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NMR measurement of LDL particle number using the Vantera® Clinical Analyzer

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ABSTRACT

Background: The Vantera Clinical Analyzer was developed to enable fully-automated, high-throughput nuclear magnetic resonance (NMR) spectroscopy measurements in a clinical laboratory setting. NMR-measured low-density lipoprotein particle number (LDL-P) has been shown to be more strongly associated with cardiovascular disease outcomes than LDL cholesterol (LDL-C) in individuals for whom these alternate measures of LDL are discordant.

Objective: The aim of this study was to assess the analytical performance of the LDL-P assay on the Vantera Clinical Analyzer as per Clinical Laboratory Standards Institute (CLSI) guidelines.

Results: Sensitivity and linearity were established within the range of 300–3500 nmol/L. For serum pools containing low, medium and high levels of LDL-P, the inter-assay, intra-assay precision and repeatability gave coefficients of variation (CVs) between 2.6 and 5.8%. The reference interval was determined to be 457–2282 nmol/L and the assay was compatible with multiple specimen collection tubes. Of 30 substances tested, only 2 exhibited the potential for assay interference. Moreover, the LDL-P results from samples run on two NMR platforms, Vantera Clinical Analyzer and NMR Profiler, showed excellent correlation ($R^2 = 0.96$).

Conclusions: The performance characteristics suggest that the LDL-P assay is suitable for routine testing in the clinical laboratory on the Vantera Clinical Analyzer, the first automated NMR platform that supports NMR-based clinical assays.

Introduction

Measurements of lipoprotein concentrations are widely used to assess and manage cardiovascular disease (CVD) risk. Lipoproteins move from the circulation into the artery wall in a gradient driven fashion. Following entry and retention in the artery wall, low-density lipoproteins (LDL) are oxidized, taken up by macrophages to form foam cells and accelerate atherosclerosis [1]. Conversely, high-density lipoproteins (HDL) demonstrate a variety of functions that antagonize atherosclerosis [2]. Traditional methods for estimating circulating concentrations of LDL and HDL measure their cholesterol content (LDL-C and HDL-C). However, the cholesterol content of lipoprotein particles varies widely among individuals and is often dependent on the metabolic state of the patient [3,4]. As a result, discordance is frequently noted between LDL-C levels and nuclear magnetic resonance (NMR) measurements of LDL particle number (LDL-P) [5–8]. As a CVD risk management tool, LDL-C values indicate which patients have lowered their risk to acceptable levels (as inferred from their treatment goal having been reached) and which have not (indicating a need for more aggressive treatment). Several large clinical studies demonstrate the limitations of this strategy in that, when alternate measures of LDL are discordant, LDL-P is more strongly associated with incident CVD events than LDL-C [5,6,9–18]. Consequently, several expert panels now recommend consideration of LDL-P levels to adjudicate and guide medical decision-making [19–21].

Proton NMR spectroscopy has been successfully applied to the measurement of lipoprotein particles, providing both concentration and average size information for very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), LDL, and HDL particles from a single serum or plasma sample [22]. This measurement is based on the proton magnetic resonance signals from terminal methyl groups on lipids within the core and shell of lipoprotein particles and the fact that the amplitude of the methyl NMR signal is proportional to the concentration of the particles. In addition, lipoproteins have distinct methyl signal

Abbreviations: NMR, Nuclear magnetic resonance; LDL-P, Low density lipoprotein particle number; LDL-C, Low density lipoprotein-cholesterol; CLSI, Clinical Laboratory Standards Institute; CV, Coefficient of variation; CVD, Cardiovascular disease; LDL, Low density lipoprotein; HDL, High density lipoprotein; HDL-C, High density lipoprotein-cholesterol; VLDL, Very low density lipoprotein; IDL, Intermediate density lipoprotein; LDT, Laboratory developed test; IVD, In vitro diagnostic; FDA, Food and Drug Administration; HDL-P, High density lipoprotein particle number; CLIA, Clinical Laboratory Improvement Amendments; LOB, Limit of blank; LOD, Limit of detection; LOQ, Limit of quantitation; SD, Standard deviation; DMSO, Dimethyl sulfoxide.

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frequency responses [23]. This attribute enables the measured plasma methyl signal envelope to be deconvoluted to provide the contributing signal amplitudes of the different-size subclasses of VLDL, LDL, and HDL, which in turn provide their particle concentrations [22].

