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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×[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.

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 μL (plus 100 μL 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*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*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.