

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

k063841

B. Purpose for Submission:

New Test System (laboratory service)

C. Measurand:

LDL-particle number, HDL cholesterol, triglycerides

D. Type of Test:

Nuclear magnetic resonance (NMR)

E. Applicant:

Liposcience Inc.

F. Proprietary and Established Names:

NMR Lipoprofile Assay

G. Regulatory Information:

1. Regulation section: Lipoprotein test system 21 CFR 862.1475
Cholesterol test system 21 CFR 862.1175
Triglyceride test system 21 CFR 862.1705
Quality control material 21 CFR 862.1660
Calibrator 21 CFR 862.1150
2. Classification: 862.1475, 862.1175, 862.1705, 862.1660: Class I, subject to limitation of exemptions (21 CFR 862.9(c)(4)); 862.1150, Class II
3. Product code: JJY (control material), CDT (triglyceride), LBS (hdl- cholesterol)
JIT (calibrator), MRR (low density lipoprotein)
4. Panel: Chemistry, 75

H. Intended Use:

1. Intended use(s):

The NMR LipoProfile[®] test, when used with the NMR Profiler, an automated NMR spectrometer, measures lipoprotein particles to quantify LDL particle number (LDL-P), HDL cholesterol (HDL-C), and triglycerides in serum and plasma using nuclear magnetic resonance (NMR) spectroscopy. LDL-P and these NMR-derived concentrations of triglycerides and HDL-C are used in conjunction with other lipid measurements and clinical evaluation to aid in the management of lipoprotein disorders associated with cardiovascular disease. The test is performed and provided as a service by Liposcience Laboratory.

2. Indication(s) for use:

See intended use, above.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

This device is cleared as a service (in-house). Samples are sent to Liposcience Laboratories to be run on the Lipoprofile II NMR, using the system's software.

I. Device Description:

The test system includes a 400 MHz proton NMR spectrometer interfaced with a commercial sample handler, an analysis server containing the software to analyze digitized spectral data, and the following reagents:

NMR Diluent - aqueous solution containing Na₂EDTA (5.0mM), CaCl₂ (1.0mM), KCL (120mM), Na₂HPO₄ (50mM), NaN₃ (0.02%), pH 7.4; NMR Wash - Triton X-100-0.1%v/v, Liqui Nox 0.1% v/v in deionized water, pH 10.0; NMR Calibrator - aqueous solution of Trimethyl Acetate (TMA) disodium salt (15.0 mM) containing Na₂EDTA (5.0 mM), CaCl₂ (3.0 mM), KCl (120 nM), D₂O 10% v/v; NMR LipoProfile Quality Control material - two levels of pooled human serum-based control material, (Control A and Control B), with pre-determined target ranges.

The control materials contain human source material. Each donor unit is tested by FDA – approved methods and found non-reactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C, and antibody to HIV-1/2, all products using human source material should be handled as potentially infectious, because no test method can offer complete assurance that infectious agents are absent. Products should be handled according to established good laboratory practices.

J. Substantial Equivalence Information:

1. Predicate device name(s):

LDL-Cholesterol: k020724, Dimension Automated LDL Cholesterol Flex Reagent
Triglycerides: k971324, Carolina Liquid Chemistries
HDL-Cholesterol: k971126, Genzyme, Liquid N-geneous HDL Cholesterol Kit
Quality controls materials: k993606 Carolina Liquid Chemistries

2. Predicate 510(k) number(s):

k020724, k971324, k971162, k993606

3. Comparison with predicate:

The device is similar in indications to the predicate assays, i.e., to quantify lipoprotein, HDL cholesterol (HDL-C), and triglycerides in serum and plasma to aid in the management of lipoprotein disorders associated with cardiovascular disease. However, the test principles, technology, and one of the measurands differ between the devices. This device measures NMR signals and uses an algorithm developed by the manufacturer, to calculate concentrations of LDL particle number, high density lipoprotein cholesterol, and triglycerides. The predicate devices utilize colorimetric enzyme assay technology on chemistry analyzers. In addition, the predicate device reports LDL-cholesterol, this device reports LDL-particle number. The predicate device is for use by clinical laboratories on clinical chemistry analyzers. Specimens to be tested with Lipoprofile are sent to Liposcience laboratories for measurement and analysis using the manufacturer's system.