NMR lipoprotein analysis has been commercially available for years as a Laboratory Developed Test (LDT) provided by a central testing facility, LipoScience, Raleigh, NC. The *NMR LipoProfile*® test, which was cleared as an in vitro diagnostic (IVD) by the Food and Drug Administration (FDA) on the NMR Profiler (2008) and Vantera Clinical Analyzer (2012), measures particle concentrations for all lipoprotein classes (VLDL, LDL, HDL) and subclasses (large, medium, small), and particle sizes for VLDL, LDL and HDL. The current IVD test reports LDL-P concentrations (nmol/L) from a serum/plasma sample with future goals to report additional lipoprotein parameters such as HDL particle number (HDL-P). The Vantera Clinical Analyzer is the first NMR device cleared by the FDA for use as a clinical instrument and is now available for distribution. It was developed to address the limiting factors of earlier NMR instruments by providing: 1) automated sample handling for high-throughput measurements (<2 min/test), 2) reduced sample volume requirements (150 μL/test), 3) quick and efficient testing without sample modification or pretreatment and 4) automated calibration and adjustment of the magnetic field homogeneity, allowing routine operation by laboratory medical technologists. Additionally, the Vantera footprint is reasonable for an NMR instrument [10.5(L) × 4(W) × 6(H) feet] making it convenient for placement in clinical laboratories. Therefore, deployment of the Vantera Clinical Analyzer should enable broader adoption of NMR-based applications and expand the number of NMR tests available in the clinical setting. The aim of this study was to assess the analytic performance of the LDL-P assay on this FDA approved and available NMR platform.

Materials and methods

Sample collection and preparation

Serum pools and controls were purchased from Solomon Park Research Laboratories. Controls were prepared by identifying serum samples with high and low lipoprotein ranges. Additional serum pools were prepared in-house from donor subjects identified at LipoScience (Raleigh, NC) or Mayo Clinic (Rochester, MN). This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), cleared by an Institutional Review Board and all donors signed consent forms. Samples were drawn into *NMR LipoProfile*® serum separator tubes (#456293/455232; Greiner Bio-One) also known as LipoTubes, allowed to clot at room temperature for 30 min, centrifuged at 3000 rpm for 15 min at room temperature, and promptly stored at 4 °C. For studies comparing collection tubes, specimens were drawn into BD Vacutainer serum collection tubes (red-top, no gel barrier) and allowed to clot as described above or K_2EDTA plasma collection tubes and processed as per the manufacturer (BD Diagnostics). Specimens (150 μL) for the Vantera were sampled directly from primary collection tubes or transferred to 13 × 75 mm tubes, then diluted automatically 1:1 with NMR diluent (50 mM sodium phosphate, 120 mM KCl, 5 mM Na_2EDTA, 1 mM $CaCl_2$, pH 7.4) onboard the Vantera. Sample preparation for NMR Profiler analysis included a 1:1 dilution with NMR diluent using a Tecan EVO autopipetter. On both platforms, the sample was delivered into the spectrometer's homogeneous magnetic field through a heated transfer line to rapidly reach the required measurement temperature. Two levels of serum controls were included at the beginning and end of each run of test samples.

Acquisition of NMR spectra and data processing

NMR spectra were acquired at the CLIA approved facilities of LipoScience on the NMR Profiler or Vantera Clinical Analyzer, both equipped with 400 MHz ^{1}H NMR spectrometers (Bruker Bio-Spin and Agilent Technologies, respectively). Data acquisition on the NMR Profiler was performed as described [24] with water suppression achieved through presaturation. Data acquisition on the Vantera was accomplished in a similar fashion with the exception that signal from water was suppressed using the WET solvent suppression technique [25]. The NMR data was acquired as 5 blocks of 2 scans on the NMR Profiler and 3 blocks of 4 scans on the Vantera for a total acquisition time of 40 and 48 s, respectively. For both systems the spectra were acquired with a sweep width of 4496.4 Hz and 9024 data points. The data was processed by zerofilling to 32 K points and multiplied by a Gaussian function to provide resolution enhancement prior to Fourier Transformation.

Deconvolution of lipoprotein signals

The methyl signal envelope appearing between 0.718 and 0.914 ppm was analyzed using the LipoProfile-3 algorithm, which models the signal as a combination of lipoprotein and serum protein subcomponent spectra [22]. The contribution of each subcomponent was determined by linear least-squares singular value decomposition constrained so concentrations could not be <0. LDL-P concentrations reported here are the sum of the IDL and large and small LDL subclass levels and reported in nmol/L units.

Vantera LDL-P trueness controls

To ensure long-term stability and consistency of Vantera LDL-P measurements, a set of "trueness controls" was developed. Trueness controls consist of a set of three serum control levels, having assigned LDL-P values determined on multiple Vantera systems and stored at −80 °C. These are routinely used to benchmark assay performance and prevent any long term drift of LDL-P values. Assigned values and uncertainty (±2*SD) for trueness controls were obtained using three Vanteras, measured for 2 runs per day (5 replicates/run) for three days. Imprecision for trueness control value assignment was typically 4–5% CV. Accurate recovery of trueness control assigned values was used to qualify labeling of all subsequent control lots.

