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Short Communication

Advantages of the lipoprotein-associated phospholipase A2 activity assay

Leslie J. Donato [a,*], Jeffrey W. Meeusen [a], Heidi Callanan [a], Amy K. Saenger [c], Allan S. Jaffe [a,b]

[a] Department of Laboratory Medicine and Pathology Mayo Clinic, Rochester, MN 55905, United States
[b] Division of Cardiology, Mayo Clinic, Rochester, MN 55905, United States
[c] Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455, United States

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ABSTRACT

Objectives: Lipoprotein-associated phospholipase A2 (Lp-PLA_2) is increased in circulation in patients at higher risk of coronary heart disease (CHD) events and stroke. Therefore, measurement of Lp-PLA_2 can be used as an adjunct to traditional cardiovascular risk factors for identifying individuals at higher risk of cardiovascular events. Recently, a reagent for measuring Lp-PLA_2 activity (diaDexus, San Francisco, CA) received FDA approval. Here we evaluate the assay performance of the Lp-PLA_2 activity assay.

Methods: Lp-PLA_2 activity assay reagent performance was evaluated on an open user-defined channel on a Cobas 6000/c501 (Roche Diagnostics, Indianapolis, IN) using a 5-point calibration curve (0–400 nmol/min/mL). Analytical performance was established for the following parameters: precision, linearity, accuracy, analytical sensitivity, analytical specificity, reference interval, reagent lot-to-lot comparison, specimen type, on-board reagent stability, and sample stability.

Results: Assay limit of detection was determined to be 7.8 nmol/min/mL with an average %CV of 2.8%. Precision studies revealed a coefficient of variation ≤1.6% between 79 and 307 nmol/min/mL and accuracy was demonstrated between 4.8–368.7 nmol/min/mL. Comparable results were generated in paired SST serum and EDTA plasma. No age association was found with Lp-PLA_2 activity at the 95th percentile however a gender association was identified resulting in gender-specific 95th percentile limits in a healthy reference population. No bias was found when comparing results from several different lots of assay reagent. Lp-PLA_2 activity results are extremely stable in both serum and EDTA plasma under refrigerate and frozen storage conditions up to 31 days.

Conclusions: Lp-PLA_2 activity assay displays accurate and precise performance characteristics on the Cobas c501 platform. The assay performance is significantly improved over the predecessor immunoassay allowing for adoption of Lp-PLA_2 activity in clinical practice.

1. Introduction

Lipoprotein-associated phospholipase A2 (Lp-PLA_2), originally known as platelet activating factor acetylhydrolase, is an inflammatory protein that is highly upregulated in atherosclerotic plaques and circulates bound mainly to low density lipoproteins [1–3]. In multivariate analysis with traditional risk factors and C-reactive protein, Lp-PLA_2 activity has been shown to be an independent predictor of coronary heart disease and stroke in the general population [4–7]. Therefore, measurement of Lp-PLA_2 may be used as an adjunct to traditional cardiovascular risk factor measures for identifying individuals at higher risk of cardiovascular disease events to enable the beginning or intensifying of risk reduction therapies. Measurement of circulating Lp-PLA_2 can be performed by quantitation of the protein concentration or by measuring the enzyme activity. The most commonly used Lp-PLA_2 concentration assay (commonly referred to as the Lp-PLA_2 mass assay) is an ELISA platform requiring significant manual processing and has significant pre- and post-analytical problems [8,9]. Thus if robust, the activity assay that can be run on an automated chemistry platform would be preferred. Accordingly, we performed an analytical evaluation of the newly FDA-approved enzymatic colorimetric Lp-PLA_2 activity assay (diaDexus Inc., San Diego, California).

2. Materials and methods

2.1. Lp-PLA_2 activity assay and patient samples

Lipoprotein-associated phospholipase A2 enzymatic colorimetric activity assay which measures the rate of hydrolysis of the substrate 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine to form the colored product 4-nitrophenol (diaDexus, San Francisco, CA) was run using an open user-defined channel on a Cobas 6000/c501 (Roche Diagnostics, Indianapolis, IN). Activity was determined using a 5-point calibration curve (0–400 nmol/min/mL).

Residual serum and EDTA plasma samples were collected from routine laboratory testing. Serum samples used for method comparison studies were obtained from diaDexus. Samples used to establish

* Corresponding author at: 200 First St. SW, Rochester, MN 55905, United States.
E-mail address: Donato.leslie@mayo.edu (L.J. Donato).

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reference intervals were collected from healthy donors defined as individuals without a history of diabetes, MI, stroke, hypertension, autoimmune diagnosis, kidney disease, liver disease, malnutrition, cancer, sarcoidosis, hyperthyroidism, amyloidosis, and had not taken lipid lowering medications within the last 90 days.

