



Validation of the analytical performance of Lumipulse G BRAHMS procalcitonin immunoassay on low sample volume

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Background: The measurement of procalcitonin (PCT) is currently considered a cornerstone in the diagnostic approach of patients with severe bacterial infections and for monitoring antimicrobial therapy. However, since low volume samples represent a challenge for PCT measurement, this study was aimed to investigate the analytical recovery of Lumipulse G BRAHMS PCT immunoassay using serially diluted lithium-heparin plasma samples.

Methods: Fifteen fresh lithium-heparin plasma samples were arbitrarily selected to cover clinically significant measuring ranges of PCT values (low PCT, 0.034–0.085 ng/mL; medium PCT, 0.092–0.638 ng/mL; high PCT, 2.022–9.187 ng/mL). The samples were diluted with Lumipulse sample diluent, to obtain 5 progressive dilutions (1:2; 1:5; 1:10; 1:20 and 1:32). The original samples and each serial dilution were then measured with Lumipulse G BRAHMS PCT immunoassay, and PCT data obtained on dilutions were compared to their theoretical values. An additional measurement of original plasma samples was performed with automatic 1:10 sample dilution set in the analyzer.

Results: The correlation between measured and theoretical PCT values was always excellent (i.e., $r=0.999$ – 1.000), whilst slopes and intercepts were also satisfactory. The mean recovery between theoretical and measured PCT values was $\pm 14\%$, $\pm 7\%$ and $\pm 1\%$ for low, mean and high sets of samples, respectively. The mean recovery of 1:10 diluted specimens was comprised within $\pm 20\%$ with manual dilution and $\pm 21\%$ with automatic dilution for low PCT values, within $\pm 5\%$ with manual dilution and $\pm 7\%$ with automatic dilution for medium PCT values, within $\pm 2\%$ with manual dilution and $\pm 1\%$ with automatic dilution for high PCT values.

Conclusions: The results of our study suggest that measuring PCT with Lumipulse G BRAHMS immunoassay using diluted (e.g., 1:10) plasma samples is a viable option to obtain accurate data and consume a much lower volume of plasma.

Keywords: Procalcitonin (PCT); sepsis; bacterial infection; antibiotics

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Introduction

Procalcitonin (PCT) is a 116-aminoacid precursor of the mature hormone calcitonin. Albeit PCT is mainly produced by the thyroid C-cells in normal conditions, an

extrathyroidal synthesis of PCT is commonplace in patients with severe bacterial infections and bacterial sepsis (1), thus contributing to enhance its blood concentration from <0.1 ng/mL up to 100 ng/mL (2,3). This considerably increase of PCT blood levels is currently considered a

cornerstone in the diagnostic approach of patients with severe bacterial infections (especially sepsis), and even for monitoring antimicrobial therapy (3,4).

The clinical significance of measuring PCT in childhood has also been clearly proven by a series of recent meta-analyses (5-7), which were all concordant to conclude that this biomarker may have a valuable role in the diagnostic approach of newborns and children with suspected bacterial sepsis (area under the curve, 0.77–0.87; sensitivity, 0.72–0.85; specificity, 0.54–0.79). Even more importantly, another recent meta-analysis published by Schuetz *et al.* concluded that PCT-guided antibiotic therapy was effective to significantly reduce antibiotic administration by approximately 50% in patients with acute respiratory infections (8), an important finding which could then be replicated in children populations by Baer *et al.* (9) and Dai *et al.* (10).

Insufficient sample volume is one of the major hurdles for performing laboratory analyses (thus including PCT) in the neonatal and childhood ages (11,12). Albeit the modern laboratory instrumentation uses very little amounts of blood, plasma or serum, the feasibility to perform all the tests requested, and which may hence be reasonably necessary to the clinical decision making, is directly dependent on the final volume of blood conveyed to the laboratory (i.e., the larger the volume, the greater the number of tests which could be performed) (13,14). The World Health Organization (WHO) clearly advises that blood sampling for routine or urgent laboratory testing in the childhood and, especially, in the neonatal age may lead to blood volume or hemoglobin depletion (15). Therefore, “minimal risk” limits have been endorsed to safeguard child safety, entailing collection of <5% of total blood volume within 1 day and <10% of total blood volume within 8 weeks, respectively. These limits obviously pose serious challenges and threats to clinical laboratories, since samples with very low volumes of blood may then be unsuitable to perform many of the various tests needed for reaching a final diagnosis of bacterial infection or for monitoring therapeutic effectiveness in patients undergoing antimicrobial treatment. Moreover, an inoculation of low blood volume is a well-known cause of false negative results of blood cultures, which may then lead to underdiagnosing sepsis in neonates or in very young children (16). The problem of insufficient samples extends far beyond the childhood age, since the collection of low volume of blood is quite frequent in patients with difficult veins (17), in critical patients who may need frequent laboratory monitoring and

may hence be at risk of iatrogenic anemia (18), as well as for reducing spurious hemolysis when samples are collected from indwelling lines (19). Although performing laboratory analyses on diluted serum or plasma samples is a potential solution to overcome this problem, the functional sensitivity of many techniques used for measuring PCT would be probably unsuitable to generate reliable data.