Assay performance testing

Sensitivity

Limits of blank (LOB), detection (LOD) and quantitation (LOQ) were calculated based on Clinical Laboratory Standards Institute (CLSI) guidelines [26]. To minimize matrix effects for the blank, serum pools were delipidated by ultracentrifugation (density = 1.22 g/mL), the bottom fraction was then removed and dialyzed against phosphate buffered saline (density = 1.006 g/mL). The LOB was determined from 60 measurements obtained from 5 delipidated pools (blank samples) tested 4 times per day for 3 days. LOB was calculated as the mean + 1.645*standard deviation (SD) of these measurements. Five serum pools containing low analyte levels were tested in 4 replicates on 3 days (n = 60). LOD was calculated as LOB + 1.65*pooled SD of the 5 individual pools. LOQ was estimated as the level of analyte giving imprecision better than a total allowable error of 20%. Serum pools containing low concentrations of LDL-P (9 pools) were tested in replicates of 4 for 3 days. Mean result and coefficient of variation (CV) were calculated for each pool. A plot of CV versus mean result was generated and fit to a power function. LOQ was estimated as the intersection of the power function fit line with CV = 20%.

Imprecision

Within-laboratory imprecision and repeatability were determined based on CLSI guidelines [27] using serum pools targeted at low, intermediate and high ranges. Testing consisted of duplicate tests run twice per day for 20 days (n = 80) on one Vantera, while within-run imprecision was calculated from 20 replicates.

Linearity

Assay linearity was evaluated based on CLSI guidelines [28] by comparing linear regression to higher order polynomial regression for results from a series of mixtures of high and low serum pools. Serum pools with elevated LDL-P were obtained by pooling serum specimens with high levels of lipoproteins and enriched by the addition of LDL isolated by ultracentrifugation [29]. Samples with intermediate LDL-P were obtained by mixing serum pools with elevated and low levels of the lipoprotein analytes in varying proportions. Four measurements were made for each mixture.

Method comparison

Consistent with CLSI guidelines [30], samples were tested in singlicate on the reference (NMR Profiler) and comparator (Vantera Clinical Analyzer) NMR systems over a period of 8 days of operation (n = 1526). Concentrations measured covered the expected reference intervals and beyond. Thirteen samples with a concentration of LDL-P <300 nmol/L and 23 samples with LDL-P >3500 nmol/L were excluded. The correlation between the results generated on the two NMR platforms was evaluated using the Deming regression analysis.

Reference interval

Healthy adult men and women between the ages of 18 and 84 were recruited by LipoScience or the Mayo Clinic. Subjects were considered ineligible based on history of heart attack, stroke, renal disease or heart failure, diabetes, taking lipid-altering drugs, having undergone procedures for CVD or cancer; body mass index ≥30, or blood pressure reading >140/90 mm Hg. A total of 452 subjects were included. Non-fasting specimens were drawn into LipoTubes and tested in singlicate on one Vantera system. Serum samples were also tested for glucose, creatinine, triglycerides, LDL-C(direct), HDL-C and total cholesterol on an AU640 (Beckman Coulter Inc.). Cumulative distributions, means, medians and 95% confidence intervals were calculated. The LDL-P reference range was estimated using non-parametric analyses with reference limits at 2.5th and 97.5th percentiles according to the nonparametric method described in CLSI guidelines [31]. Reference intervals were compared by assessing their median results by a Wilcoxon Rank Sum test.

Test for interfering substances

A total of 7 endogenous and 23 exogenous substances were tested for possible assay interference consistent with CLSI guidelines [32]. Stock solutions of substances were prepared in either H_2O (20×) or dimethyl sulfoxide-d_6 (DMSO-d_6) (80×) depending on solubility. Substances were individually added to 2 serum pools containing lipoprotein concentrations at different medical decision levels. Each serum pool was divided in half; one half was spiked with the 20× or 80× stock solution of each substance and the other half was spiked with H_2O or DMSO-d_6 as the control. Testing was performed using 8 replicates for each pool. The results of the test pool spiked with the substance were compared to the results of the control pool using a paired difference test [32]. If a statistical difference between test and control pool results was detected, this difference was checked to determine whether it was considered to be clinically meaningful (>10%). Substances for which interference was observed were tested at multiple concentrations to estimate a threshold at which interference exceeded 10%.

Comparison of collection tubes

Blood from 46 donors was drawn into three different tubes: LipoTube, BD Vacutainer serum tube (red-top, no gel barrier) or K_2EDTA plasma tube. In order to expand the range of measured values, 2 specimens with low analyte concentration were diluted (≤50%) and 2 specimens with elevated analyte were spiked with fractionated LDL (≤10% by volume). A total of 50 specimens were tested for each analyte. Results for conventional serum and EDTA plasma tubes were compared to results for the LipoTube by linear regression.

