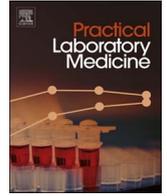




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Analytical validation of Gentian NGAL particle-enhanced enhanced turbidimetric immunoassay (PETIA)



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ABSTRACT

Objectives: This study was designed to validate the analytical performance of the new Gentian particle-enhanced enhanced turbidimetric immunoassay (PETIA) for measuring neutrophil gelatinase-associated lipocalin (NGAL) in serum samples.

Design and methods: Analytical validation of the Gentian NGAL assay was carried out on a Roche Cobas c501 and was based on assessment of limit of blank (LOB), limit of detection (LOD), functional sensitivity, imprecision, linearity and concordance with the BioPorto NGAL test.

Results: The LOB and LOD of Gentian NGAL were found to be 3.8 ng/mL and 6.3 ng/mL, respectively. An analytical coefficient of variation (CV) of 20% corresponded to a NGAL value of 10 ng/mL. The intra-assay and inter-assay imprecision (CV) was between 0.4 and 5.2% and 0.6 and 7.1% and the total imprecision (CV) was 3.7%. The linearity was optimal at NGAL concentrations between 37 and 1420 ng/mL ($r = 1.00$; $p < 0.001$). An excellent correlation was observed between values measured with Gentian NGAL and BioPorto NGAL in 74 routine serum samples ($r = 0.993$). The mean percentage bias of the Gentian assay versus the Bioporto assay was +3.1% (95% CI, +1.6% to +4.5%).

Conclusions: These results show that Gentian NGAL may be a viable option to other commercial immunoassays for both routine and urgent assessment of serum NGAL.

1. Introduction

The neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, is a single disulfide-bridged protein of 178 aminoacids with a molecular mass of 22 kDa, increasing to 25 kDa after glycosylation [1]. The protein can be detected in both serum and urine in three main molecular forms; a 25-kD monomer, a 45-kD disulfide-linked homodimer and a 135-kD heterodimer, in which the protein is covalently linked to matrix metalloproteinase 9 (MMP-9) by means of a single intermolecular disulfide bridge [1]. Although NGAL was originally detected in activated neutrophils, its expression was later identified in many other tissues and organs including the kidney, lungs, intestine and prostate. More recently, the ectopic expression of NGAL has been reported in patients with various malignancies such as kidney, colorectal, gastric, pancreatobiliary, hepatic, breast, gynecological and hematologic cancers [2].

The clinical use of NGAL has gained attention since 2003, when it was convincingly shown that the concentration of this biomarker increases considerably immediately after acute kidney injury (AKI) [3]. Since then, several studies have confirmed that

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NGAL may be considered as an early and reliable biomarker of AKI in various clinical settings [4–7].

Ideally, a candidate biomarker of AKI should have many important characteristics, such as being measurable with a noninvasive, easy, rapid and inexpensive technique and displaying high sensitivity and specificity for AKI. It should also permit early detection of AKI, provide information about severity and reversibility of disease, and predict response to treatment and clinical outcome [8]. Among the various potential biomarkers of AKI, which also include kidney injury molecule-1 (KIM-1), N-acetyl- β -d-glucosaminidase (NAG), liver fatty acid-binding protein (L-FAB), and interleukin-18 (IL-18), NGAL has been shown to be the most promising, and is the only one for which fully-automated assays have become available to date [8]. The availability of quick and reliable automated immunoassays allows rapid laboratory assessment and timely communication of test results to clinicians, enabling early diagnosis of AKI and reliable prediction of outcome. The measurement of NGAL in serum or plasma is also considered to be more convenient than measurement in urine, since urine output may be considerably reduced in patients with AKI. The reliability of urine measurement is also challenged by several preanalytical issues such as the poor standardization of urine collection procedures compared to those for venous blood and the possible contamination with leukocytes in patients with urinary infections, which may ultimately decrease the diagnostic efficiency of this biomarker [9]. The clinical significance of measuring NGAL has also been recently highlighted in conditions other than AKI, such as acute bacterial meningitis [10] and bacterial peritonitis [11]. Notably, a growing number of studies have reported that NGAL may be used as an efficient biomarker for early diagnosis and predicting the clinical outcome of certain types of cancer [12–14].

Therefore, the present study was designed to validate the analytical performance of Gentian NGAL, a new fully-automatable immunoassay for measuring NGAL in human serum and urine.