The new Lumipulse G BRAHMS PCT immunoassay (Fujirebio Diagnostics Inc., Tokyo, Japan) has been recently commercialized. Unlike some other commercial methods, the analytical characteristics of this new technique allow measuring PCT values with a 2- to 20-fold better functional sensitivity (20,21), thus offering an appealing opportunity to analyze PCT in low volume samples by measuring diluted aliquots. Therefore, this study was aimed to investigate the analytical recovery of Lumipulse G BRAHMS PCT immunoassay (Fujirebio Diagnostics Inc., Tokyo, Japan) using diluted lithium-heparin plasma samples.

Methods

A total number of 15 fresh lithium-heparin plasma samples collected in evacuated blood collection tubes (Vacutest Kima, Piove di Sacco, Padova, Italy) and referred over the same working day to the local laboratory (University Hospital of Verona) for routine PCT testing were included in this study. The samples were arbitrarily selected to cover clinically significant measuring ranges of PCT values, i.e., close to the 0.05 ng/mL diagnostic threshold for bacterial infection (“low” PCT values, 0.034–0.085 ng/mL), close to the 0.5 ng/mL diagnostic threshold for severe bacterial infection (“medium” PCT values, 0.092–0.638 ng/mL), and close to the 5–10 ng/mL diagnostic threshold for severe sepsis and septic shock (“high” PCT values, 2.022–9.187 ng/mL) (1). All lithium-heparin plasma samples were diluted with Lumipulse sample diluent, to obtain 5 progressive dilutions (i.e., 1:2; 1:5; 1:10; 1:20 and 1:32). The original samples and each serial dilution were then tested on Lumipulse G BRAHMS PCT immunoassay. The method is a two-step sandwich chemiluminescence enzyme immunoassay (CLEIA) adapted for use on the LUMIPULSE G1200 system (Fujirebio Diagnostics Inc., Tokyo, Japan). The analytical performance of this high-sensitivity PCT immunoassay have been previously described elsewhere (21). Briefly, the limit of blank (LoB), limit of detection (LoD) and functional sensitivity were found to be 0.001, 0.002 and 0.008 ng/mL, respectively, the total analytical imprecision was 2.1%, and the method was

