The aim of our study was to perform the analytical and clinical performances of the BECKMAN PCT assay in comparison with the BRAHMS PCT sensitive KRYPTOR on

KRYPTOR GOLD instrument as well as to test the analyte stability after centrifugation and the interferences of hemolysis, lipids and bilirubin.

PCT was measured using BRAHMS PCT sensitive KRYPTOR on KRYPTOR GOLD instrument (ThermoScientific, BRAHMS AG, Hennigsdorf, Germany) and with the BECKMAN PCT assay, a paramagnetic, chemiluminescent immunoassay using the ACCESS 2 Immunoassay Systems (Villepinte, France).

Imprecision studies and the limit of detection (LoD) were based on the CLSI protocols [6, 7]. A plasma pool with PCT concentration of 6.2 µg/L was used to test the low-end linearity of the assay. Analytical performances (imprecision, LoB, LoD, and linearity study) of the Beckman assay are presented in Table 1. Although the within-run coefficients of variation (CV) was slightly higher than manufacturer or Lippi's study (>2% in our study vs. <2%), the total precision CV ranged from 2.88% to 3.94%. The LoB (0.00 µg/L) and LoD (0.01 µg/L) were close to that of the manufacturer (0.001 and 0.003 µg/L, respectively). The Passing-Bablok regression of linearity showed a gradual decrease in recovery from 100.0% (assigned value 6.29 µg/L) to 81.3% (assigned value 0.01 µg/L). The CUSUM test did not show significant deviation from linearity.

We evaluated the analyte stability after centrifugation at room temperature, at 4 °C, and after three repeated freeze-thaw cycles at -20 °C on 10 samples with normal and pathological levels of PCT. Interferences of hemolysis, lipemia and hyperbilirubinemia were carried out according to methods reported in Supplementary Figure A. The stability of samples, as well as the hemolysis, lipemia and icteric related bias was determined as the difference between the average of baseline values determined from the T0 reference concentration (in fresh sample or in the pool free of interferent (T0)) and the average of values obtained from final conditions of storage or from each overload of pool (Tx). To assess the maximum allowable stability, hemolysis, lipemic and icteric index (HLI) for ACCESS PCT method, we calculated the total change limit (TCL) [8]. When the bias (in percentage) was higher than TCL values, the

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Table 1: Analytical performances of PCT BECKMAN assay on the ACCESS 2[®] analyzer.

Total CV imprecision results	PCT BECKMAN assay from our study		Data from Lippi's	
	Mean, µg/L	CV, %	Mean, µg/L	CV, %
Control level 1	0.386	2.88	0.3	4.0
Control level 2	1.730	3.94	10.92	3.1
Plasma pool	0.543	2.34	–	–
LoB	0.000			0.002
LoD	0.010			0.003
Linearity				
Theoretical values, µg/mL	Mean of observed values with PCT BECKMAN assay, µg/L (% of mean recovery)			
6.29	6.33 (100.6)			
3.14	2.99 (95.2)			
1.57	1.45 (92.1)			
0.78	0.78 (99.9)			
0.39	0.33 (85.7)			
0.19	0.17 (87.9)			
0.02	0.021 (85.4)			
0.012	0.010 (81.3)			
0.006	0.004 (65.09)			

CV, coefficient of variation; LoB, limit of blank; LoD, limit of detection.

variation was considered as significant. Supplementary Figure A reports the stability of PCT results using TCL as the goal (12.1%), mean difference exceeded the TCL after three days of storage at room temperature, whereas at 4 °C, the samples were considered stable up to 7 days. After samples had been frozen and thawed up to three times, there were no statistically significant differences with a mean percentage of variation of 0.4%, very lower the TCL value (12%). There were no significant effects on PCT values with the addition of Hb up 1,200 mg/dL, triglycerides up to 3,000 mg/dL, and bilirubin up to 31 mg/dL. The variation in each spiked plasma sample compared to baseline sample remained much lower than 10% (Supplementary Figure B).

The comparison study was performed on 387 samples from consecutive patients ranging from 0.02 to 180 µg/L admitted to the Emergency and the ICU departments of Lapeyronie Hospital (Montpellier, France). Testing was performed simultaneously measuring the PCT within 2 h from heparinized plasma on the KRYPTOR instrument and on the ACCESS 2 analyzer using the residual sample. Only KRYPTOR PCT values were used for the clinical diagnosis. Passing Bablok regression analysis, Bland-Altman plots were performed to compare the results between two methods using XLSTAT[®] software, version 2016.06.35661 (NY, USA). Clinical concordance between the methods with disease state samples was assessed using Cohen's κ-test [9]. The mean difference in PCT measurements on 387 samples was 11% (SD=24%) with a trend to a very slight overestimation of ACCESS PCT vs. KRYPTOR PCT values. However, the ACCESS PCT assay exhibited good correlation in the working range of

0.02–180 µg/L and an acceptable concordance with results from KRYPTOR with a bias of 1.62 (±6.66) µg/L. With the low PCT values (<2 µg/L), the agreement in PCT measurements was excellent ($r>0.98$ with a bias of 0.009 (±0.15) µg/L (Figure 1)). With a Kappa coefficient of 0.850 (95% CI, 0.809–0.891), the strength of agreement is considered to be almost perfect between the two methods. Overall, the discrepancies in values measured by KRYPTOR and ACCESS 2 systems would have little impact on the clinical care provided to patients (Supplementary Tables A and B).

