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► To cite this version:

Anne Marie Dupuy, Anne-Sophie Bargnoux, Valentin Montagnon, Nils Kuster, Stéphanie Badiou, et al.. Analytical evaluation of the novel VITROS BRAHMS procalcitonin immunoassay. Scandinavian Journal of Clinical and Laboratory Investigation, In press, 80 (7), pp.541-545. 10.1080/00365513.2020.1804071 . hal-02984469

HAL Id: hal-02984469

<https://hal.science/hal-02984469>

Submitted on 29 Jul 2021

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Analytical evaluation of the novel VITROS BRAHMS procalcitonin immunoassay

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ABSTRACT

To determine the analytical performance of Novel VITROS BRAHMS Procalcitonin Immunoassay on VITROS 3600 and correlation with BRAHMS PCT sensitive KRYPTOR reference method. Analytical performances including imprecision studies, linearity, limit of detection (LoD) and determination of hemolysis index were performed for VITROS BRAHMS PCT assay. Imprecision was assessed on plasma pool and internal control with 2 levels. The method comparison was performed using 162 plasma obtained from clinical departments. The total imprecision was acceptable and all CV were <5%. The LoD was in accordance with manufacturer's claims. The equation of linearity in the lower range was found to be $y = 1.0014x - 0.0091$, with $r^2 = 1$. No interference to hemoglobin up to 11 g/L was observed. Correlation studies showed a good correlation between PCT measurements using VITROS BRAHMS PCT assay against KRYPTOR system including for values lower than 2 µg/L. The novel VITROS BRAHMS PCT assay from OrthoClinical Diagnostics shows analytical performances acceptable for clinical use. In addition, the concordance with KRYPTOR method was fine at all clinical cut-offs.

KEYWORDS

Procalcitonin; data accuracy; correlation of data; cut-off; standardization

Introduction

Procalcitonin (PCT) is well established as a biomarker that reflects the presence and severity of local and systemic bacterial infections, i.e. sepsis [1] and as valuable tool to guide antibiotic therapy [2,3] allowing to tailor antibiotic treatment and to reduce antibiotic resistance [4]. Recently the importance of the PCT determination has been highlighted also for the prognosis of the COVID-19 [5].

The cut-offs of 0.25 µg/L which was used in emergency department for antibiotic therapy decision [6] and 0.5 µg/L allowing to differentiate systemic infection suggestive of sepsis from sterile causes of severe systemic inflammation [3] are the most used. Values between 0.5 and 2 µg/L are suggestive for a possible systemic infection (sepsis) with moderate risk for progression to severe systemic infection (severe sepsis) [6]. Values >2 and <10 µg/L are in favor of likely systemic infection (sepsis) with an high risk for progression to severe systemic infection (severe sepsis) [6]. Values > 10 µg/L clearly reflect an important systemic inflammatory response, almost exclusively due to severe bacterial sepsis or septic shock.

PCT clinical cut-offs and algorithms were established using the global reference method Thermo Scientific BRAHMS PCT sensitive KRYPTOR assay [7]. For this reason, several manufacturers have implemented BRAHMS PCT assays on their instruments using raw materials from BRAHMS GmbH. All BRAHMS PCT assays are calibrated

to the BRAHMS PCT sensitive KRYPTOR assay. Six license partners assays are available including Abbott (Architect and Alinity analyzers), Biomérieux (Vidas analyzer), Diasorin (Liaison analyzer), Fujirebio (Lumipulse analyzer), Roche (Cobas analyzer), and Siemens (Centaur and Atellica analyzers). To date, the company Ortho Clinical Diagnostics (Raritan, NJ) developed the BRAHMS PCT assay on VITROS[®] ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems. The analytical performance of VITROS BRAHMS PCT test on the VITROS[®] 3600, imprecision, linearity, limit of detection, determination of hemolysis index, was evaluated prior to product commercialization. In addition, clinical correlation with results from BRAHMS PCT sensitive KRYPTOR reference method (used in routine in our lab) was assessed and discordant values were analyzed according to clinical records.

Materials and methods

PCT Central laboratory method

In the central laboratory PCT was measured using BRAHMS PCT sensitive KRYPTOR on KRYPTOR GOLD instrument (ThermoScientific, BRAHMS AG, Hennigsdorf, Germany). The method consists of anti-calcitonin polyclonal antibody conjugated with europium cryptate and anti-kata-calcin monoclonal antibody conjugated with XL665.