2. Materials and methods

2.1. Assay characteristics

Gentian NGAL (Gentian, Moss, Norway) is a particle-enhanced enhanced turbidimetric immunoassay (PETIA), which can be applied to a vast array of automated clinical chemistry analyzers. According to the assay principle, urine or plasma samples are mixed with a solution containing anti-NGAL immunoparticles. The NGAL present in the sample aggregates with the anti-NGAL immunoparticles to generate immunocomplexes, which increase the turbidity of the solution. Light absorbance, measured between 480 and 560 nm, is directly proportional to the concentration of the molecule in the test sample. The NGAL concentration is calculated from a 6-point pre-diluted standard calibration curve, with calibrators values comprised between 0 and 3168 ng/mL. The manufacturers' literature states that the calibrators contain NGAL of recombinant origin, with values assigned according to Gentian's internal value transfer protocol, based on published methods [15]. In this study, the method has been applied for use on a Roche Cobas c501 module (Roche Diagnostics, Indianapolis, IN, USA). The quoted NGAL serum/plasma reference range is 13.7–104.6 ng/mL, the total sample volume needed for the assay is 3 μ L and test results are available in 10 min.

2.2. Analytical performance

The analytical validation of Gentian NGAL PETIA consisted of calculation of limit of blank (LOB), limit of detection (LOD) and functional sensitivity, and estimation of inter-assay and intra-assay imprecision, linearity, and correlation with another widely used NGAL PETIA (BioPorto Diagnostics).

The calculation of the LOB and LOD was carried out as described by Armbruster and Pry [16], as follows: [LOB] = mean value + 1.645* standard deviation (SD) of 10 consecutive replicates of saline; [LOD] = LOB + 1.645* SD of 10 replicates of a routine serum sample with the lowest measurable NGAL concentration. The functional sensitivity of the assay was defined as the lowest NGAL concentration which could be measured with an imprecision (CV) \leq 20%. This value was determined by performing nine serial dilutions in sample buffer (i.e., from 1:2 to 1:512) of a routine serum sample with NGAL concentration of 1485 ng/mL. Each dilution was measured in 10 replicates and the imprecision was calculated for each dilution. A model fit was constructed to extrapolate the NGAL value associated with a CV of 20%.

The imprecision studies were performed using three serum pools selected for low (\sim 40 ng/mL), intermediate (\sim 260 ng/mL) and high (\sim 1465 ng/mL) NGAL concentrations. Within- and between-run imprecision was respectively assessed in 20 consecutive runs and over 10 consecutive working days, using the same calibration curve and identical reagent lots. Results were reported as coefficient of variation (CV%). The total analytical imprecision was calculated according to the formula proposed by Krouwer and Rabinowitz [17].

The linearity of the assay was tested as follows: a routine serum sample with high NGAL concentration (1420 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 another routine serum sample with a low NGAL concentration (37 ng/mL), to produce samples with a clinically significant range of NGAL concentrations in serum. The serial dilutions were then measured in duplicate and theoretical values were calculated from the measured values of undiluted specimens.

2.3. Method comparison

The comparison study was performed using routine serum samples referred from the emergency department to the laboratory for assessment of serum creatinine, over one working morning. All samples were tested within 2 h of collection. The results of the Gentian NGAL assay were compared with those obtained on the same sample with BioPorto NGAL Test (BioPorto Diagnostics A/S,

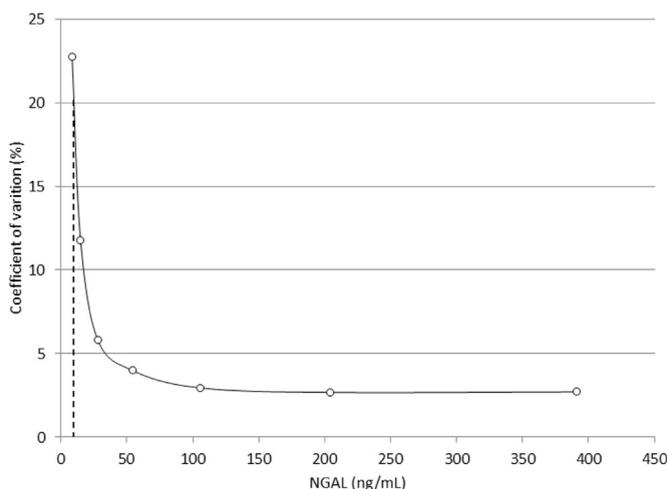


Fig. 1. Calculation of the functional sensitivity (concentration at CV = 20%) of Gentian NGAL.