K. Standard/Guidance Document Referenced (if applicable):

1. CLSI. *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. CLSI document EP6-A, 2003.
2. CLSI. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition*. CLSI document EP9-A2, 2002.

L. Test Principle:

The test system involves measurement of the 400 MHz proton NMR spectrum of a plasma or serum sample, deconvolution of the composite signal at ~0.8 ppm to produce the signal amplitudes of the lipoprotein subclasses that contribute to the composite plasma signal, and conversion of these subclass signal amplitudes to lipoprotein subclass concentrations. The 0.8 ppm plasma NMR signal arises from the methyl group protons of the lipids carried in the VLDL, LDL, and HDL subclasses of varying diameter. The NMR signals from the various lipoprotein subclasses have unique and distinctive frequencies and lineshapes, each of which are accounted for in the deconvolution analysis model. LDL subclass particle concentrations, in units of nanomoles of particles per liter (nmol/L), are summed to give the reported total LDL particle concentration (LDL-P). By employing conversion factors that assume that the various lipoprotein subclass particles have cholesterol and triglyceride contents characteristic of normolipidemic individuals, HDL cholesterol and triglyceride concentrations are also derived.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All analytical studies described below were performed on patient samples or pools.

a. Precision/Reproducibility:

Within-run precision:

Two pools of samples were analyzed in 20 replicates on the same run. The observed within-run precision is shown below.

	<u>Pool 1 -</u>			<u>Pool 2 -</u>		
	<u>TG</u> <u>(mg/dL)</u>	<u>HDL-C</u> <u>(mg/dL)</u>	<u>LDL-P</u> <u>(nMol/L)</u>	<u>TG</u> <u>(mg/dL)</u>	<u>HDL-C</u> <u>(mg/dL)</u>	<u>LDL-P</u> <u>(nMol/L)</u>
Mean	<u>188.8</u>	<u>41.4</u>	<u>2221.8</u>	<u>74.6</u>	<u>57.1</u>	<u>1041.8</u>
SD	<u>2.0</u>	<u>0.5</u>	<u>49.1</u>	<u>1.2</u>	<u>0.4</u>	<u>47.7</u>
CV%	<u>1.1</u>	<u>1.3</u>	<u>2.2</u>	<u>1.5</u>	<u>0.7</u>	<u>4.6</u>

Inter-assay precision:

The same pools used to test within-run precision were run on 20 different runs over 4 days. Results are shown below:

	<u>Pool 1 -</u>			<u>Pool 2 -</u>		
	<u>TG</u> <u>(mg/dL)</u>	<u>HDL-C</u> <u>(mg/dL)</u>	<u>LDL-P</u> <u>(nMol/L)</u>	<u>TG</u> <u>(mg/dL)</u>	<u>HDL-C</u> <u>(mg/dL)</u>	<u>LDL-P</u> <u>nMol/L)</u>
Mean	<u>219.1</u>	<u>42.4</u>	<u>1924.8</u>	<u>79.5</u>	<u>55.5</u>	<u>1052.9</u>
SD	<u>2.92</u>	<u>1.17</u>	<u>66.7</u>	<u>1.7</u>	<u>0.9</u>	<u>68.4</u>
CV%	<u>1.33</u>	<u>2.75</u>	<u>3.5</u>	<u>2.1</u>	<u>1.5</u>	<u>6.5</u>

Precision near the upper limits of the assay:

In addition, 3 serum pools were prepared, each having a concentration at least as high as the upper limit of analytical measurement range for each analyte. Each pool was analyzed in 10 separate runs (conducted on 10 separate NMR analyzers) on a single day. Observed percents CV ranged from 2-4%.

b. Linearity/assay reportable range:

Two pools (one high and one low) were prepared from patient samples. The target values of the high and low pools were derived from the averaged values of 6 different runs. The

high and low pools were then mixed and diluted in different proportions to produce multiple HDL-C, triglyceride, and LDL-P levels. Preparations were kept at 2-8°C, and analyzed on 6 different runs. Results are tabulated below.