Detection is based on TRACE (Time Resolved Amplified Cryptate Emission). Results were obtained within 19 min on the KRYPTOR® system.

PCT on VITROS 3600

The VITROS BRAHMS PCT test was evaluated with the VITROS BRAHMS PCT Reagent Pack and the VITROS BRAHMS PCT Calibrators on the VITROS 3600 Immunodiagnostic System using Intellicheck® Technology based on two-step immunoassays with an automated luminescent detection. VITROS method uses a 2 levels calibration. Controls are liquid-frozen, three levels with target values at 0.5, 2.0 and 60.0 µg/L. Results were obtained within 24 min.

Analytical performances and method comparison

Imprecision studies were based on the CLSI EP15 protocol (five measurements in duplicate twice per day on 3 levels for 5 consecutive days) [8] using plasma pool of low PCT value (0.45 µg/L) and on two control materials stored at -20°C . The limit of detection (LoD) was determined according to the current CLSI standard [9]. Both analyte free and low concentration samples were tested. A buffer matrix (sample A) was used as the analyte free sample, and dilutions of pooled plasma (sample B) were made using the buffer, such that the concentrations of the diluted sample B approximates 3 and 4 times the assay's sensitivity claimed by the manufacturer. Ten replicates of the sample A and 10 replicates of both low concentration samples (3X and 4X) were run. The LoD was calculated as $\text{LoD} = \text{LoB} + 1.645 \sigma\text{S}$, where σS is the standard deviation of the population of the low concentration sample measurements. The interference to hemolysis was performed according to Delgado's approach [10]. We selected two simultaneous 3.5 mL lithium heparin tubes from five donors who had a PCT value around 0.5 µg/L. One of the 2 tubes was directly centrifuged (10 min, 2000g), while the other was subjected to three freeze-thaw cycles to induce hemolysis and subsequently centrifuged. Both plasma samples were mixed in different proportions to create a hemolysis gradient.

A plasma pool with PCT concentration of 6.0 µg/L was used to test the low-end linearity of the assay. The pool was diluted at the following final concentrations: 3.0, 1.5, 1.0, 0.75, 0.37, 0.18, 0.06 and 0.03 µg/L. Each concentration was measured in duplicate.

The method comparison study was performed on samples obtained from 162 consecutive patients admitted to the Emergency Department and the Intensive Care Unit departments of Lapeyronie University Hospital (Montpellier, France). The samples spanned the analytical range of the BRAHMS PCT KRYPTOR GOLD reference method (0.02–50 µg/L). Testing was performed simultaneously measuring the PCT from heparinized plasma on the KRYPTOR GOLD® instrument and on the VITROS 3600 analyzer using the residual sample. Only BRAHMS PCT KRYPTOR GOLD values were used for the clinical diagnosis.

In addition, we measured on the VITROS 3600, external quality assessment (EQA) specimens stored at -80°C from the 2018 ProBioQual EQA program (ProBioQual, Lyon, France) and we compared values to other systems using BRAHMS PCT antibodies

Statistical analysis

Deming regression analysis was used to compare the results from the VITROS 3600 PCT assay with those from the BRAHMS PCT KRYPTOR used in our daily practice. The scatter of differences was visualized by means of Bland-Altman plots [11]. The CUSUM test was used to detect deviation from expected values in linearity studies. Additionally, we took account of the concept of acceptable difference limit, which was calculated according to ISO 5725-6 [12] as follows: Acceptable difference limit = $Z \times \sqrt{2(CV_1^2 + CV_2^2)^{1/2}}$, where $Z = 1.96$ as determined using the 95% confidence interval for bi-directional changes, and $\sqrt{2}$ was a comparison of two results, CV_1 and CV_2 were respectively the imprecision of each assay (both expressed in %). Statistical analyses were performed using XLSTAT® software, version 2016.06.35661 (NY, USA).

In addition, to better evaluate the clinical concordance between methods, the population was divided into 5 categories as follows: category 1, PCT values < 0.25 µg/L; category 2, PCT values between 0.25 and 0.5 µg/L; category 3, PCT values between ≥ 0.5 and 2 µg/L, category 4, PCT values between ≥ 2 and 10 µg/L and category 5, PCT values ≥ 10 µg/L. Clinical concordance between the methods with disease state samples was then assessed using Cohen's κ -test with values < 0 as indicating no agreement, 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial and 0.81–1 as almost perfect agreement [13,14].

