

Analytical evaluation of the novel Lumipulse G BRAHMS procalcitonin immunoassay

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ABSTRACT

Objectives: This study was designed to evaluate the analytical performance of the novel Lumipulse G1200 BRAHMS procalcitonin (PCT) immunoassay.

Design and methods: This analytical evaluation encompassed the calculation of the limit of blank (LOB), limit of detection (LOD), functional sensitivity, intra- and inter-assay imprecision, confirmation of linearity and a comparison with the Vidas BRAHMS PCT assay.

Results: The LOB, LOD and functional sensitivity were 0.0010 ng/mL, 0.0016 ng/mL and 0.008 ng/mL, respectively. The total analytical imprecision was found to be 2.1% and the linearity was excellent ($r=1.00$) in the range of concentrations between 0.006–75.5 ng/mL. The correlation coefficient with Vidas BRAHMS PCT was 0.995 and the equation of the Passing and Bablok regression analysis was [Lumipulse G BRAHMS PCT] = $0.76 \times$ [Vidas BRAHMS PCT] + 0.04. The mean overall bias of Lumipulse G BRAHMS PCT versus Vidas BRAHMS PCT was -3.03 ng/mL (95% confidence interval [CI]: -4.32 to -1.74 ng/mL), whereas the mean bias in samples with PCT concentration between 0–10 ng/mL was -0.49 ng/mL (95% CI: -0.77 to -0.24 ng/mL). The diagnostic agreement was 100% at 0.5 ng/mL, 97% at 2.0 ng/mL and 95% at 10 ng/mL, respectively.

Conclusions: These results attest that Lumipulse G BRAHMS PCT exhibits excellent analytical performance, among the best of the methods currently available on the diagnostic market. However, the significant bias compared to the Vidas BRAHMS PCT suggests that the methods cannot be used interchangeably.

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1. Introduction

The exact definition of sepsis has changed considerably over time, and remains controversial. This is probably due to the fact that sepsis develops as a continuum of severity, ranging from local infection, through bloodstream propagation to septic shock, which can ultimately generate multiple organ dysfunction syndrome (MODS) and death. According to the most recent definitions endorsed by the third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) in February 2016 [1], sepsis is a clinical syndrome characterized by biological and biochemical abnormalities due to development of dysregulated inflammatory response to infections, whereas septic shock has been identified as a subset of sepsis in which profound circulatory, cellular and metabolic abnormalities substantially increase the risk of mortality. Notably, the expert panel reiterated the concept that sepsis is the leading cause of death from infections, especially when not promptly recognized and appropriately managed [1]. Globally, the mortality can be as high as 10–20% in patients with sepsis, 20–50%

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in those with severe sepsis and 40–80% in patients with septic shock [2]. These worrying figures reinforce the well-known concept that an early diagnosis is critical for reversing the otherwise unfavorable clinical outcome in septic patients.

The issue of the early diagnosis of sepsis has engaged the minds of many physicians over the past decades [3]. In fact, recent epidemiological data suggest that the frequency curve of sepsis has only modestly bent backward, since this condition may still develop in approximately 2% of all hospitalized patients, with a frequency that remains dramatically high, up to 30%, in intensive care unit (ICU) patients [2]. It is hence obvious that the availability of early and accurate diagnostic and prognostic biomarkers should be regarded as a valuable opportunity for effective clinical management of severe bacterial infections [4].

Procalcitonin (PCT) is the 116 amino acid precursor of the hormone calcitonin. Under physiological conditions, PCT is synthesized and secreted by the C-cells of the thyroid gland, but its blood concentration remains virtually undetectable (< 0.1 ng/mL) [5]. In severe bacterial infections and sepsis, however, the extrathyroidal production of PCT is dramatically enhanced, so that its concentration in blood may increase by several orders of magnitude, up to 100–1000 ng/mL in the most severe cases [6]. Because of its peculiar kinetics in patients with bacterial infections, the measurement of PCT is currently considered as the biochemical gold standard for diagnosing and monitoring sepsis and other severe bacterial infections such as community-acquired pneumonia [7,8]. More specifically, a PCT concentration < 0.5 ng/mL has been identified as the most accurate diagnostic threshold for ruling out bacterial infections, slightly elevated PCT concentrations (i.e., between 0.5 and 2.0 ng/mL) are commonly observed in bacterial infections which have triggered a minor systemic inflammatory response, whereas values > 10 ng/mL are almost exclusively suggestive of severe sepsis or septic shock [5]. In addition to its consolidated role in the diagnosis of severe bacterial infections, the use of PCT estimation is rapidly broadening in clinical practice. Recent evidence suggests that PCT-guided algorithms may be effective for reducing the duration of treatment and doses of antibiotics, as well as for decreasing the overall mortality in critically ill patients with bacterial infections [9].