Stability testing

Three separate studies of 10 donors (n = 30) drawn in LipoTubes were used to assess LDL-P stability. Samples were stored at 4 °C and aliquots tested daily. Daily mean results for all donors were evaluated with acceptable differences falling within ±10% of the day 0 (draw day) mean.

Results

Assay performance

The NMR LDL-P assay was assessed for its analytical performance on the Vantera Clinical Analyzer including the determination of the ability to reliably detect and quantify LDL particles in serum. The average value obtained when testing replicate blanks (LOB) was determined to be 0 nmol/L, while the analytical sensitivity or limit of detection (LOD) was calculated to be 41 nmol/L. Testing of nine serum pools, with LDL-P concentrations ranging from 85 to 261 nmol/L, gave a functional sensitivity or limit of quantitation (LOQ) of 132 nmol/L with a CV of <10%. Controls with three levels of lipoprotein analytes (low, intermediate and high) were tested for inter-assay (within-lab) precision, intra-assay (within-run) precision and repeatability. The CVs ranged from 3.9 to 5.3% for within-lab precision, 2.6–4.9% for repeatability and 2.7–5.8% for within-run precision (Table 1). In order to test the ability of the LDL-P assay to detect LDL particles, the measured values were plotted against the actual or expected concentrations. The measured LDL-P values demonstrated a linear relationship with the expected values within the range of 225–4320 nmol/L with a correlation coefficient of $R^2 = 0.99$ (Fig. 1A).

Method comparison

In order to ensure that the performance of the LDL-P assay on the Vantera Clinical Analyzer was similar to the well characterized LDL-P assay on the NMR Profiler, a method comparison was performed. Specimens outside the reportable range of 300–3500 nmol/L were excluded from the analysis leaving the total number of specimens analyzed to 1490. The linear regression (data not shown) for the LDL-P data,

Table 1
Within-laboratory imprecision, repeatability and within-run imprecision for LDL-P measured on the Vantera Clinical Analyzer.

	LDL-P (nmol/L)		
	Low	Intermediate	High
Within-laboratory[a]			
Mean	989	1267	1944
SD	52.2	50.1	75.1
CV	5.3%	4.0%	3.9%
Repeatability[a]			
Mean	989	1267	1944
SD	48.8	32.6	63.4
CV	4.9%	2.6%	3.3%
Within-run[b]			
Mean	843	1310	1838
SD	48.5	39.1	50.3
CV	5.8%	3.0%	2.7%

[a] Based on CLSI EP5-A2 tested using 3 controls, 2 runs per day in duplicate for 20 days (total n = 80).
[b] Based on 1 run of 20 tests.