Gentofte, Denmark), on the same Roche Cobas c501 (Roche Diagnostics). The technical and analytical characteristics of the BioPorto assay have been described elsewhere [18]. Briefly, Bioporto NGAL Test is another PETIA for quantitative assessment of NGAL in human urine, EDTA plasma and serum samples, that can be adapted for use on a variety of automated clinical chemistry analyzers. The intra-assay, inter-assay and total imprecision for the BioPorto NGAL Test was found to be 1.0–2.3%, 1.2–2.0% and 2.2%, respectively. The BiPorto NGAL Test has also been shown to display a highly significant agreement with the current reference chemiluminescent immunoassay (Abbott Architect NGAL; Abbott Diagnostics, Lake Forest, IL, USA) ($r = 0.925$; $p < 0.001$) [18].

2.4. Statistics and ethical approval

The linearity of the Gentian NGAL assay was tested by calculation of Spearman's correlation coefficient (r) and using Passing and Bablok regression analysis. The agreement of values obtained using Gentian NGAL and BiPorto NGAL was tested with Mann-Whitney test for paired samples, Spearman's correlation, Passing and Bablok regression analysis and Bland and Altman plot analysis. The statistical analysis was performed using Analyse-It (Analyse-it Software Ltd, Leeds, UK), a statistical add-in for Microsoft Excel. The evaluation of Gentian NGAL was performed using pre-existing serum samples collected in evacuated blood tubes containing gel and clot activator (Vacutest 3.5 mL, 75 × 13 mm; Kima, Padova, Italy), which were referred for routine laboratory testing. The entire study was carried out for purposes of ISO 15189:2012 accreditation, in accordance with the Declaration of Helsinki, under the terms of relevant local legislation, and was approved by the local Institutional Review Board (University Hospital of Verona, Verona, Italy).

3. Results

The LOB and LOD of Gentian NGAL were found to be 3.8 ng/mL and 6.3 ng/mL, respectively. The estimation of functional sensitivity is shown in Fig. 1. A 20% CV of the assay corresponded exactly to a NGAL serum concentration of 10 ng/mL. The data obtained from the imprecision studies using serum pools with low, medium and high NGAL values are shown in Table 1. The intra-assay and inter-assay imprecision (CV) for high and low serum pools ranged between 0.4 and 5.2% and between 0.6 and 7.1%. The total analytical imprecision (CV) was 3.7%. The linearity of the assay was virtually perfect over NGAL concentrations between 37 and 1420 ng/mL ($r = 1.00$; $p < 0.001$). The Spearman correlation coefficient was 1.00 ($p < 0.001$) and the equation of the Passing and Bablok regression line was $y = 1.00x + 0.5$.

An excellent correlation was observed when comparing values measured with Gentian NGAL and BioPorto NGAL in 74 routine samples with NGAL concentrations between 90 and 1501 ng/mL ($r = 0.993$; 95% CI, 0.988–0.995; $p < 0.001$). The equation of the Passing and Bablok regression line was $[\text{Gentian NGAL}] = 0.99 \times [\text{BioPorto NGAL}] + 28$. The results of the Bland and Altman plot

Table 1

Intra- and inter-assay imprecision of Gentian NGAL.

Serum pool	Intra-assay		Inter-assay	
	Conc. (ng/mL)	CV	Conc. (ng/mL)	CV
Pool low	39.1 ± 2.0	5.2%	30.0 ± 2.1	7.1%
Pool medium	257.9 ± 2.7	1.1%	241.3 ± 3.0	1.2%
Pool high	1465.4 ± 5.4	0.4%	1414.8 ± 9.1	0.6%

NGAL, neutrophil gelatinase-associated lipocalin; CV, coefficient of variation. Conc, NGAL concentration in pool.

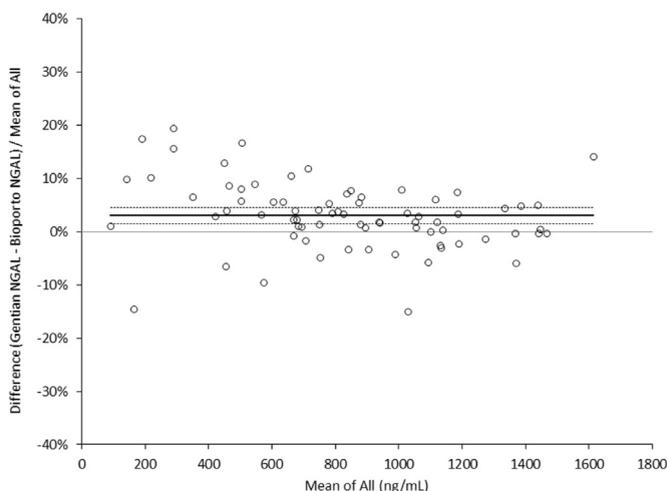


Fig. 2. Bland and Altman plot analysis of correlation between BioPorto NGAL test and Gentian NGAL (horizontal lines show mean bias and 95% CI of bias).