Due to the steadily increasing use of PCT measurement for diagnosis and management of bacterial infections, the number of PCT immunoassays available on the diagnostic market has increased in recent years, with automated immunoassays replacing the former manual techniques. The most widely used methods in clinical laboratories are now represented by time resolved amplified cryptate emission (TRACE), enzyme-linked fluorescent (ELFA), chemiluminescent enzyme (CLEIA), electro-chemiluminescent (ECLIA) and immunoturbidimetric immunoassays [10,11]. Recently, a novel automated CLEIA immunoassay for PCT measurement has been adapted for use on the LUMIPULSE G system. The aim of this study was to evaluate the analytical performance of this novel method.

2. Materials and methods

2.1. Description of the immunoassay

The Lumipulse G BRAHMS PCT immunoassay (Fujirebio Diagnostics Inc., Tokyo, Japan) is a two-step sandwich immunoassay adapted to be used on the LUMIPULSE G1200 system for quantitative determination of PCT in human serum and plasma. The assay is based on CLEIA technology. Briefly, the PCT molecules present in the test sample bind to monoclonal anti-PCT mouse antibodies and to capture anti-calcitonin mouse antibodies coated on polystyrene beads, thus generating stable immune complexes. The polystyrene beads are then washed to eliminate unbound material and incubated with another monoclonal anti-calcitonin mouse antibody conjugated with alkaline phosphatase. After a second wash, the substrate 3-(2'-spiroadamantyl)-4-methoxy-4-(3'-phosphoryloxy)-phenyl-1,2-dioxetane (AMPPD) is added to the reaction mixture and the resulting luminescence is monitored at 477 nm. The intensity of the luminescent reaction is directly proportional to the concentration of PCT in the test sample. According to manufacturer's data, the assay displays an analytical sensitivity of 0.0048 ng/mL, a functional sensitivity of 0.0079 ng/mL and linearity comprised between 0.02–85.10 ng/mL. The sample volume needed for the assay is 60 μ L (plus 100 μ L of dead volume on the analyzer) and results are available in 30 min.

2.2. Evaluation of the analytical characteristics

The analytical characteristics of Lumipulse G BRAHMS PCT were evaluated by calculation of the limit of blank (LOB), limit of detection (LOD) and functional sensitivity. More specifically, the LOB was calculated as the value corresponding to the sum of the mean and $1.645 \times$ standard deviation (SD) of 10 consecutive replicates of saline, as described elsewhere [12]. The LOD was calculated as the sum of the LOB and $1.645 \times$ SD of 10 replicates of an inpatient serum pool with the lowest measurable PCT value [12]. The functional sensitivity was defined as the lowest PCT concentration that could be determined with a coefficient of variation (CV) $\leq 10\%$. This value was calculated by preparing serial dilutions in sample buffer (i.e., 1:2; 1:4; 1:8; 1:16 and 1:32) of an inpatient serum sample with a PCT concentration of approximately 0.20 ng/mL. After measuring each dilution in 10 replicates, the CV was calculated for each dilution. A model fit was then developed to extrapolate the PCT value with 10% imprecision.

2.3. Imprecision studies

The imprecision studies were carried out using three serum pools with low (i.e., ~0.2 ng/mL), intermediate (i.e., ~3.2 ng/mL) and high (i.e., ~21.6 ng/mL) concentrations of PCT. Each serum pool was obtained by pooling 30 anonymized serum samples collected in evacuated blood tubes (Vacutest 3.5 mL, 75 × 13 mm, containing gel and clot activator; Kima, Padova, Italy) referred to the laboratory for routine PCT testing. The pools were then thoroughly mixed and divided into 21 identical aliquots of 2 mL, which were stored at -70°C . The intra-assay imprecision was evaluated by 20 sequential measurements of one stored aliquot of each pool, whereas the inter-assay imprecision was assessed by measurement of the remaining stored aliquots over 20 consecutive working days (i.e., one aliquot of each pool per day). The results were reported as CV.