The hemolysis related bias was determined as the difference between the average of baseline values determined in the pool free of hemolysis (T0) and the average of values obtained from each overload of pool (Tx). To assess the maximum allowable hemolysis index (HI) for VITROS BRAHMS PCT method, we calculated the total change limit (TCL) described by Oddoze et al. [15] using the formula $[(2.77 \times \text{CVa})^2]^{1/2} + [(0.5 \times \text{CVb})^2]^{1/2}$, where CVa is analytical imprecision and CVb is within-subject variation. The allowable HI for PCT was defined as the last HI value without bias higher than the cut-off criteria, using TCL as the significant limit of bias. When the bias (in percentage) was higher than TCL, values, the variation was considered as significant. For all comparisons, p -value $< .05$ was considered statistically significant. In the presence of discordant results between two methods, the samples were re-analyzed on the two instruments and the clinical record was checked.

Results

Analytical performances (imprecision, LoD, linearity and hemolysis interference study) of the VITROS BRAHMS PCT assay are presented in Table 1. The within-run coefficients of variation (CV) gave satisfactory results (all CVs

<3.0%). The total precision CV ranged from 2.2% to 4.6%. The LoD was close to that of the manufacturer. No hemolysis interference was observed for VITROS BRAHMS PCT assay at the cut-off 0.5 µg/L even at an HI value of 11 g/L. The percentage variation from the baseline value free of hemolysis and the overloaded plasma with hemolysate was below 10% which was the calculated TCL. Our data extend the lack of interference to hemoglobin to 11 g/L while the manufacturer only tested up to 6 g/L.

The VITROS BRAHMS PCT assay displayed acceptable linearity over the most clinically relevant range for PCT concentrations with a trend of gradual decrease in recovery. The Passing-Bablok regression showed a break in the dilution series at the second lowest sample value (0.06 µg/L). The CUSUM test did not show significant deviation from linearity.

Deming regression analyses between the PCT measurements of 162 plasma samples on the KRYPTOR GOLD vs the VITROS 3600 are reported in Figure 1. The mean difference in PCT measurements for the entire measurement range was 7% (SD = 16%). We observed a trend to overestimate low PCT values (in the range 0.2–2 µg/L) and to underestimate high values (>10 µg/L) with the VITROS 3600 method; however, the PCT assay on the VITROS 3600 exhibited good correlation in the working range of 0.02–50 µg/L and an acceptable concordance with results from KRYPTOR GOLD with a bias of −0.12 (±0.98) µg/L. We focused on the low PCT values between 0.02 and 2 µg/L to target the cut-off 0.5 µg/L. The agreement in PCT measurements was excellent between the VITROS 3600 and KRYPTOR GOLD systems as indicated by the correlation coefficient >0.97 (Figure 1). Bland Altman analysis further confirmed this good agreement (Figure 1) with a bias of −0.06 (±0.10) µg/L. We calculated the acceptable difference limit threshold to be 27% and found 14 (8.6%) inconsistent results between plasma samples measurements. Thus, PCT measurements with the KRYPTOR GOLD and VITROS 3600 systems were classified as significantly different. However this test should be taken with caution. Effectively, the interpretation of acceptable difference limit test has to consider that the analyzers with the highest imprecision have increased acceptable difference limit thresholds. So the poor informative value of the acceptable difference limit in this setting represents a limitation of this method comparison.

The Kappa coefficient determined to be 0.878 (95%CI, 0.746–0.892) and consequently, the strength of agreement is considered to be almost perfect between the two methods. Overall, the discrepancies in values measured by KRYPTOR GOLD® and VITROS 3600 systems would have little impact on the clinical care provided to patients.

The EQA samples with low PCT values (<5 µg/L) measured with VITROS 3600 were close to those reported from other diagnostic devices including Vidas® from Biomérieux, Architect® from Abbott, Cobas® from Roche, Advia Centaur® from Siemens, Liaison® from Diasorin and Lumipulse® from Fujirebio using BRAHMS antibodies;

however, for high values VITROS BRAHMS PCT assay exhibited an underestimation

Discussion

The analytical performance of the VITROS BRAHMS PCT assay on the VITROS 3600 system was acceptable. The VITROS BRAHMS PCT assay showed equally good analytical performance compared to other systems using BRAHMS PCT antibodies. The clinical concordance with BRAHMS PCT on KRYPTOR GOLD allows the VITROS BRAHMS PCT assay to be considered a satisfactory solution. Due to the lack of standardization we focused on systems using BRAHMS PCT antibodies rather than on methods using their own antibodies such as Radiometer, Beckman, Diasys or Diazyme.