HDL-C:

Target Value (mg/dL)	7.0	14.1	28.1	29.7	32.0	36.0	47.7	67.4	87.0	106.7	160.0
Observed Average	7.0	13.9	28.4	29.5	32.1	36.0	47.7	66.4	86.1	106.2	161.1
%bias	-0.4	-1.1	1.0	-0.7	0.1	0.2	-0.1	-1.4	-1.1	-0.5	0.7

Triglycerides:

Target value (mg/dL)	5.4	10.9	21.8	43.6	79.5	133.4	223.2	492.7	941.8	1390.9
Observed average	5	11.3	22.1	48.6	88	143.1	232	513.7	964.9	1414
% bias	-8.8	3.6	1.4	11.4	10.6	7.3	3.9	4.3	2.4	1.7

LDL-P:

Target value (nMol/L)	297.3	594.6	663.0	765.6	936.6	1450	2305	3160	4015
Observed average	300.6	594.4	624.0	799.6	959.0	1622	2498	3306	4074
% Bias	1.11	-0.03	-5.87	4.44	2.39	11.91	8.37	4.63	1.48

Reportable ranges, based on the lower limit of detection and validation by method comparison are:

LDL-P: 300-3500 nmol/L

HDL-C: 7-140 mg/dL

Triglycerides: 5-1100 mg/dL

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

TMA (Trimethylacetic acid, Sodium salt) is used as the NMR calibrator for the NMR clinical analyzers. TMA is used routinely as a calibrator once daily during instrument startup to establish daily normalization factors. It also serves as a quality assessment tool to ensure quality NMR spectra are produced by the NMR analyzer. New calibrator material is run in parallel with the existing calibrator in five separate runs.

The stability of the TMA calibrator material and storage conditions was studied over an 18-month period. The TMA calibrator was stored at room temperature and at 4 deg C, in both plastic vials and glass bottles. Aliquots were taken and TMA signal methyl integrals were measured once every two months during the 18 month study period. There were no significant changes in the integral of the stored TMA material during the study period for the different storage conditions. The coefficient of variation over the study period (where

measurements were made in 6 different NMR analyzers) for the TMA integrals were less than 1%.

Control material consists of human serum at two levels (A and B). To assign values, new controls are run on every available NMR clinical analyzer in-house during a two week period in parallel with the then existing controls. Means, Standard Deviations and %CVs are computed and new targets are established.

Stability for control material over 17 months was determined. The percents deviation, of observed mean values relative to the target mean values, for control materials across multiple NMR analyzers were under 5%.

d. Detection limit:

Serial dilutions of sample pools as well as a blank solution (NMR diluent) were run on 10 different instruments, two times each per instrument to collect 20 data points. Data were compiled and analyzed to determine bias and %CV for each dilution.

The lower limits of the reportable range, defined by the limits of detection, are: LDL-P: 300-nmol/L; HDL-C: 7 mg/dL , Triglycerides: 5 mg/dL.

The limits of quantitation, determined to achieve total error < 20%, are: LDL-P: 300-nmol/L; HDL-C: 10 mg/dL , Triglycerides: 25 mg/dL.

e. Analytical specificity:

Twenty drugs representing classes of five major categories of medications that are likely to be taken by the target population were selected and evaluated for their possible interference with the NMR assay. Pure drugs were purchased and prepared in solutions which were spiked into 6 different pools of plasma samples to attain levels similar to the highest possible blood concentrations as shown in the following table. Mean percent differences for each substance were <5%.