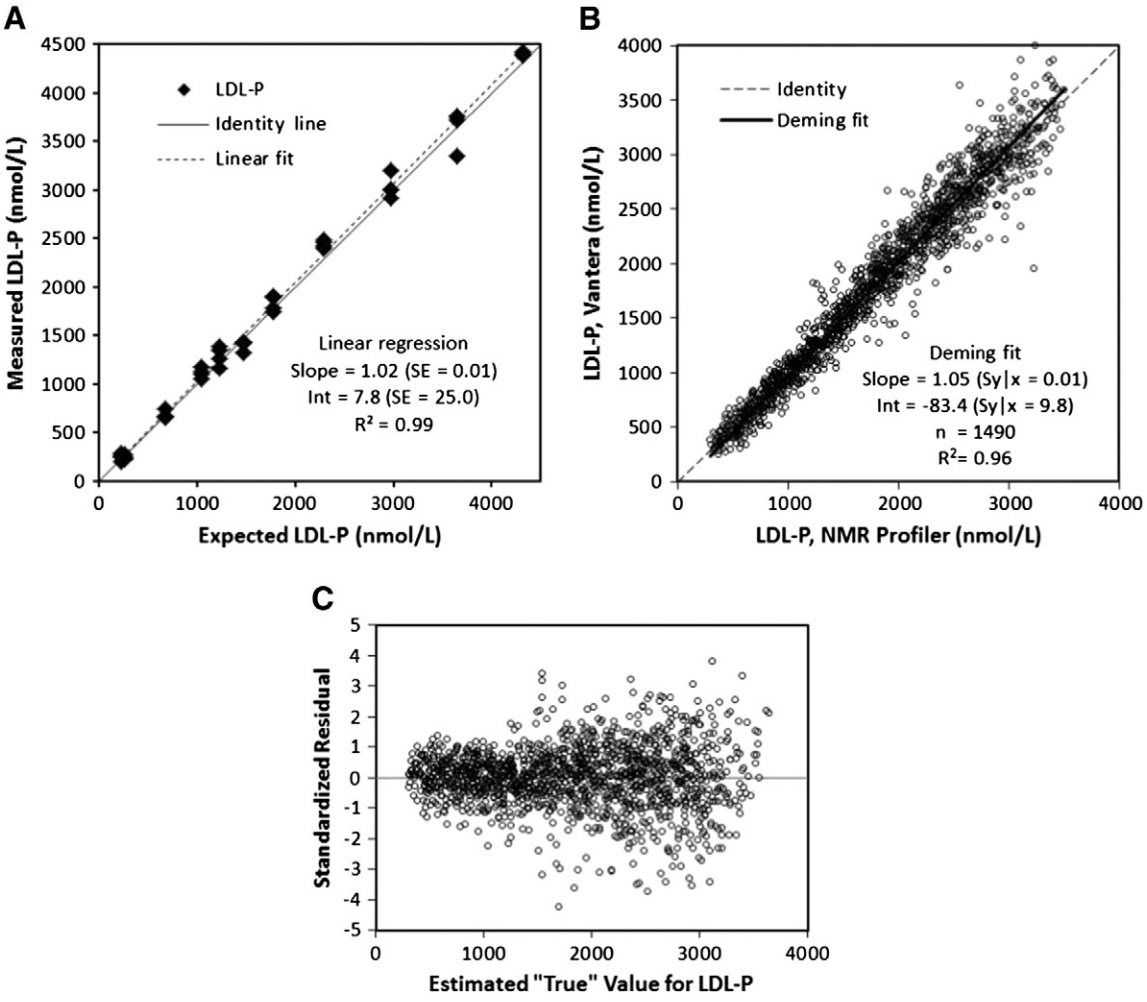


Fig. 1. A) Results of linearity testing for the Vantera LDL-P assay. B) Comparison of Vantera and NMR Profiler methods for detection and quantification of LDL-P. C) Residual plot of LDL-P assays.

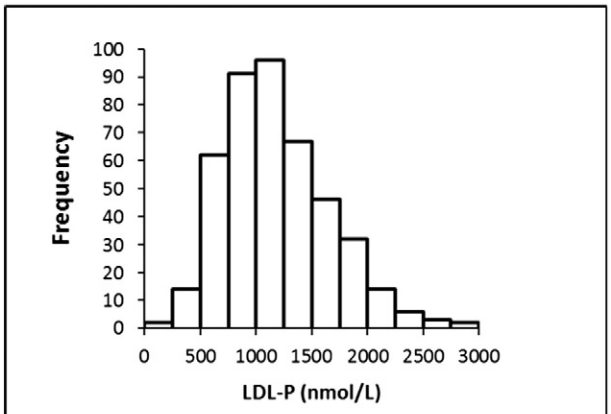
with the NMR Profiler results on the x-axis, produced a slope of 1.03, intercept of −36.6 and correlation coefficient (R^2) of 0.96 and the Deming regression (Fig. 1B) gave a slope of 1.05, intercept of −83.4 and R^2 value of 0.96. The residuals, differences between the LDL-P values and the regression line, were plotted against the estimated concentration for LDL-P. The points on the plot were randomly dispersed around the horizontal axis, suggesting that the results of the two assays were linearly related, and there appeared to be only one significant outlier (Fig. 1C).

Reference interval determination

A population of healthy individuals (n = 452) was used to determine the reference interval for the LDL-P assay. Fig. 2 shows the distribution of LDL-P values in this population. The mean LDL-P value was 1193 ± 472 nmol/L and the median was 1127 nmol/L, which defined the 95 percentile range for reference interval as 457–2282 nmol/L (Fig. 2). The reference interval for LDL-P showed significant differences between genders (men = 372–2365; women = 480–2057 nmol/L) as did the mean (men = 1279 ± 491; women = 1148 ± 456 nmol/L) (p = 0.002). For comparison, the LDL-C results from the same reference population were juxtaposed to the LDL-P results (Fig. 2). The mean LDL-C was 113 ± 34 mg/dL. According to the National Cholesterol Education Panel's Adult Treatment Panel (ATP) III guidelines [33], LDL-C levels below 100 mg/dL (<20th percentile based on the Framingham Offspring Study and NHANES III Survey) are considered "optimal" for patients who are at high risk for CVD. In this reference population, we found that an LDL-C value of roughly 100 mg/dL (40th percentile) corresponded to an LDL-P value of approximately 1000 nmol/L (Fig. 2).