2.4. Linearity

An anonymized routine serum sample with high PCT concentration (i.e., 75.5 ng/mL) was serially diluted at fixed ratios (1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3; 8:2; 9:1) with an anonymized routine serum sample with low PCT value (i.e., 0.006 ng/mL), to cover the most clinically significant range of PCT concentrations in health and disease. Serial dilutions were analyzed in duplicate and the theoretical values were calculated from the measured value of the undiluted specimen. Linearity was assessed by calculation of Passing and Bablok regression analysis and Spearman's correlation coefficient (r).

2.5. Method comparison

The comparison studies were performed using 100 consecutive anonymized serum samples referred to the laboratory for routine testing, each of which was divided into two identical aliquots and simultaneously analyzed with both the Lumipulse G BRAHMS PCT and the ELFA immunoassay Vidas BRAHMS PCT (BioMérieux, Marcy l'Etoile, France). According to the manufacturer's data sheet, the measuring range of the latter method is between 0.05 and 200 ng/mL, and the inter- and intra-assay imprecision are between 1.9–4.6% and 3.6–7.0%, respectively. The correlation between the two methods was assessed with Passing and Bablok regression analysis and Spearman's correlation coefficient (r). The mean bias and its 95% Confidence Interval (95% CI) compared to Vidas BRAHMS PCT were estimated by Bland and Altman plot analysis. The agreement (and kappa statistics) of values was assessed at the three relevant diagnostic PCT thresholds of 0.5 ng/mL, 2.0 ng/mL and 10 ng/mL. A receiver operating characteristics (ROC) curve analysis was also used to define the agreement at the same PCT cut-offs.

2.6. Statistics and ethical approval

The significance level of the statistical analyses was set at $p < 0.05$. The statistical analysis was performed with Analyze-it (Analyze-it Software Ltd, Leeds, UK). The analytical evaluation of the Lumipulse G BRAHMS PCT was entirely based on anonymized pre-existing serum samples referred for routine PCT testing. Therefore, no 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.

3. Results

3.1. Analytical characteristics

The LOB and LOD of Lumipulse G BRAHMS PCT, calculated from the raw data as previously described, were found to be 0.0010 ng/mL and 0.0016 ng/mL, respectively. The calculation of the functional sensitivity of the immunoassay is shown in Fig. 1. The 10% imprecision obtained after measurement of serial dilutions of a serum sample with PCT concentration of 0.20 ng/mL corresponded to a PCT value of 0.008 ng/mL, thus perfectly matching the manufacturer's stated functional sensitivity.

3.2. Imprecision studies

The results of the imprecision studies are shown in Table 1. The intra-assay and inter-assay imprecision for the serum pools with low, medium and high PCT concentrations ranged between 0.9–1.8% and between 1.4–1.9%, respectively. The total analytical imprecision, calculated according to Krouwer and Rabinowitz [13] was found to be 2.1%.

3.3. Linearity studies

The Lumipulse G BRAHMS PCT immunoassay displayed excellent linearity over the range of the most relevant PCT concentrations in health and disease (0.006–75.5 ng/mL). More specifically, the equation of the Passing and Bablok

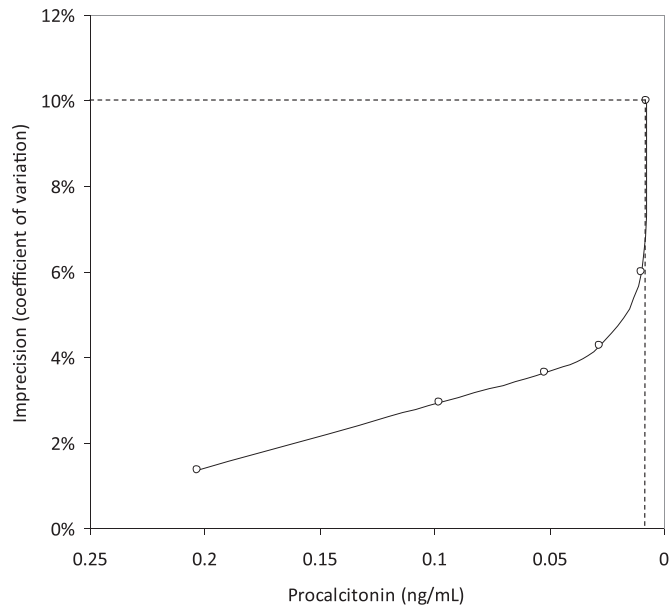


Fig. 1. Calculation of the functional sensitivity of the Lumipulse G BRAHMS procalcitonin (PCT) immunoassay.

Table 1

Analytical imprecision of Lumipulse G BRAHMS PCT.