Drugs	Concentration (uMol/L)
Simvastatin	30
Fenofibrate	70
Nicotinic acid Sod salt	2200
Acetylsalicylic acid	880
Acetaminophen	1050
Naproxen Sod	470-
Ibuprofen	550
Piroxicam	10
Hydrochlorothiazide	27
Triamterene	31
Furosemide	38
Metoprolol	23
Nifedipine	37

Enalapril maleate	13
Hydralazine	60
Isosorbide dinitrate	80
Clopidogrel	28
Glipizide	7
2,4- Thiazolidinedione	61

Potential Interferent	Concentration
Bilirubin	20 mg/dL
Creatinine	20 mg/dL
Hemoglobin	500 mg/dL
Urea	300 mg/dL
Uric acid	40 mg/dL

f. Assay cut-off:

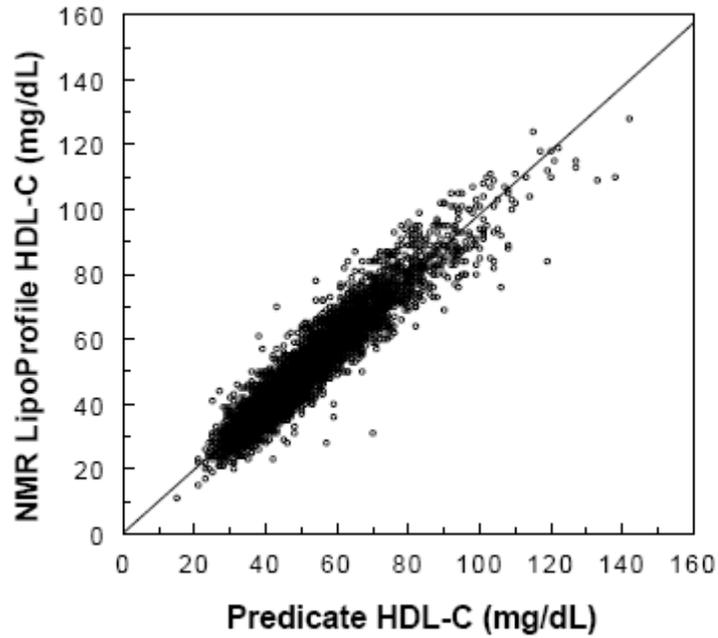
Not applicable; this is a quantitative assay.

2. Comparison studies:

a. Method comparison with predicate device:

HDL-C and triglycerides were evaluated by split sample comparison to the predicate devices. Samples were from apparently healthy men and women, ages ranging from 44-84 (mean 61). Points on the plot, as well as calculations of correlation coefficients and standard errors are based on singlicate measurements. The HDL-C analysis includes 5362 plasma samples with values ranging from 15 to 140 mg/dL. The triglyceride analysis includes the same 5362 samples with triglyceride values ranging from 21 to 400 mg/dL.

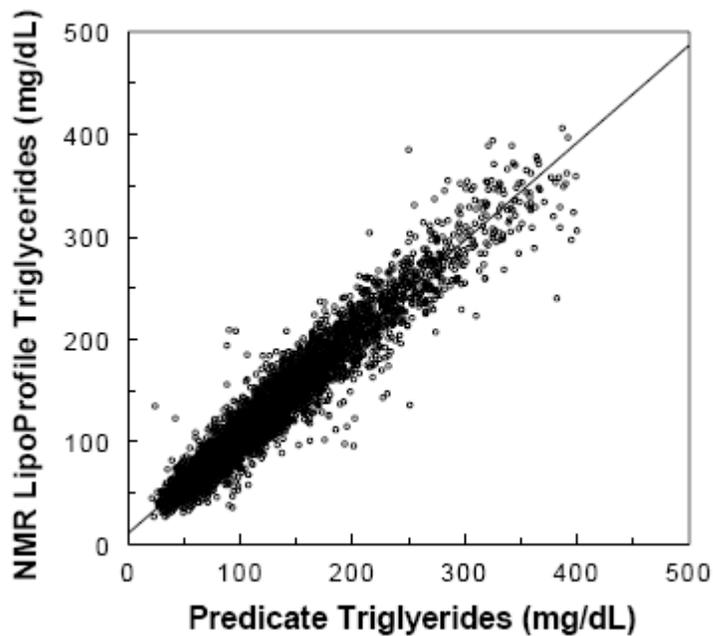
HDL-C:



Regression Statistics

	Coefficient	Standard Error	Lower 95%	Upper 95%	R ²
					0.897
Intercept	0.2577	0.2430	-0.2283	0.7437	
X Variable	0.9828	0.0045	0.9737	0.9918	

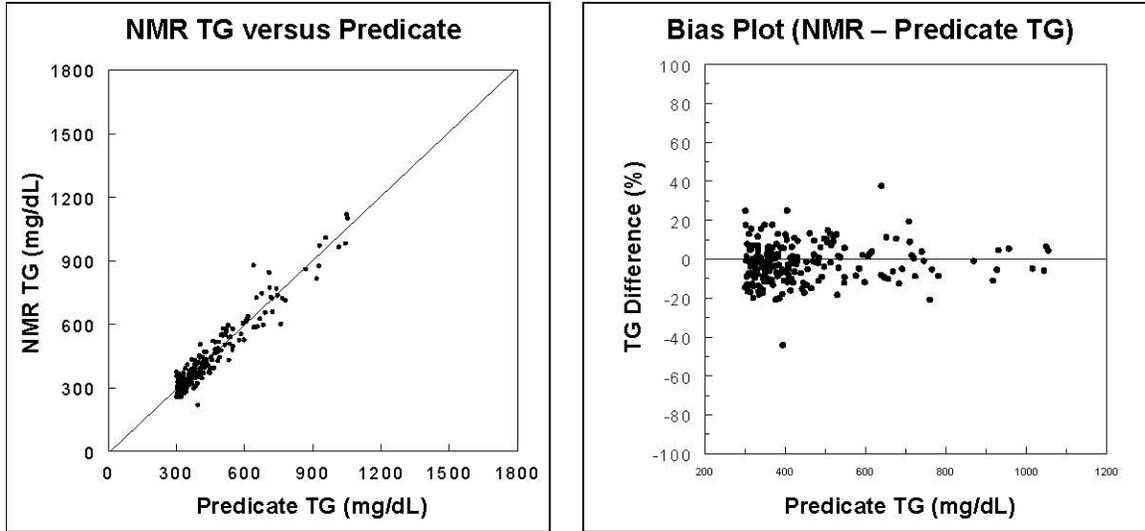
Triglycerides:



Regression Statistics

	Coefficient	Standard Error	Lower 95%	Upper 95%	R ²
					0.929
Intercept	10.7278	0.5026	9.7226	11.7329	
X Variable	0.9524	0.0036	0.9452	0.9596	

An additional method comparison study for triglycerides was performed to evaluate samples across the entire assay reportable range. The study evaluated 199 patient specimens. Results are shown below.



Regression Statistics

	Coefficient	Standard Error	Lower 95%	Upper 95%	R ₂
X Variable	1.01	0.02	0.97	1.05	0.924
Intercept	-12.56	9.93	-32.42	7.29	

b. Matrix comparison:

The various specimen tubes recommended by the manufacturer for use with this assay are serum drawn in gel barrier NMR LipoTubes (Greiner, Inc. Part #456293), or red-top collection tubes; or plasma drawn into EDTA or heparin collection tubes. Three studies were conducted to validate the equivalence of NMR LipoProfile results using these serum and plasma specimens. In all 3 studies, blood was collected in the specified collection tubes, centrifuged within 1 hour, and immediately tested. The study comparing serum (red-top) to EDTA plasma tested individual specimens from 38 volunteers, the study comparing serum (red-top) to serum (NMR LipoTube) tested individual specimens from 20 volunteers, and the study comparing serum (NMR LipoTube) to heparin plasma tested individual specimens from 35 volunteers. Samples tested spanned most of the assay range. Average biases observed were less than 5% for triglycerides and HDL-C, and

ranged between 4-8% for LDL-P. No concentration-dependent trends were observed.

