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Evaluation of a particle enhanced turbidimetric assay for the measurement of neutrophil gelatinase-associated lipocalin in plasma and urine on Architect-8000: Analytical performance and establishment of reference values

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ABSTRACT

Objectives: Neutrophil gelatinase-associated lipocalin (NGAL) is a promising biomarker for acute kidney injury. NGAL can be measured in both blood and urine. Apart from kidney injury, NGAL levels in both plasma and urine can be influenced by various pathological situations. Accurate evaluation and comparison of results deriving from clinical studies require robust assays, appropriate specimen handling and reference intervals that will reflect its levels in a healthy population for both biological matrices.

Methods: We report the analytical validation of a latex particle-enhanced turbidimetric immunoassay (PETIA) aimed to measure NGAL in plasma and urine on an automated biochemistry analyzer (ABBOTT-Architect-8000). Assay performance characteristics were evaluated using standard protocols. Urine and plasma specimen storage requirements were determined and reference ranges for blood and urine were determined using healthy controls.

Results: The assay is precise (total CV% < 4.8%), and sensitive (limit of quantification: 8.4 ng/mL for plasma and 9.0 ng/mL for urine), showing no hook effect. Calibration is stable for at least 30 days. The assay showed excellent linearity over the studied interval (20–4450 ng/mL). The analyte is stable at 4 °C for at least 5 days, and at 20 °C for 4 h. Gender specific reference ranges for plasma (male: 38.7–157.6 ng/mL, female: 24.4–142.5 ng/mL) and unisex for urine (<9.0–49.41 ng/mL) are proposed.

Conclusion: Our data indicate that NGAL can be measured with adequate precision and sensitivity on automated biochemistry analyzers and its measurement could easily be added to a standard panel to screen kidney diseases.

1. Introduction

Neutrophil gelatinase-associated lipocalin (NGAL) is a small molecule of 178 amino acids that belongs to the superfamily of lipocalins, a group of proteins that specialize in transporting small hydrophobic molecules [1,2]. NGAL is known to exist in three molecular forms: a 25 kDa monomer which is the predominant form released by tubular epithelial cells, a 45 kDa homodimer predominantly secreted by neutrophils and a 145 kDa NGAL/matrix metalloproteinase-9 (MMP9) covalently complexed heterodimer [1,3]. NGAL is a molecule with diverse biologic activities. Recent research has described NGAL as a potential early marker of acute kidney injury (AKI) especially when the timing of insult is certain. The expression of NGAL rises 1000-fold in humans in response to renal tubular injury and it appears rapidly in blood and urine, making the measurement of the levels of this molecule a potential useful biomarker for the early diagnosis of AKI in both biological fluids [4].

However in most research studies, the measurement of NGAL in serum, plasma and urine samples has been performed with either research ELISAs or immunoblotting systems. Very few of these studies reported sufficient data on the analytical performance of the methods used for the NGAL measurement. Lack of these data makes difficult the accurate evaluation of measurements and the reliability of the results and since these methods are not standardized the comparison

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; MMP9, matrix metalloproteinase-9; uNGAL, urinary NGAL; pNGAL, plasma NGAL; AKI, acute kidney injury; PETIA, particle enhanced turbidimetric immunoassay; CMIA, chemiluminescent microparticle immunoassay; eGFR, estimated glomerular filtration rate; uCr, urinary creatinine.

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of the results is difficult between studies when different methods or kits from different manufacturers are used.

Pedersen et al. recently evaluated the analytical performance of an ELISA commercial kit able to measure NGAL in both plasma and urine and that could be used in clinical studies [5]. These methods are manual, difficult to use on a 24 hour–7 day basis and not recommended for routine clinical practice.

Commercial kits that could be used on automated analyzers were introduced recently. A commercial chemiluminescent microparticle immunoassay (CMIA) became recently available from Abbott. This method is using the Architect immunochemistry platform and is designed for the measurement of NGAL in urine samples only. The analytical characteristics of this method have been evaluated by Grenier et al. [6] A point-of-care immunoassay based on whole blood measurement is also available (Triage NGAL-Test, Alere) [7]. More recently a CE-IVD particle enhanced turbidimetric immunoassay (PETIA) was introduced (The NGAL Test™-Bioporto, Gentofte, Denmark). This method is intended for the measurement of NGAL in both plasma and urine and has been licensed for application on a variety of automated clinical chemistry analyzers. So far the performance of this method has been evaluated only on Beckman-Coulter and Roche Cobas analyzers [8,9].

In this study, we report the evaluation of the analytical performance of this PETIA assay on the Architect c8000 platform. We also report reference intervals for both urinary and plasma NGAL for this method in adult population. To our knowledge this is the first study that reports reference intervals for NGAL in plasma and urine using the same reference population.