	Intra-assay				Inter-assay			
	n	Mean (ng/mL)	SD (ng/mL)	CV	n	Mean (ng/mL)	SD (ng/mL)	CV
Pool low	20	0.240	0.002	0.9%	20	0.258	0.004	1.4%
Pool medium	20	3.252	0.059	1.8%	20	3.459	0.066	1.9%
Pool high	20	21.60	0.24	1.1%	20	23.11	0.36	1.6

SD, standard deviation; CV, coefficient of variation.

regression analysis was $y = 1.00x + 0.14$, and Spearman's correlation coefficient was 1.00 (95% CI, 1.00–1.00; $p < 0.001$).

3.4. Method comparison

An excellent correlation was found when comparing data obtained with Lumipulse G BRAHMS PCT and Vidas BRAHMS PCT. More specifically, Spearman's correlation coefficient was 0.995 (95% CI, 0.993–0.997; $p < 0.001$), and the equation of the Passing and Bablok regression analysis was $[\text{Lumipulse G BRAHMS PCT}] = 0.76 \times [\text{Vidas BRAHMS PCT}] + 0.04$ (Fig. 2). The result of the Bland and Altman plot analysis is shown in Fig. 3. The mean overall bias of Lumipulse G BRAHMS PCT versus Vidas BRAHMS PCT was -3.03 ng/mL (95% CI: -4.32 to -1.74 ng/mL), whereas the mean bias in samples with PCT concentration between 0–10 ng/mL ($n = 78$) was -0.49 ng/mL (95% CI: -0.77 to -0.24 ng/mL). The diagnostic agreement of the two methods at three relevant diagnostic PCT thresholds was 100% (kappa statistic: 1.00; 95% CI: 1.00–1.00) at 0.5 ng/mL, 97% (kappa statistic: 0.94; 95% CI, 0.87–1.01) at 2.0 ng/mL and 95% (kappa statistic: 0.87; 95% CI, 0.75–0.98) at 10 ng/mL. The area under the curve (AUC) was 1.00 (95% CI: 1.00–1.00) at 0.5 ng/mL, 0.98 (95% CI: 0.94–1.00) at 2.0 ng/mL and 0.91 (95% CI: 0.83–0.98) at 10 ng/mL, respectively.

4. Discussion

The measurement of serum biomarkers such as PCT has become part of standard clinical practice for identifying and managing bacterial infections [4,5]. The concentration of PCT may assist physicians in deciding whether or not the presumed infection is truly bacterial, thus enabling a more accurate diagnosis and more appropriate treatment, but it is also increasingly used to guide antibiotic stewardship [6–8]. Therefore, the availability of simple, rapid, high-throughput and highly accurate methods for measuring PCT is now virtually essential for effective patient management [5]. Several commercial immunoassays have been developed in the past decades for the routine measurement of PCT in clinical laboratories, encompassing rather different techniques and characterized by varying analytical performances [10,11].

The results of this evaluation of the new Lumipulse G BRAHMS PCT confirm that the method exhibits excellent analytical

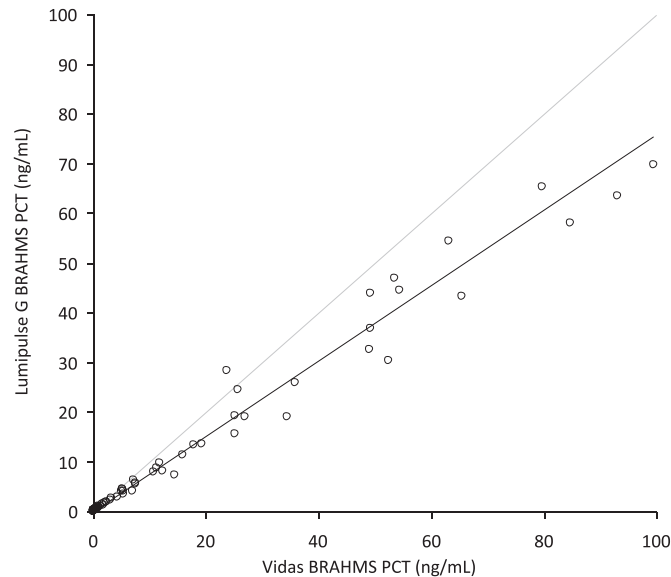


Fig. 2. Passing and Bablok regression analysis of procalcitonin (PCT) values obtained with Lumipulse G BRAHMS PCT and Vidas BRAHMS PCT. Regression line and line of identity are shown.