3. Clinical studies:

a. Clinical Sensitivity:

See section c, below for clinical study descriptions.

b. Clinical specificity:

See section c, below for clinical study descriptions.

c. Other clinical supportive data (when a. and b. are not applicable):

Since there is not a predicate device that measures LDL-P, descriptions and results of 3 clinical studies were provided. (Literature references for these studies are listed in the product package insert).

Study 1

Samples from the nested case-controlled EPIC study (the European prospective Investigation into Cancer and Nutrition) were evaluated. The study population included men and women, inhabitants of Norfolk, UK, aged 45-79 years. Non-fasting serum samples were collected at the beginning of the study (baseline). Participants were followed for 6 years for the development of CAD, defined as CAD death, MI and angina. The samples tested by the Lipoprofile assay were from cases (n=1003) who developed CAD during the 6 year follow-up period and controls (n=1885) matched for age, gender, and enrollment time who did not develop CAD. None of the cases or controls were taking any lipid altering medication.

Conditional logistic regression was used to calculate odds ratios for future (incident) CAD, adjusted for smoking and blood pressure. Odds ratios for development of CAD by quartiles are shown in the table below.

	Quartiles				p [§]
	1	2	3	4	
LDL-P					
Range (nmol/L)	<1278	1278-1525	1526-1812	>1812	
Univariable Odds Ratio (95% CI)*	1.00	1.23 (0.97-1.56)	1.48 (1.17-1.87)	2.00 (1.58-2.59)	<0.0001
Multivariable Odds Ratio (95% CI)**	1.00	1.13 (0.89-1.44)	1.21 (0.94-1.54)	1.37 (1.04-1.83)	0.02

*Univariable odds ratios and 95% confidence intervals were calculated by conditional logistic regression, taking into account matching for gender, age, and enrollment time and adjusted additionally for smoking and systolic blood pressure. **Multivariable odds ratios were from a model adjusted additionally for HDL-C and triglycerides. §p value for linear trend.

Study 2:

Samples from a randomized, double-blind, placebo-controlled trial were evaluated by the Lipoprofile assay. Participants in the study were apparently-healthy female health-care professionals, aged 45 years and older, who were free of self-reported CVD. Evaluations were conducted on 27,673 baseline non-fasting EDTA plasma samples from participants. During the study participants were followed for incident CVD, which included non-fatal MI, percutaneous coronary intervention, coronary artery bypass grafting, non-fatal ischemic stroke, and cardiovascular death. Over the (mean) follow-up period of 11 years, 1015 CVD events occurred. Odds ratios associated with quartiles for LDL-P observed in this study are shown below.

	Quintiles					p [§]
	1	2	3	4	5	
LDL-P						
Range (nmol/L)	<963	963-1165	1166-1387	1388-1703	>1703	
HR (95% CI)*	1.00	1.37 (1.01- 1.85)	1.35 (1.01- 1.81)	1.80 (1.36- 2.38)	2.51 (1.91- 3.30)	<0.001

*Hazard ratios and 95% confidence intervals were calculated by Cox proportional hazard regression models adjusted for age, randomized treatment assignment, smoking status, menopausal status, postmenopausal hormone use, blood pressure, diabetes, and body mass index. §p value for linear trend.

Study 3:

Samples from men enrolled in the prospective case-controlled Veterans Affairs HDL Intervention Trial (VA-HIT) were evaluated using the Lipoprofile assay. Fasting

plasma samples were collected at baseline and after 7 months from men younger than 74 years (mean age 64.2) with an established diagnosis of CHD and low levels of LDL-C and HDL-C. Case subjects were men who experienced a CHD event (cardiac death or non-fatal MI) during the mean 5.1 year follow-up period. Control subjects were men matched for age who remained free of CHD events during follow-up. There were 354 CVD events and 697 age-matched controls. Samples from these individuals were evaluated as described below.