analysis are shown in Fig. 2. The mean percentage bias of Gentian NGAL versus BioPorto NGAL was +3.1% (95% CI: +1.6% to +4.5%). The difference between NGAL concentrations obtained with the two assays was found to be statistically significant ($p < 0.001$).

4. Discussion

The measurement of biomarkers of tubular injury for diagnosis and prognosis of AKI is becoming very popular in clinical laboratories. Among the various available biomarkers, NGAL has been found to have the best diagnostic and prognostic efficiency [8], and is also the only AKI biomarker for which fully-automated immunoassays are currently available. The clinical applications of this biomarker have recently broadened to include diagnosis and monitoring of other human diseases, such as cancers [2] and bacterial infections [10,11]. In the case of AKI, a first meta-analysis, published in 2009 by the NGAL Meta-analysis Investigator Group and including 19 studies and 2538 patients (487 of whom [19.2%] developed AKI) showed that the area under the receiver operating characteristics curve (AUC/ROC) for predicting AKI was as high as 0.82 (95% CI, 0.73–0.89) [5]. The measurement of NGAL was also found to be useful for predicting renal replacement therapy (AUC/ROC, 0.78; 95% CI, 0.65–0.92) and in-hospital mortality (AUC/ROC, 0.71; 95% CI, 0.53–0.75). Notably, the diagnostic accuracy of plasma or serum NGAL was found to be similar to that of urine NGAL. A more recent (2016) meta-analysis, including 24 studies and 4066 patients, substantially confirmed these findings, concluding that the AUC of NGAL was as high as 0.86 ± 0.02 for early diagnosis of AKI [6]. In the same year, an additional meta-analysis of ten prospective studies involving nearly 30,000 participants also concluded that, among the various urinary tubular injury biomarkers, only NGAL may be an independent predictor of end stage renal disease in patients with chronic kidney disease [7].

Until recently, the availability of commercial methods for measuring NGAL in serum, plasma or urine was limited, mainly consisting of a fully-automated immunoassay (BioPorto NGAL Test) for use on conventional clinical chemistry analyzers [18,19] and another assay available only on the immunochemistry platform Abbott Architect [20]. Now that Gentian NGAL PETIA has been introduced to the market, we have validated its analytical performance on the Roche Cobas c501, a widely used clinical chemistry analyzer. Our results suggest that Gentian NGAL assay displays excellent analytical performance. The estimated LOD, LOB and the analytical sensitivity were optimal for the reference range of serum NGAL (13.7–104.6 ng/mL). The total imprecision (3.7%) was slightly worse than that of the BioPorto NGAL Test (2.2%) but comparable to that of the Abbott Architect NGAL (2.9–3.7%). Much better imprecision results could have been achieved calculating total imprecision on data obtained only on medium and high serum pools (1.2%). The linearity was excellent over a range of NGAL concentration between 37 and 1420 ng/mL. The comparison with BioPorto NGAL Test, the current reference immunoassay, was acceptable, yielding a clinically insignificant bias (~3%) compared to the intra-individual biological variability of this biomarker (11%) [20].

5. Conclusions

The result of our analytical validation show that Gentian NGAL may be a viable option for both routine and urgent assessment of serum NGAL. Additional advantages are: the very fast turnaround time (results are available in approximately 10 min compared to 35 min for the Abbott Architect NGAL and nearly 2 h with manual ELISAs), acceptable imprecision and optimal linearity in the clinically significant range of NGAL concentrations. The possibility of widespread implementation of this test on a wide array of clinical chemistry platforms may also permit the concomitant measurement of NGAL and other biomarkers of renal function (e.g., creatinine or blood urea nitrogen) on a single blood sample, so optimizing laboratory workflow and saving economic resources. Finally, the bias observed when comparing Gentian NGAL results with those obtained using BioPorto NGAL Test was modest (less than biological variation), thus providing a solid background for further studies addressing the possibility of using both methods

interchangeably within a network of clinical laboratories.

Conflict of interest

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

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None.

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