FOCUSED REPORT

Method Validation of Dipeptidyl Peptidase 3 Assay

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Background: Dipeptidyl peptidase 3 (DPP3) is a peptidase released from dying cells. It cleaves proteins in the renin–angiotensin pathway, which can result in hemodynamic instability. At elevated concentrations DPP3 is associated with worse outcomes, particularly in patients with shock. Herein we describe the assay performance of a DPP3 assay (4TEEN4 Pharmaceuticals GmbH) in human plasma.

Methods: DPP3 concentration was measured using the DPP3 immunoluminometric assay (4TEEN4 Pharmaceuticals GmbH) and the signal was read using a luminometer (Berthold Centro LB963). Analytical performance was established for precision, linearity, accuracy, detection limit, analytical specificity, reference interval, kit lot-to-lot comparison, specimen type, and sample stability.

Results: Limit of detection was verified at 1.6 ng/mL in EDTA plasma with a coefficient of variation (CV) of <10%. Precision studies revealed a CV ≤ 6% at 28.5 ng/mL and 59.4 ng/mL and comparability with a manufacturer performed assay was demonstrated between 7.7 and 195.2 ng/mL. An upper 97.5% limit of 22 ng/mL without age or sex associations was verified in healthy donors. The assay was not susceptible to interference from lipemia or bilirubin. However, measured DPP3 concentrations increased linearly with increasing hemolysis. DPP3 concentrations are stable in EDTA plasma for up to 24 h and at least 11 months when stored ambient or at −80°C, respectively.

Conclusions: DPP3 can be measured precisely in EDTA plasma using the immunoluminometric DPP3 assay. Given the potential clinical use of DPP3 in critical care patients, caution should be taken to avoid inducing pre-analytical hemolysis during sample collection.

INTRODUCTION

Dipeptidyl peptidase 3 (DPP3) is an intracellular zinc-dependent aminopeptidase involved in the metabolism of a variety of biologically active peptides (1, 2). It is present in all cells, is released from dying cells, and is involved in modulation of the renin–angiotensin system by cleaving angiotensin II, angiotensin III, angiotensin 1–7, and angiotensin 1–5, which can lead to hemodynamic instability. DPP3 reduces myocardial contractility in animal models and, conversely, inhibition of DPP3 restores contractility of myocardial cells (3, 4). Elevated concentrations of circulating DPP3 presage subsequent

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IMPACT STATEMENT

Elevated circulating DPP3 is prognostic for worse outcomes in critically ill patients, especially those with established shock. Here we show that DPP3 can be measured accurately and precisely using an immunoluminometric assay and is suitable for clinical use.

shock in patients with acute coronary syndromes and are prognostic for worse outcomes in critically ill patients, and especially those with established shock (5). Therefore, accurate measurement of blood concentrations of DPP3 is critical if this marker is to be used to guide patient care decisions (6). Herein we perform an analytical evaluation of a DPP3 immunoassay kit using human EDTA plasma.

MATERIALS AND METHODS

DPP3 Concentration Assay and Patient Samples

The method is an immunoluminometric assay (ILMA) for the measurement of DPP3 protein concentration in human EDTA plasma. The patent for the DPP3 assay is held by 4TEEN4 Pharmaceuticals GmbH and the reagents were produced by SphingoTec, V01 released September 2022. The method utilizes 2 monoclonal antibodies targeting different epitopes on DPP3. One antibody is immobilized on the inside of microtiter plate wells and the other is attached to a luminescent tracer molecule. After washing unbound tracer molecules from the well, sphingotest® lightning reagents are applied and measurement of luminescence is performed on a luminometer (Berthold Centro LB963). The assay is calibrated by weighted native human DPP3 (DPPCA.20002) under ISO13485 certification (7). The residual samples used in this study were utilized for quality improvement and method validation and the work described did not require Institutional Review Board (IRB) approval.

Assay Performance Studies

Analyte stability was tested in 10 residual EDTA plasma samples (DPP3 concentration range: 15 to 81 ng/mL) that were separated by centrifugation and aliquoted for various storage durations and temperatures. Ambient stability (22 to 25°C) was assessed for 4, 8, 24, and 72 h of storage. Refrigerated stability (1 to 8°C) was assessed for 1, 7, and 14 days of storage. Acceptability was defined as a mean percent difference of ≤20% compared to time zero. Frozen stability at −20°C was assessed for 1, 7, 14, and 30 days of storage. The number of acceptable freeze/thaw cycles was assessed by thawing aliquots to ambient temperature and refreezing. Acceptability was defined as mean percent difference of ≤20% compared to the initial concentration before freezing.

Assay precision was analyzed according to CLSI EP05-A3 ($5 \times 5 \times 2$ method) (8). Briefly, DPP3 was measured in 2 pooled residual EDTA samples (average concentration 28.5 ng/mL and 59.4ng/mL) in 5 replicates for 5 days to assess both intra- and inter-assay precision. Acceptance criteria was an average %CV of ≤20%.