Logistic regression models were used to evaluate the associations of CHD events with concentrations of LDL-P measured at baseline and during the trial (7 month time point), adjusted for non-lipid covariates. The table below shows odds ratios for a new CHD event associated with a 1-standard deviation (SD) increment of LDL-P in the subjects treated with placebo (n=546).

	<i>Baseline</i>	<i>On-Trial</i>
LDL-P		
Odds Ratio (95% CI)*	1.31 (1.09-1.57)	1.19 (0.99-1.43)
p value	0.004	0.054

*Odds ratios and 95% confidence intervals were calculated for a 1-SD increment of LDL-P using logistic regression models adjusted for age, hypertension, smoking, body mass index, and diabetes.

4. Clinical cut-off:

See expected values, below.

5. Expected values/Reference range:

In order to determine the distribution of LDL-P levels expected in a representative sampling of the general population, plasma samples (n=5,362) were analyzed from apparently healthy men and women (mean age 61, ranging from 44 to 84 years) enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA), a large epidemiologic study sponsored by the National Heart, Lung, and Blood Institute. This reference population consisted of men (n=2,529) and women (2,833) with the following ethnic make-up: 1467 African-American (27.4%); 2039 Caucasian (38.0%); 658 Chinese (12.3%); and 1198 Hispanic (22.3%). The following table provides the concentrations of LDL-P by percentile in this reference population:

Distribution of LDL-P Observed in a Reference Population - Multi-Ethnic Study of Atherosclerosis (MESA)

Percentile	All (n=5362)	Men (n=2529)	Women (n=2833) LDL-P
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	LDL-P (nmol/L)	LDL-P (nmol/L)	(nmol/L)
5	770	800	760
10	870	900	850
20	1000	1040	970
30	1100	1150	1060
40	1190	1250	1150
50	1280	1330	1230
60	1380	1430	1330
70	1480	1530	1440
80	1610	1640	1570
90	1790	1820	1760
95	1980	1990	1970

HDL Cholesterol and Triglycerides

The following reference values for patient classification have been recommended by the NCEP for HDL cholesterol and triglycerides for the assessment and management of CVD risk. Each laboratory should verify the validity of these reference values for the population it serves.

HDL Cholesterol, mg/dL Classification	
<i>Low</i>	<i>High</i>
<40	≥60

Triglycerides, mg/dL Classification			
<i>Normal</i>	<i>Borderline-high</i>	<i>High</i>	<i>Very high</i>
<150	150-199	200-499	≥500

N. Instrument Name: NMR Profiler

O. System Descriptions:

1. Modes of Operation:

The NMR Profiler is a 400 MHz proton nuclear magnetic resonance spectrometer.

2. Software:

The digitized spectrum is stored in computer memory and the Lipoprofile analysis software performs fitting (deconvolution), determines amplitudes and converts these to particle number or concentration units.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes

3. Specimen Identification:

Bar code of source tube

4. Specimen Sampling and Handling:

The NMR Profiler is interfaced with a sample handler. Samples are manually placed in the sample handler rack, in the same sequence as bar coded source tubes. Source tubes are sequentially scanned by the Liposcience laboratory technician. This information is stored in the database and in a file required as input to the NMR Profiler. Each spectrum is stored in the file system in a manner that preserves the identity of the sample from which the spectrum was collected.

5. Calibration:

The instrument is calibrated with an aqueous solution of Trimethyl Acetate (TMA) a disodium salt (15.0 mM) containing Na₂EDTA (5.0 mM), CaCl₂ (3.0 mM), KCl (120 nM), D₂O 10% v/v.

6. Quality Control:

NMR LipoProfile Quality Control material include two levels of pooled human serum-based control material, with pre-determined target ranges.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.