Interfering substances

Thirty substances were tested for their potential to interfere with the LDL-P assay. Results (Table 2) showed that interference was not detectable or <10% at the highest level tested for 24 of 30 substances tested. Six substances showed potential interference with the LDL-P assay in the initial screening and were tested at multiple concentrations to identify levels at which interference was significant. Four substances (albumin, fenofibrate, ibuprofen and naproxen) were considered to be low risk for eliciting analytical interference. Albumin suppressed measured LDL-P at the upper limit of the tested range, with 10% interference estimated to occur at 59 mg/mL exogenous albumin. The recommended test ranges for albumin, based on CLSI guidelines, are 35–50 mg/mL [32]. Fenofibrate elicited an apparent increase in measured LDL-P at concentrations >52 μM, which is above the expected therapeutic concentration (41.6 μM) [32]. Similarly, ibuprofen and naproxen elicited an apparent increase in measured LDL-P at concentrations >1228 and 2170 μM, well above therapeutic concentrations of 48.5–340 and 130–521 μM, respectively [32]. The remaining two substances presented a greater risk for interference because the concentration at which interference occurred was within the therapeutic range. Salicylic



	LDL-P (nmol/L)			LDL-C (mg/dL)
Percentile	All (n=452)	Men (n=158)	Women (n=294)	All (n=452)
5	539	528	542	63
10	643	713	638	75
20	784	883	749	84
30	909	1004	863	94
40	1009	1087	970	102
50	1127	1241	1070	109
60	1248	1366	1202	118
70	1396	1505	1322	129
80	1572	1676	1482	140
90	1894	1941	1818	157
95	2047	2169	1986	169
Median	1127	1241	1070	109
Mean (SD)	1193 (472)	1279 (491)	1148 (456)	113 (34)
95% reference interval	457–2282	372–2365	480–2057	57–187

Fig. 2. Distribution of LDL-P values from 452 reference range study participants (top). Means, medians, and reference intervals for LDL-P and LDL-C for all participants in the reference range study as well as for just the men and women from the same population (bottom).

acid at concentrations >1.3 mM (929 mg) resulted in 10–15% lower LDL-P values (therapeutic range of 0.7–2.8 mM or 500–1500 mg) [32]. Clopidogrel hydrogen sulfate at concentrations >40 μg/mL (201 mg) resulted in an approximate 10–20% increase in LDL-P (therapeutic range ≤120 μg/mL or ≤600 mg). As per CLSI standards for interference testing, clopidogrel hydrogen sulfate concentrations tested are representative of parent drug concentrations and are not reflective of active metabolite, therapeutic concentrations.

Comparison of collection tubes

Various tube types were tested for their compatibility with the NMR LDL-P assay. The results performed on specimens collected in LipoTubes were plotted against those obtained in conventional red-top serum tubes (no gel barrier) (Fig. 3A). A linear regression analysis was performed and the resulting line gave a slope of 0.97 ($R^2 = 0.96$). Moreover, BD red-top, no gel serum tubes showed no significant bias based on either 95% confidence intervals around the correlation slope and intercept, or estimation of bias from Bland–Altman residual plots (data not shown). For the LDL-P assay performed on specimens collected in EDTA plasma tubes (Fig. 3B) the resulting linear regression produced a slope of 0.94 ($R^2 = 0.97$). The LDL-P assay results in EDTA plasma tubes, however, were modestly (3–7%) lower than results from specimens collected in LipoTubes.

Stability

Thirty serum samples were evaluated for reproducibility in quantification over time with acceptable measurements falling within ±10% of the day 0 mean. Results showed that the mean LDL-P values were stable out to day 6 with changes of <10% (Table 3). However, on day 7 a change of 10.2% was observed.

Discussion

Because of commonly encountered variability in the amount of cholesterol carried in LDL particles, either for physiologic reasons (e.g. metabolic syndrome or type 2 diabetes) or as a result of statin therapy, LDL-C is frequently discordant with LDL-P [4,34,35]. When discordance is present, LDL-P is a stronger predictor of coronary heart disease risk, as well as on-trial LDL-related risk among patients treated with lipid-altering therapy. As a result, several expert panels recommend the use of LDL-P as a target of therapy to guide management and adjudicate response to pharmacotherapy in patients with increased CVD risk [19–21]. The *NMR LipoProfile*® test, which reports LDL-P values, was cleared by the FDA for use on the Vantera Clinical Analyzer.

The ability to reliably quantify lipoprotein particle concentrations of clinical serum/plasma specimens via NMR has been established for several years using highly specialized equipment and trained operators [22,24,36]. In the past, this technology has not been amenable to testing outside a central laboratory facility, in part because of the lack of an NMR platform that was compatible with typical clinical laboratory operations. The Vantera Clinical Analyzer includes a number of features to address this gap: 1) an operator interface developed specifically for the clinical setting, 2) simplified set-up, calibration, maintenance and operation 3) high-throughput, fully-automated sample processing (>35 samples/h) and 4) a smaller footprint. Results from this study demonstrate the successful development of an automated NMR-based assay for LDL-P that showed the precision, sensitivity, measurement range and linearity suitable for routine clinical measurements. The types of tubes for sample collection and stability further support and reconfirm previously described observations [22]. Furthermore, the reference range verification study provides a framework for understanding the relationship between particle-based and cholesterol-based lipoprotein measurements. Lastly, a previously published comparison of NMR-measured LDL-P values with LDL apoB (after removing VLDL by ultracentrifugation) revealed a strong correlation between the two measures of LDL particle concentration ($r = 0.93$) [22], suggesting that NMR-measured LDL-P may be a simple, inexpensive means of assessing the number of atherogenic LDL particles without the results being confounded by the inclusion of VLDL particles.

