Evaluation of a new accurate and reliable particle enhanced immunoturbidimetric assay for the detection of Procalcitonin in the management of sepsis

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BACKGROUND

The *Third International Consensus Definitions for Sepsis and Septic Shock* [1] redefined sepsis as the life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis is considered as a major public healthcare issue affecting more than 30 million people worldwide, associated with a mortality of potentially 6 million people and accounts for almost $24 billion in health care expenditures only in the US, annually [2]. As the quick and accurate sepsis management reduces a possible negative clinical course and the economic burden [3], a multiplicity of plasma biomarker have been developed [4,5] to overcome the long turn-around time of the blood culture [4]. They can also go beyond the limitations of the score systems (e.g. SIRS, SOFA, and qSOFA), which can mainly predict the outcomes of sepsis but are less useful for its diagnosis and for the antibiotic stewardship [6]. Due to its broad market availability and its good clinical accuracy, procalcitonin (PCT) has revealed to be an indispensable tool for the management of sepsis in clinical chemistry [7]. However, the immunoassays so far commercially available are based on heterogeneous technologies (e.g. CLIA), what have often the disadvantage of relatively low time-to-result and need dedicated instruments to be run. Here we present the method evaluation on Cobas c501 of the new polyclonal based particle enhanced immunoturbidimetric assay (PETIA) of DiaSys, which can be easily applied to every high throughput clinical chemistry instrument. This technology is fast and easy-to-use and the new test from DiaSys demonstrates performances suitable for its clinical use.

METHODOLOGY

3 lots of reagent *PCT FS* were evaluated in parallel using a Cobas c501 analyzer. The reagent stability was analysed in terms of 37 °C stress and on-board stability, by evaluating the calibrators´ absorbance and the recovery of low and high controls. The precision of *PCT FS* was evaluated as intra-day at cut-off (20-repetition in-series, using a 0.5 ng/mL sample) and inter-day (5 days, 2 runs a day in duplicate, using samples 0.5, 2 and 10 ng/mL). The Limit of Blank (LOB) was measured analyzing a NaCl-solution in quintuplicate over 3 days. The Limit of Quantitation (LOQ) was defined as the lowest concentration with a total error (TE) lower than 40 %, measured on 5 concentrations (ranging 0.5 - 0.15 ng/mL) in 3 days, 3 replicates a day. The linearity of the PETIA assay was measured between 0 and 50 ng/mL (acceptance limit 10%) and the prozone (antigen excess) up to 1000 ng/mL (acceptance limit = absorbance of calibrator 5 plus 3 %). The following interferences were measured as recovery of increasing spiked samples in comparison to the reference value without interfering material (±10 %): Bilirubin, Rheumatoid Factor, Hemolysis, Triglyceride, Ascorbate, Calcitonin, α-CGRP, β-CGRP, Imipenem, Cefotaxime, Noradrenaline, Dobutamine, Furosemide, Vancomycin and Dopamine. Finally, a method comparison vs. Roche-BRAHMS PCT on Cobas e411 was performed, comparing 148 commercially available samples through Passing & Bablok regression over the whole analytical range of *PCT FS* (LOQ – 50 ng/mL).

RESULTS

The result of the evaluation is reported as median of the 3 lots analyzed. The 37 °C reagent stability was > 19 days, the on-board and the calibration stability 12 weeks. The intra-day precision (CV%) at cut-off was 6.22 %. The inter-day precision was 6.15 %, 3.93 %, and 3.19 % at 0.5, 2 and 10 ng/mL, respectively. LOB and LOQ were 0.02 and 0.2 ng/mL, respectively. The reagent displayed a linear recovery up to 50 ng/mL. No prozone effect was detected up to 1000 ng/mL. The PETIA didn´t show interference with any of the tested material. Calcitonin did interfere for concentrations > 20 ng/mL. The Passing & Bablok regression versus the reference test showed an excellent Spearman´s correlation coefficient and a slightly lower recovery vs. the reference test (slope = 0.919, intercept = 0.041, R = 0.983).