The levels of imprecision were comparable with those reported for the PCT assay on the BRAHMS PCT KRYPTOR GOLD® claimed by the manufacturer and other systems using BRAHMS antibodies [16,17]. Imprecision is excellent, with all CVs < 5%.

Our data extend the manufacturer's claim of lack of interference to hemoglobin of 6 g/L to 11 g/L. This result is of great interest particularly for samples from pediatrics units or the ED where the rate of hemolyzed samples is known to be significant.

Our data showed excellent analytical correlation with PCT on KRYPTOR GOLD® and were in accordance with those obtained by other systems using BRAHMS PCT assays. Effectively, the regression analysis between BRAHMS PCT KRYPTOR GOLD® vs Abbott-Architect® [17], Biomérieux-Vidas® [18], Roche-Cobas e601® [18] and Siemens-Centaur® [18] yielded slopes at 0.91, 1.40, 1.12 and 0.80 respectively and correlation coefficients between 0.97 and 0.98. The study conducted in our lab between Lumipulse® G600II BRAHMS PCT from Fujirebio and BRAHMS PCT KRYPTOR CompactPlus® reveals a regression line of the same order ($y = 1.07x + 0.0447$, $r > 0.99$) [10].

Recently, a study conducted by Chambliss et al. [19] compared at low PCT values (<2 µg/L) the analytical correlation between BRAHMS PCT KRYPTOR® vs Abbott-Architect i2000®, Biomérieux-MiniVidas®, and Roche-Cobas e411®. The slope with the correlation coefficient was found at 0.80, 1.18 and 0.79 with 0.88, 0.98, and 0.79 respectively. With a slope in the working range of 0.02–50 µg/L at 0.89 and in the range <2 µg/L at 1.04, the VITROS BRAHMS PCT assay was very well aligned with BRAHMS PCT KRYPTOR GOLD. The overestimation noted around the cut-off of 0.5 µg/L on 13 samples remains acceptable since the values are very close to 0.5 µg/L (ranging from 0.39 to 0.49 µg/L for KRYPTOR vs 0.51 to 0.56 µg/L for VITROS), except for one sample for which we had the KRYPTOR value at 0.49 µg/L for a VITROS value at 0.76 µg/L. The review of the clinical record for this patient sample showed that PCT was requested for monitoring a fever in a patient with recent pneumonia. All the evaluations

Table 1. Analytical performance of VITROS BRAHMS PCT assay on VITROS 3600 analyzer.

	In our study			Manufacturer's data	
	Mean, µg/L	CV, %		Mean, µg/L	CV, %
<i>Repeatability imprecision</i>					
Low plasma pool	0.45	2.05		0.48	1.5
High plasma pool	1.92	1.77		1.92	1.5
<i>Intermediate precision</i>					
Plasma pool	0.45	2.25	Plasma pool	0.48	3.7
Control level 1	0.51	3.28	Control level 1	0.48	3.4
Control level 2	1.92	4.64	Quality control level 2	1.93	4.0
LoD	0.012			0.007	
HI, g/L	>11			>6	
Linearity	Theoretical values, µg/L		Mean of observed values, µg/L (% of mean recovery)		
	6.07		6.07 (100)		
	3.03		3.03 (100)		
	1.51		1.50 (99)		
	0.75		0.72 (97)		
	0.37		0.35 (95)		
	0.18		0.17 (95)		
	0.06		0.05 (98)		
	0.03		0.03 (113)		

CV: coefficient of variation; HI: hemolysis index; LoD: limit of detection.