There is no reference measurement procedure for DPP3 so assay accuracy (comparability to a manufacturer performed assay) was assessed by comparison of DPP3 concentration in 40 residual EDTA plasma samples to the same samples measured at 4TEEN4 Pharmaceuticals using the same assay. Linear regression was performed using the 4TEEN4 Pharmaceuticals results as the reference.

Assay linearity was assessed using 4 mixing studies that utilized samples spanning the analytical measuring range. One study utilized residual

Table 1. Analytical performance characteristics.

Parameter	Package insert (11)	Study results
Precision	Repeatability: CV ≤ 5.3%	Within-run: mean (SD; %CV) *acceptance criteria: CV ≤ 20% 28.5 ng/mL (1.1; 3.7%) 59.4 ng/mL (3.4; 5.7%)
	Reproducibility: CV ≤ 7.0%	Between-run: mean (SD; %CV) *acceptance criteria: CV ≤ 20% 28.5 ng/mL (1.5; 5.1%) 59.4 ng/mL (4.4; 7.4%)
Accuracy (comparability)	Not stated	Method comparison (Mayo Clinic vs 4TEEN4) n = 40, range = 7.7 to 195.2 ng/mL (measured at Mayo Clinic) Linear regression: $y = 1.04x + 0.50$
Linearity	1.6 to 200 ng/mL	Four mixing studies (low, high [ng/mL]) (0, 128.3), (0, 340.7), (0, 199.0) (1.7, 207.3) Average (range) % difference from expected = 0.9% (−11%, 9%)
Limit of quantitation	1.6 ng/mL	Residual patient plasma measured 20 times Mean (SD; %CV): 3.0 ng/mL (0.1; 2.6%) and 0.5 ng/mL (0.1; 8.9%)
Reference interval	Upper 97.5% limit: ≤22 ng/mL	n = 41 (19 males and 22 females, ages: 23 to 66 years) range: 3.9 to 21.0 ng/mL
Reagent lot-to-lot comparison	Not stated	Residual EDTA plasma (n = 33) tested on 2 lots of reagent Slope and intercept generated from Passing–Bablok analysis. Range, ng/mL 5.8 to 187.8 Slope 1.01 Intercept −0.68 Mean (range) difference, ng/mL −0.4 (−5.7 to 5.6)
Analytical specificity	Limit triglycerides: 2000 mg/dL (22.6 mmol/L) Bilirubin: 40 mg/dL (0.68 mmol/L)	Concentration maximum % difference Triglycerides 1000 mg/dL (11.3 mmol/L) 5.5% Bilirubin 50 mg/dL (0.86 mmol/L) 8.3%
Sample stability	25°C: 48 h 2 to 8°C: 48 h −20°C: not stated −80°C: not stated Freeze–thaw: 5 cycles	Acceptable stability if results from 9 of 10 samples were within 20% of the original result. 25°C: 24 h 2–8°C: 14 days −20°C: 30 days −80°C: 11 months Freeze–thaw: 1 cycle
Heterophile interference	Human anti-mouse antibody: 645.4 ng/mL	Range % difference (n = 10) Heterophile blocking tube −22.7% to 13.6% Heterophile blocking reagent −13.2% to 1.5%
Sample type	Plasma only	Paired EDTA plasma and serum (n = 10) Concentration range (plasma): 24.5 to 65.3 ng/mL Linear regression: $y = 0.09x + 25.6$; $R^2 = 0.009$ Percent difference (min, max): −76%, +77%

EDTA samples with low and high concentrations of DPP3 (1.7 ng/mL, 207.3 ng/mL) and 3 studies utilized the zero calibrator as the low sample and 3 residual EDTA plasma samples for the high sample (128.3, 199.0, and 340.7 ng/mL). Mixed samples were at the following ratios of high:low concentrations: 0:100, 25:75, 50:50, 75:25, and 100:0. Linearity was deemed acceptable if the mixed

samples yielded 80% to 120% recovery compared to the measured concentrations of the high and low samples and linear regression analysis of the measured values yielded a slope of 0.9 to 1.10 and an $R^2 > 0.9$.

The limit of quantitation (LOQ) was determined by running 2 pools of residual plasma samples with low DPP3 concentrations 20 times. The package insert LOQ of 1.6 ng/mL was deemed acceptable if the CV of the replicate measurements at or below the package insert limit was ≤20%.

A lot-to-lot evaluation was performed by running 33 EDTA plasma samples with concentrations ranging from 5.8 to 187.8 ng/mL on 2 different lots of reagent. Samples were stored at −80°C for 11 months between testing. Regression analysis was performed using Passing–Bablok.