Figure 1: Method comparison

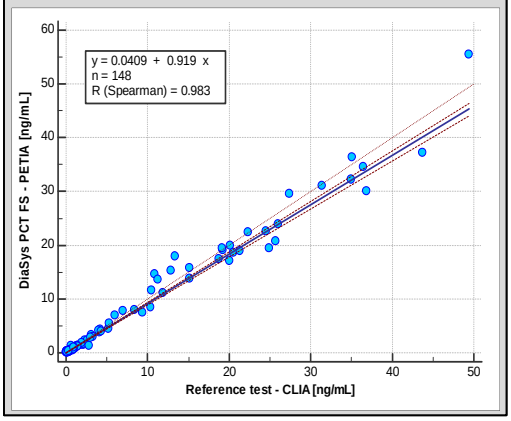

Figure 2: Prozone

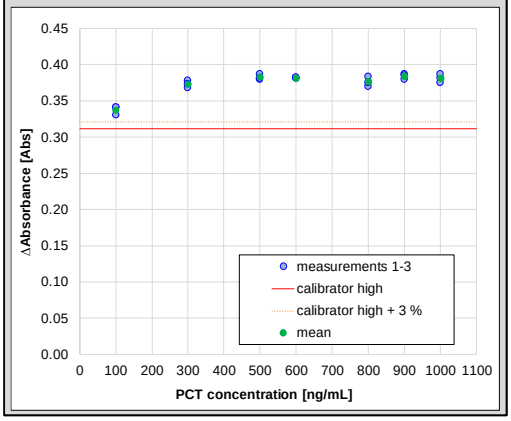

Figure 3: Linearity

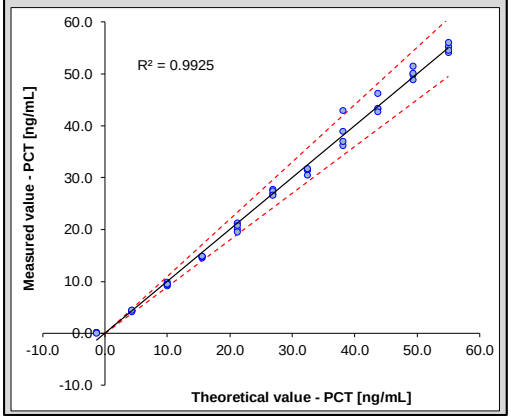

Figure 4: Stability (as calibrators´ signals)

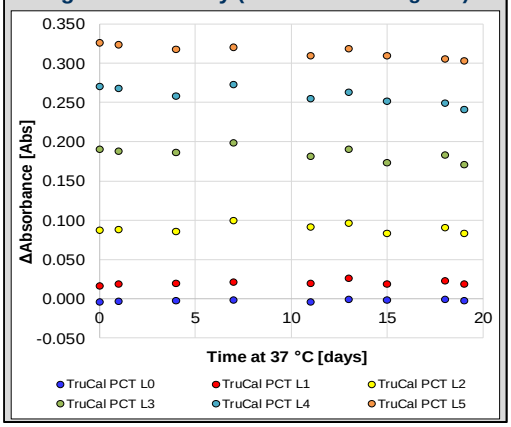

Table 1: Interferences

Interfering substance	No interference up to
Ascorbic acid	150 mg/dL
Bilirubin (conjugated)	60 mg/dL
Bilirubin (unconjugated)	60 mg/dL
Hemoglobin	1000 mg/dL
Lipemia (Triglycerides)	1500 mg/dL
Rheumatoid factor	1000 IU/mL
α-CGRP (human)	10 µg/mL
β-CGRP (human)	10 µg/mL
Calcitonin (human)	20 ng/mL
Cefotaxime	180 mg/dL
Dobutamine	22.4 µg/mL
Dopamine	26 mg/dL
Furosemide	4 mg/dL
Imipenem	0.5 mg/mL
Noradrenalin	4 µg/mL
Vancomycin	3 mg/mL

Table 2: Precision

Type of precision	Results of evaluation
Within run	CV = **6.53 %** (at 0.45 ng/mL)
	CV = **4.17 %** (at 1.98 ng/mL)
	CV = **3.74 %** (at 9.73 ng/mL)
Between run	CV = **7.34 %** (at 0.50 ng/mL)
	CV = **5.00 %** (at 1.87 ng/mL)
	CV = **3.56 %** (at 9.48 ng/mL)

CONCLUSION

The present work proves that the new DiaSys PETIA reagent *Procalcitonin FS* has superior performances, comparable to the reference test in the relevant analytical range. The new PETIA is accurate, fast and easy-to-use in sepsis management and can be potentially applied to all the most common clinical chemistry analyzers.

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