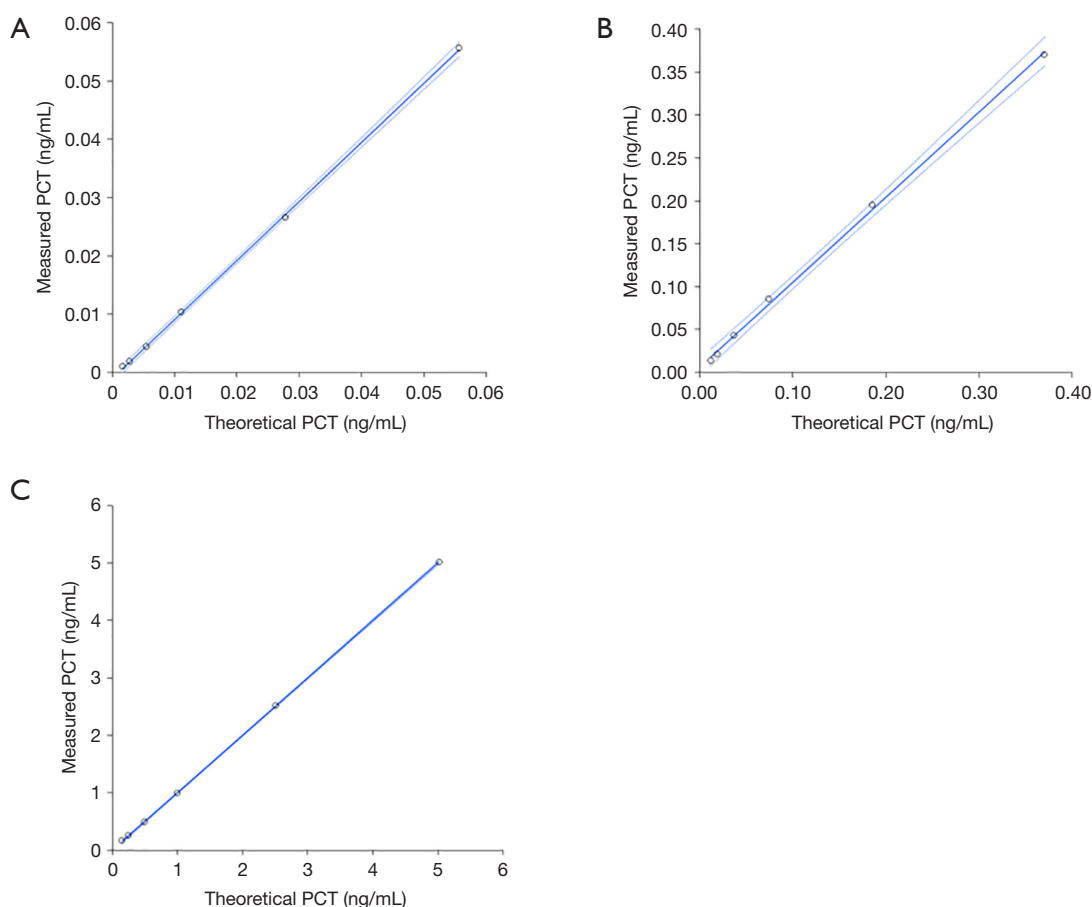


Figure 1 Linear regression analysis of mean theoretical and man measured values PCT values using Lumipulse G BRAHMS PCT in plasma samples with (A) low PCT values (0.034–0.085 ng/mL); (B) medium PCT vales (0.092–0.638 ng/mL); (C) high PCT values (2.022–9.187 ng/mL). The straight line defines the mean theoretical value, whilst the dotted lines comprise the 95% confidence interval (95% CI) of the correlation. PCT, procalcitonin.

found to be linear within a range of PCT concentrations comprised between 0.006 and 75.5 ng/mL. An additional measurement of the original undiluted 15 plasma samples was performed by using the automatic 1:10 dilution set in the analyzer.

All measurements were consecutively performed using the same LUMIPULSE G1200 system analyzer, an identical lot of reagents and within a single analytical session. The PCT results obtained on the dilutions were compared to their theoretical values. The analytical performance on diluted samples was then assessed with linear regression analysis, Pearson's correlation and percent (%) recovery. The mean absolute bias [and 95% confidence interval (CI)] was calculated with Bland and Altman plot analysis. The statistical analysis was performed with Analyse-it (Analyse-

it Software Ltd, Leeds, UK). This study was completely based on pre-existing plasma samples referred for routine PCT testing, so that no patients' informed consent was needed. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local Ethical Committee (University Hospital of Verona; protocol number: 971CESC, 25 July 2016).

Results

The results of this study are reported in *Figure 1*, which shows the mean theoretical PCT values of each set of plasma samples (i.e., displaying “low”, “mean” and “high” values) plotted against the mean measured PCT values of the same plasma samples. Overall, the correlation was always

Table 1 Linear regression analysis and Pearson's correlation of PCT values obtained measuring progressive dilutions (i.e., 1:2; 1:5; 1:10; 1:20 and 1:32) of routine plasma samples compared to their theoretical values.

Plasma samples	n	Slope (and 95% CI)	Intercept (and 95% CI)	Correlation
"Low" PCT samples	5	1.014 (0.993 to 1.036)	-0.001 (-0.002 to -0.000)	r=1.000 (P<0.001)
"Medium" PCT samples	5	0.993 (0.949 to 1.037)	0.006 (-0.001 to 0.014)	r=0.999 (P<0.001)
"High" PCT samples	5	1.001 (0.994 to 1.007)	-0.002 (-0.018 to 0.013)	r=1.000 (P<0.001)

Table 2 Mean differences between the theoretical and measured procalcitonin values (ng/mL)