2.2. Assay performance studies

A full analytical evaluation of the Lp-PLA_2 activity assay was performed with the pre-FDA approved reagents and results were verified with FDA-approved reagents. Inter- and intra-assay precision was assessed by repeated measurements (n = 20) of pooled residual serum containing low, mid, and high Lp-PLA_2 activity on the same day (intra) or on 20 different days (inter). Two calibrations were performed during the inter-assay precision study. Accuracy was assessed by comparing results of 40 serum samples (range: 5.5–364.6 nmol/min/mL) run at diaDexus on a Beckman AU400 analyzer. Linearity was determined by serial recovery studies (mixing of serum samples with high and low Lp-PLA_2 activity [range 77–356.8 nmol/min/mL] at ratios: 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10) and measured results were compared to expected calculated results. Reference intervals were established using 256 healthy individuals (117 male, 139 female; age 23–86). Reagent lot-to-lot comparisons were performed using residual patient serum run on four different lots of Lp-PLA_2 activity reagent. Three lots were pre-FDA approved reagents (calibrator 1112134–1112140/reagent 1112131–1112132 [lot 1], calibrator 1201224–1201230/reagent 1201221–1206015 [lot 2], calibrator 1204160–1204165/reagent 1205055–1206015 [lot 3]) while the fourth lot was the first FDA-approved lot (500757 [lot 4]). Specimen type comparisons were performed by collecting paired serum (both clot tube [red top] and serum separator tube [SST]) and EDTA plasma. Specimen stability was assessed by collecting serum (red top and SST) and EDTA plasma, removing matrix from the cells within 2 h of collection, and storing aliquots at ambient, refrigerate, and frozen temperatures. To assess freeze/thaw stability serum (red top and SST) and EDTA plasma were collected, separated from cells within 2 h of collection and stored frozen at −20 °C for >24 h for each freeze/thaw cycle (range 120–226 nmol/min/mL). Analytical specificity was assessed by adding potential interfering substances (hemoglobin, lipids, and bilirubin to obtain various hemolysis (H-index), lipemia (L-index), and icterus (I-index) indices, respectively on the Cobas analyzer) to residual serum pools at three different Lp-PLA_2 activity values. Laboratory prepared hemolysate (10.0 g/dL hemoglobin) was used to obtain various H-index values in three samples (120, 188, and 380 nmol/min/mL). Intralipid (20% fat emulsion) was used to obtain increased lipid content (L-index) in three samples (106, 178, and 264 nmol/min/mL). Bilirubin (1000 mg/dL) was used to obtain increased icterus (I-index) in three samples (101, 180, and 292 nmol/min/mL). Stability of the reagents on the analyzer was assessed by analyzing 3 concentrations of pooled serum once per day for 24 days using the same reagent cassette over a period of 3 months.

Table 1
Analytical performance characteristics of the Lp-PLA2 activity assay.

Parameter	Package insert	Study results	
Imprecision	Within run: CV ≤ 1.5% Between run: CV ≤ 3.8%	Within run: mean (SD; %CV) 78.7 nmol/min/mL (0.49; 0.6%) 114.0 nmol/min/mL (0.49; 0.4%) 307.3 nmol/min/mL (1.78; 0.6%)	Between run: mean (SD; %CV) 118.7 nmol/min/mL (1.53; 1.3%) 199.5 nmol/min/mL (2.98; 1.5%) 266.9 nmol/min/mL (3.1; 1.2%)
Accuracy	Not stated	Method comparison (Mayo Clinic vs. diaDexus) n = 40, range = 4.8–368.7 nmol/min/mL (on Cobas c501) Passing Bablock regression: y = 0.99x + 1.2	
Linearity	6 to 382 nmol/min/mL Slope (range 0.98 to 1.04) Intercept (range −0.40to −0.03)	3 serum mixing studies (low, high [nmol/min/mL]) (77.0, 317.7), (99.9, 356.8), (88.7, 263.3) Average (range) % difference from expected = 1.8% (0.8%, 7.5%)	
Limit of quantitation	10 nmol/min/mL	Residual patient serum with lowest Lp-PLA2 activity measured 20 times Mean (SD; %CV): 7.8 nmol/min/mL (0.22; 2.8%)	
Reference interval	Upper 95% limit: Males: 295 nmol/min/mL Females: 264 nmol/min/mL	n = 256 (117 males and 139 females, ages: 23–86y, range: 106–352 nmol/min/mL) Upper 95% reference limit (95% confidence interval) - Males ≤284.1 nmol/min/mL (262.2, 306.1 nmol/min/mL) - Females ≤228.5 nmol/min/mL (217.3, 239.7 nmol/min/mL)	
Reagent on-board stability	Not stated	3 serum pools measured once per day on 24 days (over a total of 61 days) Pool 1 Pool 2 Pool 3 Mean (nmol/min/mL) 117.9 194.0 242.2 % CV 1.3% 1.6% 1.6%	
Reagent lot-to-lot comparison	Not stated	Residual serum (n = 20) tested on 3 lots of pre-FDA approved reagent (lots 1–3) and one lot of FDA-approved reagent (lot 4). Slope and intercept generated from Passing Bablock analysis. Lot 2 vs. Lot 1 Lot 3 vs. Lot1 Lot 4 vs. Lot 1 Range (nmol/min/mL) 99.5–382.3 99.3–385.0 10.3–368.7 Slope 0.99 1.0 0.99 Intercept 1.0 1.3 1.2 Mean % Difference − .08 1.74 -0.8	
Analytical specificity	Limit Hemoglobin 100 mg/dL Triglycerides 400 mg/dL Bilirubin 20 mg/dL	Concentration Maximum % Difference Hemoglobin 250 mg/dL −16.9% Triglycerides 750 mg/dL −17.9% Bilirubin 25 mg/dL −5.4%	
Specimen type	Acceptable: - EDTA plasma (+/− gel) - Serum (+/− gel)	Paired serum (SST) and EDTA plasma (n = 20); range: 98–274 nmol/min/mL Mean % difference (min, max): −1.1% (−2.5%–0.4%) Passing Bablock regression: y = 1.0x–1.5	