2. Material and methods

2.1. Collection of patient samples and healthy controls

This study was approved by KAT Hospital's scientific and ethical committee and was performed according to the declaration of Helsinki. Patient samples were collected from the routine at the Clinical Biochemistry Department and were used anonymously.

To determine the reference range of this assay, 200 plasma and urine samples from healthy adult individuals (137 males and 63 females) were used to determine NGAL. These were selected from a group of eligible blood donors. Informed consent was obtained from each participant. Their personal medical history was recorded and exclusion criteria were diabetes, hypertension, cardiovascular disease, recent infection, inflammation, anemia, neoplastic diseases, kidney and liver disease and recent surgery. Obese subjects (BMI > 30 kg/m^2) as well as those with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 were excluded from analysis. Also subjects with albuminuria (albumin-to-creatinine ratio > 30 mg/mmol) or leukocyturia (>3 cells/per high power field or with positive leukocyte esterase in urine dipstick) were also excluded. Blood samples from patients and from healthy controls were collected in EDTA tubes. Random urine samples were collected in sterile containers without preservatives. Mid-stream voids were used to minimize contamination. All samples (blood and urine) were centrifuged, aliquoted and stored at −80 °C until tested.

2.2. Method description

The NGAL Test™ is a particle-enhanced turbidimetric immunoassay (PETIA) for the quantitation of NGAL in human urine, heparin and EDTA plasma on a variety of automated clinical chemistry analyzers. On the Abbott Architect c8000 analyzer, 2.4 μL of patient sample is automatically mixed with 40 μL reaction buffer (R1). After a short incubation the reaction is started with the addition of 120 μL from an immunoparticle suspension (R2), which contains polystyrene microparticles coated with monoclonal antibodies to human NGAL. The presence of NGAL in the sample causes aggregation of immunoparticles (turbidity) and is quantified by the intensity loss of the transmitted light due to the scattering properties of the aggregated particles. The light absorption of the reaction is measured at 572 nm after 10 min total reaction time. The NGAL concentration in the sample is then calculated by interpolation on an established calibration curve. A six point spline calibration method is used with calibrators covering the range from 50 to 5000 ng/mL. Test results are reported in ng/mL. Samples with test results above the highest calibrator are automatically flagged and displayed as >5000 ng/mL and automatically rerun on the instrument with a 1:5 dilution using as diluent the saline solution contained in the instrument.

2.3. Calibration and assay precision

Calibration stability was determined by calibrating the assay on the analyzer and running 3 synthetic controls, daily for one month. Standard Levey–Jennings graphs were created for all controls. The synthetic controls had been prepared by the manufacturer using recombinant human NGAL in HEPES-buffer containing a preservative. Target values were assigned at the following levels: 50, 204 and 490 ng/mL. The assay precision was also evaluated using two additional urine and two plasma panels. The panels were prepared by pooling urine or plasma samples selected from patients with and without AKI in order to obtain samples with low and high levels of NGAL in plasma and urine. Aliquots of each plasma and urine panel were prepared and stored at −80 °C. Each control (synthetic and panels) was measured in duplicate twice a day with at least 2 h interval between each run. This procedure repeated for 20 consecutive days. For each sample, within-run coefficient of variation (CV), between-run CV and total CV were calculated.

2.4. Limit of quantification (LoQ)

LoQ study was performed in order to determine the lowest actual amount of NGAL in urine and plasma that could be reliably detected with a CV ≤20%. A urine sample with a concentration of 605.5 ng/mL and a plasma sample with a concentration of 608.9 ng/mL were used. Their concentration was determined by measuring each sample 6 times. Sample dilutions were prepared with saline (1:5, 1:19, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45 and 1:50). The diluted samples were run in triplicates in three different runs. The mean value and the total CV% for each diluted sample were calculated. The CV% for each sample was plotted against concentration and a reciprocal curve fit was used to determine the NGAL concentration corresponding to a 20% CV. This value defined the limit of quantification.

2.5. Assay linearity

Linearity was evaluated according to NCCLS guide EP6-A. A plasma sample and a urine sample with high levels of NGAL concentration (3748 and 4450 ng/mL respectively) and a plasma and a urine sample with very low concentration of NGAL (27 and 20 ng/mL respectively) were used. The concentration of NGAL in these samples was determined by measuring each sample 6 times. A series of dilutions was prepared by mixing different portions of the high and low concentration samples to cover most of the measurement range of the assay (4450 to 20 ng/mL for urine and 3748 to 27 ng/mL for plasma). Linearity was evaluated by plotting the measured values of the diluted samples (along with the undiluted high and low samples) against their expected values (polynomial regression analysis). All samples were run in triplicates.

2.6. High dose hook effect

The possible presence of a hook effect was examined using a sample spiked with purified NGAL with a final concentration of 40,000 ng/mL. The spiked sample was used to prepare serial dilutions ranging from 40,000 ng/mL to 500 ng/mL. A native urine sample from a patient with severe oliguric AKI was also used. This native urine sample had a

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