Dilutions	Sample low			Sample medium			Sample high		
	Theoretical	Measured	Mean difference	Theoretical	Measured	Mean difference	Theoretical	Measured	Mean difference
1:2	0.028	0.027	0.001	0.185	0.195	-0.010	2.508	2.515	-0.006
1:5	0.011	0.010	0.001	0.074	0.085	-0.011	1.003	0.992	0.012
1:10	0.006	0.004	0.001	0.037	0.043	-0.006	0.502	0.488	0.014
1:20	0.003	0.002	0.001	0.019	0.021	-0.003	0.251	0.255	-0.004
1:32	0.002	0.001	0.001	0.012	0.014	-0.002	0.157	0.164	-0.007

excellent (i.e., comprised between 0.999 and 1.000), whilst slopes and intercepts were also satisfactory (*Table 1*). The mean differences (i.e., recovery) between the theoretical and measured PCT values were comprised between $\pm 14\%$, $\pm 7\%$ and $\pm 1\%$ for low, mean and high PCT values, respectively (*Table 2*). The mean recovery of the 1:10 diluted specimens was comprised within $\pm 20\%$ (mean absolute bias, -0.012 ng/mL; 95% CI, -0.022 to -0.001 ng/mL) with manual dilution and within $\pm 21\%$ (mean absolute bias, -0.012 ng/mL; 95% CI, -0.016 to -0.007 ng/mL) with automatic dilution for the low PCT set of plasma samples, within $\pm 5\%$ (mean absolute bias, 0.056 ng/mL; 95% CI, -0.102 to 0.213 ng/mL) with manual dilution and within $\pm 7\%$ (mean absolute bias, -0.023 ng/mL; 95% CI, -0.043 to -0.003 ng/mL) with automatic dilution for the medium PCT set of plasma samples, within $\pm 2\%$ (mean absolute bias, -0.138 ng/mL; 95% CI, -0.743 to 0.467 ng/mL) with manual dilution and within $\pm 1\%$ (mean absolute bias, -0.023 ng/mL; 95% CI, -0.387 to 0.341 ng/mL) with automatic dilution for the high PCT set of plasma samples, respectively.

Discussion

The clinical significance of measuring PCT for diagnosing both localized and systemic infections, as well as for guiding

antimicrobial therapy, is now increasingly recognized (4). The mounting focus on this biomarker has been paralleled by commercialization of a considerable number of semi- or fully-automated immunoassays, which have made its measurement available in the vast majority of clinical laboratories worldwide. Some challenges remain, however. Unlike clinical chemistry tests, for which 2–10 μL may be sufficient for measuring a large number of analytes, the sample volume needed for measuring PCT in serum or plasma ranges between 20 to 200 μL , thus making its assessment rather challenging in low volume samples. Therefore, the availability of analyzers characterized by limited sample aspiration and methods with extraordinarily favorable functional sensitivity would enable the use of dilutions rather than the original samples, thus allowing to measure PCT and contextually saving precious biological materials for additional testing.

The results of our study show that the use of plasma sample dilutions other than the original undiluted plasma specimens is a viable option with Lumipulse G BRAHMS PCT immunoassay for saving serum or plasma volume and contextually producing robust and reliable data. Overall, dilution of samples up to 1:32 was associated with recoveries comprised between $\pm 1\%$ and $\pm 14\%$, thus fulfilling the conventional 20% imprecision acceptance limit set for most PCT immunoassays (22). In particular,

the recovery of the target dilution automatically available in LUMIPULSE G1200 system (i.e., 1:10) yielded data highly comparable to those obtained with external manual dilution of plasma samples (i.e., 1–21% vs. 2–20%). Notably, the sample volume needed for measuring PCT with Lumipulse G BRAHMS PCT immunoassay is 60 μ L, so that a 1:10 dilution entails using only 6 μ L of plasma or serum. The slightly worse recovery observed for plasma samples with very low PCT values (i.e., comprised between 0.034–0.085 ng/mL) at the 1:10 dilution is not clinically meaningful, since in both cases, entailing manual and automatic dilution, the bias was 0.012 ng/mL, a functional PCT value that is neither measurable with the vast majority of other currently available commercial PCT automated immunoassays (20).

Conclusions

The results of our study suggest that measuring PCT with Lumipulse G BRAHMS PCT immunoassay using diluted (e.g., 1:10) plasma is a viable option to obtain accurate test results and for consuming a much lower volume of plasma.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/jlpm.2018.07.10>). Giuseppe Lippi serves as the unpaid Editor-in-Chief of *Journal of Laboratory and Precision Medicine* from November 2016 to October 2021. Martina Montagnana serves as the unpaid Associate Editor-in-Chief of *Journal of Laboratory and Precision Medicine* from November 2016 to October 2021. The other authors have no conflicts of interest to declare. The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was carried out in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the local Ethical Committee (University Hospital of Verona; protocol number: 971CESC, 25 July 2016). Individual informed

consent was waived.

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