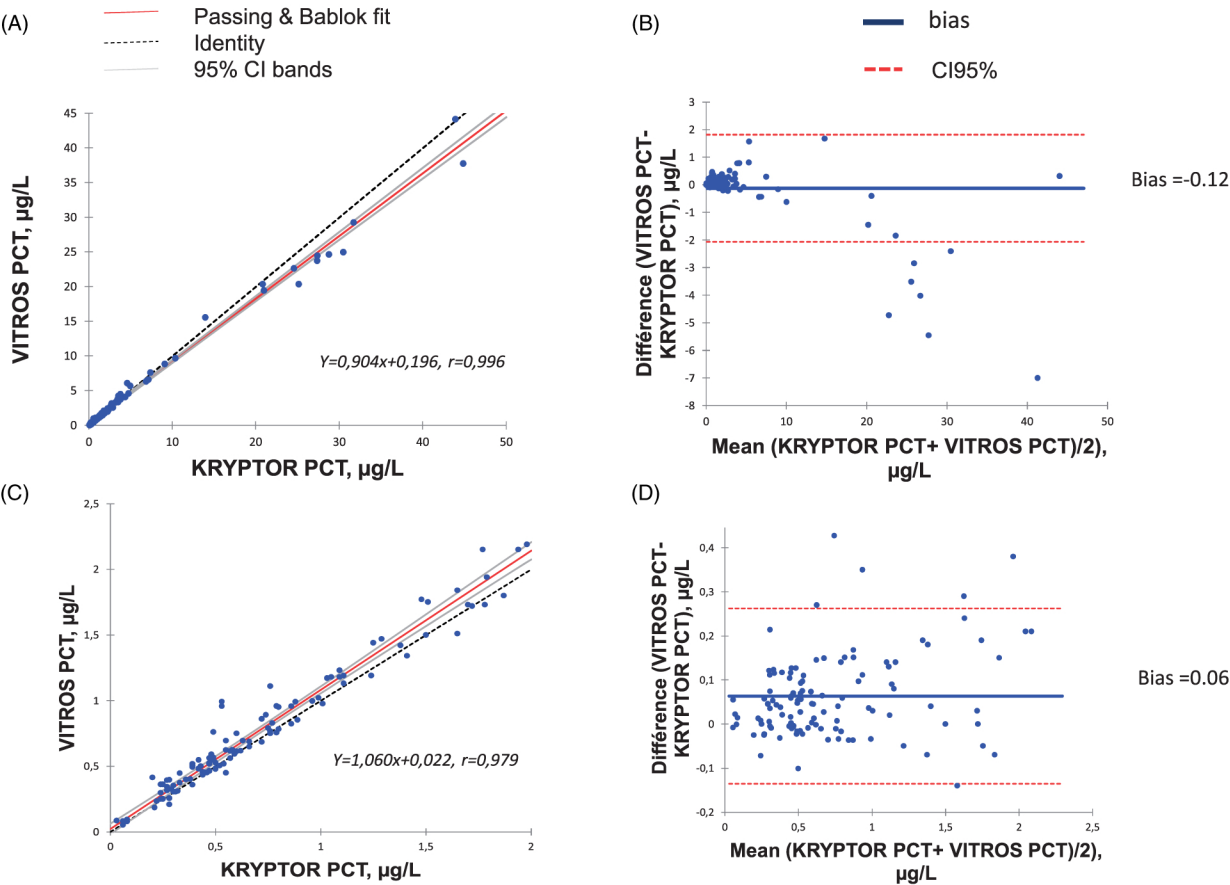


Figure 1. (A) Deming regression analysis on 162 plasma samples in the range 0.02–50 µg/L of KRYPTOR PCT against VITROS PCT and (B) Bland-Altman analysis, (C) Deming regression analysis on 117 plasma samples in the range 0.02–2 µg/L of KRYPTOR PCT against VITROS PCT and (D) Bland-Altman analysis.

between KRYPTOR and other analyzers using BRAHMS PCT assays as well as the EQA analysis demonstrated the lack of standardization of calibration between methods. Even using the same antibodies, the correlation between KRYPTOR and other analyzers was not completely concordant. Effectively, we noted the overestimation between KRYPTOR and Vidas and the underestimation with Abbott

and Roche [19]. In addition, the results of EQA analysis highlighted an underestimation of VITROS values for high PCT concentrations. This underestimation at high values on EQA samples, but also observed in the comparison study on the patient samples, have few impact on clinical care. The differences in EQA determinations could be due 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.

Conclusion

The clinical concordance with BRAHMS PCT on KRYPTOR GOLD© allows the VITROS BRAHMS PCT assay to be considered a satisfactory solution for the determination of PCT in human plasma with acceptable analytical performance and good analytical correlation with the KRYPTOR method, the gold standard.

Acknowledgements

Reagents, calibrators, quality controls for the VITROS B-R-A-H-M-S PCT assay that were used in this study were kindly provided by Ortho Clinical Diagnostic France. The authors thank ProBioQual for kindly providing their data.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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