The upper reference limit was verified using methods recommended in CLSI EP28-Ed3-IG by collecting EDTA plasma samples from 41 unique healthy subjects (22 women, 19 men) ranging from 23 to 66 years of age (9). Exclusion criteria included the presence of diabetes, kidney disease/failure, liver disease, malignant neoplasms, malnutrition, hyperlipidemia, or anyone on lipid-lowering medications. Measured DPP3 concentrations were compared to the 97.5th percentile of a healthy reference interval (n = 120 healthy subjects) established previously of 22 ng/mL (90% confidence interval [CI], 18–34 ng/mL) (10–12). The package insert upper 97.5% limit of the reference interval was deemed transferable to our population if at least 90% of measured results were below the upper limit.

Assay interference testing was performed using residual EDTA plasma samples with DPP3 concentrations within the measuring range and adding various amounts of exogenous interferant to simulate lipemia, icterus, and hemolysis. To simulate lipemia, various amounts of Intralipid (lipid emulsion, Millipore Sigma) were added to samples with an ending interferent concentration ranging from 0 to 1000 mg/dL. To simulate icteric samples, various amounts of bilirubin (bilirubin conjugate, ditaurate, disodium salt, Millipore Sigma) were added to EDTA plasma samples with an ending interferent concentration ranging from 0 to 50 mg/dL. To simulate hemolyzed specimens, various amounts of laboratory-prepared hemolysate (approximately 10.0 g/dL hemoglobin) were added to EDTA plasma samples to achieve an H-index interferent concentration of 0 to 1000. Heterophile interference was evaluated by measuring DPP3 before and after treatment using heterophile blocking tubes (HBT) or heterophile blocking reagent (HBR) (Scantibodies Laboratories, Inc.). Residual paired EDTA plasma and serum were collected from 10 individuals and DPP3 was measured in both sample types. Data analyses including regression evaluations were performed using Excel Analyze-IT.

RESULTS

Table 1 shows the results of the analytical validation studies. Within-run repeatability studies showed <6% CV and between-run reproducibility showed <8% CV at both analyte concentrations tested. Comparability studies compared to the reference showed a slope of 1.04 and intercept of 0.50 ng/mL by linear regression using samples that spanned the analytical measuring range. The assay was determined to be linear, spanning the concentration range of 0 to 341 ng/mL, with the percent difference ranging from −11% to 9% for all samples tested. The LOQ listed in the package insert of 1.6 ng/mL was verified using residual patient samples of near and below this concentration (3.0 and 0.5 ng/mL, respectively).

In the healthy donor cohort, there were no statistically significant associations with age or sex and DPP3 concentration. The median and range for all samples was 7.0 ng/mL and 3.9 to 21.0 ng/mL, respectively. Results in all samples tested were below the package insert upper 97.5th percentile limit of 22 ng/mL (10–12).

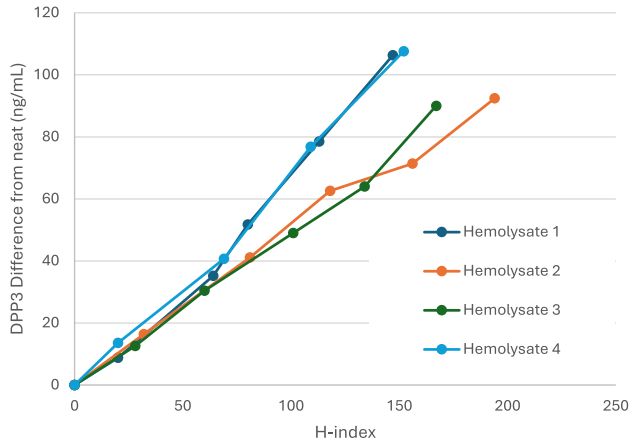

Fig. 1. Increase in measured DPP3 concentration with increasing hemolysis.
One residual EDTA plasma sample with DPP3 concentration of 21.9 ng/mL and 4 different preparations of hemolysate were used and show that measured DPP3 increases linearly with increasing hemolysate concentration (H-index).

DPP3 results were stable in plasma of up to 24 h stored at ambient temperature, 14 days when stored at 2 to 8°C, 30 days when stored at −20°C, 11 months when stored at −80°C, and after one freeze–thaw cycle. DPP3 results in paired EDTA plasma and serum samples (n = 10) displayed significantly disparate results with a range of percent difference in concentration of −76% to 77% and R^2 of 0.009 by linear regression.

Interferences from lipemia and icterus were not observed up to an L-index of 1000 or an I-index of 50 as measured on a Roche Cobas analyzer. Significant interference due to hemolysis was observed, with measured DPP3 concentrations increasing linearly with increased H-index. The addition of different preparations of hemolysate caused consistently increased DPP3 concentrations in one residual patient sample (Fig. 1). Similarly, the hemolysis-induced change in DPP3 concentration was similar when hemolysate was added to 3 residual patient samples with different endogenous concentrations of DPP3 (17, 37, and 131 ng/mL). A total of 10 samples were pretreated with 2 different heterophile binding reagents. Pre-incubation with HBT showed larger nonspecific changes in DPP3 results (−22.7% to 13.6%) compared to the changes observed with HBR (−13.2% to 1.5%).