Because NMR technology supports the simultaneous determination of multiple analytes across the NMR spectrum, it provides an attractive tool for profiling a range of biological molecules and metabolites and has widespread applications related to personalized diagnostics. NMR has been applied in the research setting to identify serum/plasma markers of prediabetes/diabetes, cancer, inflammation, Alzheimer's disease and CVD [37–39]. The ability to test complex clinical specimens requires that interference from drugs and other substances is not a critical issue. NMR technology is immune from the type of interferences common to chemical methods (e.g. turbidity). Since all the NMR-based lipoprotein information are extracted from the terminal methyl lipid signals, only substances that contain a methyl signal that appear in the lipid region of 0.7–0.9 ppm can potentially cause interference with the lipoprotein analysis. Dramatic changes in the matrix including ionic strength and pH can have an effect on the chemical shift of the lipid signal, though this is unlikely to occur with samples collected following standard specimen requirements. However, the in vitro method, which is recommended by CLSI for determining common interfering substances, may cause the types of artificial changes to the spectra that are unlikely to occur after drug administration in vivo. Additionally,

Table 2
Summary of interference testing for the NMR LDL-P assay measured on the Vantera Clinical Analyzer.

Substance	Drug name	Test concentration	Concentration eliciting interference
Bilirubin, unconj.	–	200 μg/mL (342 μM)	–
Bilirubin, conj.	–	289 μg/mL (342 μM)	–
Creatinine	–	50 μg/mL (442 μM)	–
Hemoglobin	–	2 mg/mL	–
Protein-albumin[a]	–	0–60 mg/mL	Interfered > 59 mg/mL
Urea	–	2.6 mg/mL (43 mM)	–
Uric acid	–	235 μg/mL (1.4 mM)	–
Acetaminophen	Tylenol	200 μg/mL (1.3 mM)	–
Acetylsalicylic acid	Aspirin	0–660 μg/mL (3.6 mM)	–
Atorvastatin	Lipitor	52 μg/mL	–
Clopidogrel hydrogen sulfate	Plavix	0–360 μg/mL	Interfered > 40 μg/mL
Enalaprilat dihydrate	Vasotec	0.3 μg/mL (0.9 μM)	–
Fenofibrate	Tricor	0–45 μg/mL (125 μM)	Interfered > 19 μg/mL (52 μM)
Furosemide	Lasix	60 μg/mL (181 μM)	–
Glipizide	Glucotrol	2.0 μg/mL (4.5 μM)	–
Heparin	Lovenox	3000 U/L	–
Hydralazine hydrochloride	Apresoline	180 μg/mL	–
Ibuprofen sodium salt	Advil	0–560 μg/mL (2425 μM)	Interfered > 280 μg/mL (1228 μM)
Isosorbide dinitrate	Isordil	150 ng/mL (636 nM)	–
Menhaden oil	Fish oil	2.4 mg/mL	–
Metformin hydrochloride	Glucophage	600 μg/mL	–
Metoprolol tartrate	Lopressor	13 μg/mL (18.7 μM)	–
Naproxen sodium	Aleve	0–550 μg/mL (2170 μM)	Interfered > 550 μg/mL (2170 μM)
Nicotinic acid sodium salt	Vitamin B3	0–1.2 mg/mL	–
Nifedipine	Adalat	0.4 μg/mL (1.2 μM)	–
Pioglitazone hydrochloride	Actos	27 μg/mL	–
Piroxicam	Feldene	60 μg/mL (181 μM)	–
Pravastatin	Pravachol	48 μg/mL	–
Salicylic acid	–	0–599 μg/mL (4.3 mM)	Interfered > 185 μg/mL (1.3 mM)
Simvastatin	Zocor	48 μg/mL	–

Test concentrations were obtained from EP7-A2, Appendix C, where available.
[a] Test concentration range reflects added albumin (does not include endogenous albumin).