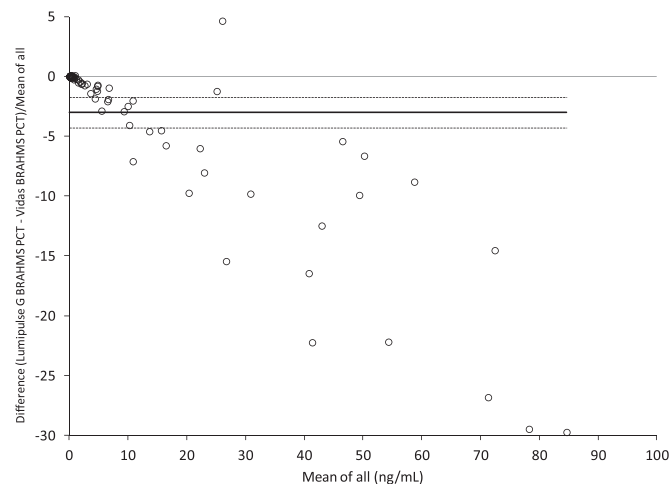


Fig. 3. Bland and Altman plot analysis of procalcitonin (PCT) values obtained with Lumipulse G BRAHMS PCT and Vidas BRAHMS PCT.

performance, making it one amongst the best and most sensitive techniques commercially available for PCT measurement. To the best of our knowledge, no previous commercial technique has been shown to exhibit values of LOD, LOB and functional sensitivity better than those observed with this immunoassay. Notably, the LOD (0.0010 ng/mL) and LOB (0.0016 ng/mL) obtained in our study were found to be 4- to 5-fold lower than those declared by the manufacturer, whereas the functional sensitivity was virtually identical (i.e., 0.008 ng/mL versus 0.0079 ng/mL). Importantly, such an excellent functional sensitivity would enable the early and accurate detection of minor variations of PCT concentration in serum or plasma, thus providing extremely useful information for antibiotic treatment and therapeutic monitoring. On the other hand, however, it remains to be established whether having such a low detection limit would also carry some disadvantages, especially in terms of false positive results. An accurate definition of reference range and diagnostic thresholds would appear to be mandatory to overcome potential problems emerging from the considerably enhanced analytical sensitivity of this novel immunoassay. The manufacturer's claimed linearity in the relevant clinical range of serum PCT concentrations was confirmed in our study, which showed a virtually perfect correlation coefficient (i.e., $r=1.00$) in the range of PCT values between 0.006–75.5 ng/mL. This would probably allow extension of the measuring range specified by the manufacturer (0.02–100 ng/mL) to values lower than 0.02 ng/mL, thus potentially enhancing its clinical usefulness for diagnosis and monitoring of minor or localized bacterial infections. Notably, the total imprecision of Lumipulse G BRAHMS PCT was also found to be better than the manufacturer's claim (1.9–3.8%), but also lower than that found in the evaluation of other commercial immunoassays [10,11]. However, despite the excellent correlation of PCT values with Vidas BRAHMS PCT, a

significant bias was observed. The mean overall bias of Lumipulse G BRAHMS PCT versus Vidas BRAHMS PCT was -3.03 ng/mL, whereas the mean bias in samples with PCT concentration between 0–10 ng/mL was lower (-0.49 ng/mL). Although no clear indication is provided by the manufacturers, the significant bias is probably attributable to the fact that the two methods use different calibrators and better agreement between commercial methods will only be possible when a reference calibration material will be developed and universally accepted. The gradual trend toward underestimation with increasing PCT concentration by Lumipulse G BRAHMS PCT compared to Vidas BRAHMS PCT was also confirmed by our agreement analysis, inasmuch as the concordance at specific cut-offs gradually decreased from 100% at 0.5 ng/mL, to 97% at 2.0 ng/mL and 95% at 10 ng/mL. This suboptimal agreement, combined with the significant underestimation of PCT values with Lumipulse G BRAHMS PCT, confirms that harmonization of the different PCT methods has not yet been reached, and the different immunoassays should not be used interchangeably for purposes of longitudinal patient monitoring.

5. Conclusions

In conclusion, the results of this evaluation of Lumipulse G BRAHMS PCT demonstrate that this technique exhibits excellent analytical performance, among the best of the methods currently available on the diagnostic market for routine PCT measurement. However, the significant bias shown by comparing the two immunoassays studied emphasizes that longitudinal patient monitoring should be always performed using the same immunoassay.

Conflict of interest

None of the authors have any conflict of interest to report.

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