DISCUSSION

Our data show that DPP3 concentrations can be precisely measured in human EDTA plasma samples using the DPP3 immunoluminometric assay (4TEEN4 Pharmaceuticals GmbH). The assay is linear and robust across a concentration range of 1.6 ng/mL to nearly 200 ng/mL with the 97.5th percentile reference interval of 22 ng/mL. No differences related to sex or age were observed. However, results obtained in serum were significantly different from those obtained in EDTA plasma. There may be multiple reasons for the difference such as release of DPP3 during clotting but given that all clinical studies have used EDTA plasma to establish clinical cutpoints, it would be advisable to use EDTA plasma for clinical use unless future studies prove utility for serum measurements. Two different reagent lots were available during our evaluation. The lot-to-lot comparison showed no detectable drift in DPP3 results

demonstrating consistency in kit manufacturing across these two lots. If heterophile interference is suspected, pretreating samples with HBR would be more acceptable compared to HBT given the amount of nonspecific binding to HBT found in our study.

While lipemia and bilirubin did not appreciably alter DPP3 results, hemolysis significantly and linearly increased measured DPP3 concentrations. The increase was observed even at the level of the smallest change in H-index measured (20 units) and continued linearly up to the highest H-index tested (H-index of 1000, data not shown). This sensitivity to hemolysis is likely the result of intracellular release of DPP3 from red blood cells. The contribution of hemolysis to measured DPP3 is of particular importance given the patient populations in which measured DPP3 may be of most clinical utility, namely patients with various shock states. In this clinical setting, it may be difficult to distinguish between intravascular hemolysis that is pathologic to the disease process and preanalytical hemolysis that can occur during sample collection.

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REFERENCES

1. Jaffe AS, Donato LJ. DPP3 in cardiogenic shock. Clin Chem 2024;70:1200–1.
2. Ye P, Duan W, Leng YQ, Wang YK, Tan X, Wang WZ. DPP3: from biomarker to therapeutic target of cardiovascular diseases. Front Cardiovasc Med 2022;9: 974035.
3. Deniau B, Rehfeld L, Santos K, Dienelt A, Azibani F, Sadoune M, et al. Circulating dipeptidyl peptidase 3 is a myocardial depressant factor: dipeptidyl peptidase 3 inhibition rapidly and sustainably improves haemodynamics. Eur J Heart Fail 2020;22:290–9.
4. Garcia B, Ter Schiphorst B, Santos K, Su F, Dewachter L, Vasques-Novoa F, et al. Inhibition of circulating dipeptidyl-peptidase 3 by procizumab in experimental septic shock reduces catecholamine exposure and myocardial injury. Intensive Care Med Exp 2024;12:53.
5. Wenzl FA, Bruno F, Kraler S, Klingenberg R, Akhmedov A, Ministrini S, et al. Dipeptidyl peptidase 3 plasma levels predict cardiogenic shock and mortality in acute coronary syndromes. Eur Heart J 2023;44:3859–71.
6. Rehfeld L, Funk E, Jha S, Macheroux P, Melander O, Bergmann A. Novel methods for the quantification of dipeptidyl peptidase 3 (DPP3) concentration and activity in human blood samples. J Appl Lab Med 2019;3:943–53.
7. Kaufmann P, Muenzner M, Kastorf M, Santos K, Hartmann T, Dienelt A, et al. A novel and highly efficient purification procedure for native human dipeptidyl peptidase 3 from human blood cell lysate. PLoS One 2019;14:e0220866.

8. CLSI. CLSI EP05-A3: Evaluation of precision of quantitative measurement procedures. 3rd Ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2014.
9. CLSI. CLSI EP28IG: Verification of reference intervals in the medical laboratory implementation guide. 1st Ed. Wayne (PA): Clinical and Laboratory AQ9 Standards Institute; 2022.
10. Innelli P, Lopizzo T, Paterno G, Bruno N, Radice RP, BertiniP, et al. Dipeptidyl amino-peptidase 3 (DPP3) as an early marker of severity in a patient population with cardiogenic shock. Diagnostics (Basel) 2023;13: 1350.
11. Instructions for use — sphingotest® DPP3 [package insert]. Hennigsdorf (Germany): SphingoTec GmbH; 2021.
12. Picod A, Azibani F, Harjola VP, Karakas M, Kimmoun A, Levy B, et al. Targeting high circulating dipeptidyl peptidase 3 in circulatory failure. Crit Care 2025;29: 340.