clopidogrel is an inactive pro-drug that is rapidly converted to the active form (30–60 min) after administration [40]. The clopidogrel metabolites do not contain methyl groups that would arise within the lipid region of the NMR spectrum nor are they hydrophobic enough to bind to the lipoprotein particles and affect their structures. Therefore, they are unlikely to interfere with lipoprotein particle quantitation. Unfortunately, the clopidogrel metabolites were not available for testing at the time this study was conducted. Furthermore, the therapeutic range for clopidogrel was calculated using 600 mg as the worst case scenario. The FDA approved loading dose, suggested for treatment of patients with unstable angina and non-STEMI, is 300 mg and the routine daily dose is 75 mg. Given that the majority of the drug is rapidly metabolized to its active form and lower daily maintenance dose, the ≥201 mg concentration of the inactive parent compound is not likely to be present in specimens collected from patients routinely taking clopidogrel. Similarly, the recommended daily doses of aspirin are 81–160 mg for CVD and 325–650 mg for various other indications. The results of this study revealed that a patient would have to achieve a circulating concentration of salicylic acid, the active form of aspirin, ≥929 mg in order to elicit an effect on the LDL-P results. The reported therapeutic

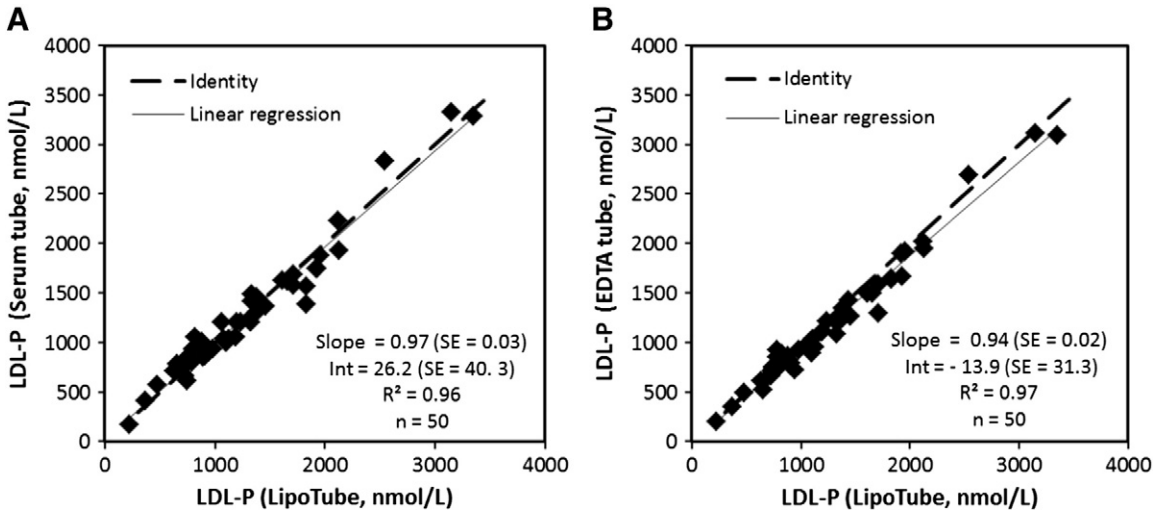


Fig. 3. Comparison of collection tubes for LDL-P. A) LDL-P results for BD Vacutainer serum tubes (no gel barrier) compared to the LipoTube (gel barrier). B) LDL-P results for EDTA plasma tubes compared to the LipoTube (gel barrier).

Table 3
Stability of LDL-P measured by NMR assay on the Vantera Clinical Analyzer.

Day	N	Mean LDL-P (nmol/L)	%bias
0	30	1210	n/a
1	30	1210	0.0
2[a]	20	1201	2.2
3[a]	20	1234	5.0
4	30	1314	8.6
5	30	1295	7.0
6	30	1323	9.3
7[b]	29	1337	10.2

[a] Days 2 & 3 not observed in 1 of 3 studies (n = 20). %bias calculated relative to mean day 0 LDL-P of 1175 nmol/L.
[b] Day 7 missing 1 subject due to short draw (n = 29). %bias calculated relative to mean day 0 LDL-P of 1213 nmol/L.

range for salicylic acid encompasses topical acne treatment. Therefore, patients are not likely to experience circulating salicylic acid concentrations ≥929 mg with routine, daily aspirin or topical salicylic acid treatment. In conclusion, although in vitro testing in this study indicated some sources of potential interference, this does not prohibit reliable LDL-P quantitation since most interferences were observed at concentrations above the therapeutic window or were not likely to affect NMR spectra obtained from patients taking normal therapeutic doses of these substances.

The successful development of a method to measure LDL-P on a fully automated platform allows NMR technology dissemination into the routine, clinical laboratory setting and creates the opportunity for NMR-based testing across a broader range of clinical applications. In fact, several leading national reference laboratories and large hospital system laboratories have successfully integrated the Vantera into their clinical lab operations. Current efforts are focused on expanding the menu of NMR-based tests that are available for physicians to make informed decisions on patient care, especially in an era when personalized medicine, using tests that are inexpensive and widely available, is becoming highly desirable.

Conflicts of interest

All authors are employees of LipoScience, Inc.

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