We measured on the ACCESS 2, external quality assessment (EQA) specimens stored at –80 °C from the 2019 to 2020 ProBioQual EQA program (ProBioQual, Lyon, France) and we compared values to KRYPTOR. Using the EQA samples (n=16), PCT values with Beckman method were higher than those reported by KRYPTOR instrument especially for EQA levels higher than 3 µg/L (Supplementary Figure C). Effectively, we observed on the 387 samples a bias at 1.62 (±6.66) µg/L in the entire measurement range and 0.009 (±0.152) µg/L for values lower than 2 µg/L and a bias at 15.76 (±8.40) µg/L for EQA values >3 µg/L.

Overall, the analytical performances of the BECKMAN PCT assay on the ACCESS 2 system were acceptable with an excellent imprecision (all CV <4%), good linearity and LoD largely lower than 0.25 µg/L. Our data highlighted the lack of interference to hemoglobin up to 1,200 mg/dL and confirm the absence of interference with lipids and bilirubin, which is of great interest for emergency, ICU and pediatrics departments for which the samples are frequently hemolyzed, turbidimetric, and icteric. The determination of optimal stability is

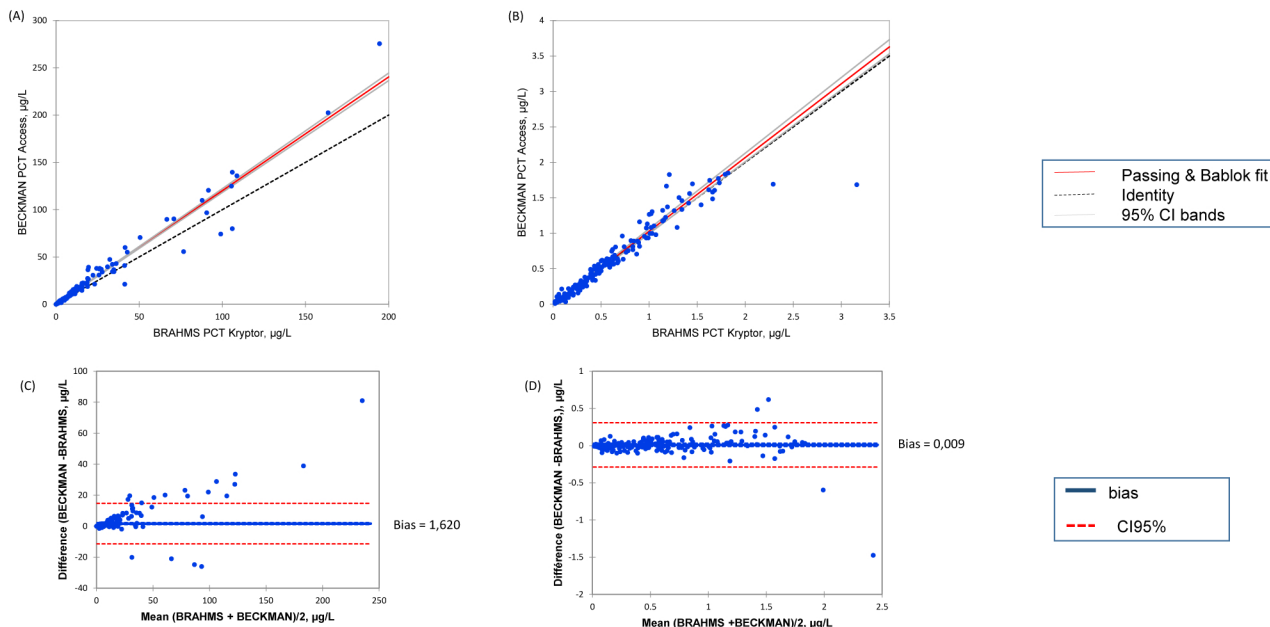


Figure 1: Passing-Bablok regression analysis on plasma samples in the range (A) 0.02–180 µg/L and (B) 0.02–2 µg/L of BRAHMS PCT Kryptor against BECKMAN PCT assay on ACCESS 2. Bland-Altman analysis between BRAHMS PCT vs. BECKMAN PCT assay on (C) all measuring range and (D) on <2 µg/L values.

useful to know for centralized automated platforms with blood testing carried out at a distance from clinical departments, leading to the transport of samples and thus exposing the samples to associated variable environmental conditions. PCT results were not affected by three frozen-thawed cycles, which can be useful for assaying PCT a posteriori on a Biobank stored at -20°C .

No reference methods for PCT quantitation are yet available, and comparison studies could be difficult to interpret [10]. Since all the clinical cut-offs were defined according to the first fully automated PCT assay, the BRAHMS PCT KRYPTOR, we decided to perform the correlation and clinical performances against this method. Our data showed excellent analytical correlation with PCT on KRYPTOR with very good strength of agreement and were in accordance with those obtained by Lippi's et al. study [5].

The slight overestimation remains acceptable. At cut-off 0.5 µg/L, no over- or underestimation was observed (ranging from 0.43 to 0.50 for KRYPTOR vs. 0.47 to 0.56 for ACCESS), with no clinical impact. The results of EQA analysis highlighted an overestimation of ACCESS values for high PCT concentrations. This overestimation at high values on EQA samples, and on the patient samples, have few impact on clinical care, but demonstrated the lack of standardization of calibration between methods [10, 11]. The differences in EQA determinations could be due not only to antibodies but

also to matrix effects of EQA samples or differences in measurement methods; however, these results highlighted the urgent need of standardization since the implementation of this dosage on new platforms is constantly growing.

In conclusion, the novel ACCESS PCT assay from Beckman shows analytical performances acceptable for clinical use as well as a fine concordance at all clinical cut-offs. Moreover, the discordance between the assays observed with the EQA system clearly suggested that standardization of PCT assays and commutability of EQA materials are necessary steps to understand the lack of comparability between results obtained by different PCT assays.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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