510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k111516

B. Purpose for Submission:

Modification of a previously cleared device to change software architecture for NMR LipoProfile Test (LDL-P, HDL-C and triglycerides) and NMR curve fit algorithm for LDL-P

C. Measurand:

LDL-P (low density lipoprotein particle number), HDL cholesterol, triglycerides

D. Type of Test:

Nuclear magnetic resonance (NMR)

E. Applicant:

LipoScience Inc.

F. Proprietary and Established Names:

NMR LipoProfile Test

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRR	I, meets limitations per 21 CFR 862.9 (c)(4)	21 CFR §862.1475 – Lipoprotein test system	Chemistry (75)
LBS	I, meets limitations per 21 CFR 862.9 (c)(4)	21 CFR §862.1175 – Cholesterol test system	Chemistry (75)
CDT	I, meets limitations per 21 CFR 862.9 (c)(4)	21 CFR §862.1705 – Triglyceride test system	Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for use:

   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 human serum and plasma using nuclear magnetic resonance (NMR) spectroscopy. LDL-P and these NMR-derived concentrations of HDL-C and triglycerides are used in conjunction with other lipid measurements and clinical evaluation to aid in the management of lipoprotein disorders associated with cardiovascular disease. This test is performed and provided as a service by LipoScience Laboratory.

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 NMR Profiler, using software version Gen2.1.

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 Na_2EDTA (5.0mM), $CaCl_2$ (1.0mM), KCL (120mM), Na_2HPO_4 (50mM), NaN_3 (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 Na_2EDTA (5.0 mM), $CaCl_2$ (3.0 mM), KCl (120 nM), D_2O 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.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   NMR Profiler and NMR Lipoprofile Assay

2. Predicate K number(s):

   k063841

3. Comparison with predicate:

Similarities		
Item	Predicate device (k063841)	Proposed device
Indications for use	Measures lipoprotein particles to quantify LDL particle number (LDL-P), HDL cholesterol (HDL-C), and triglycerides in human serum and plasma using nuclear magnetic resonance (NMR) spectroscopy. LDL-P and these NMR-derived concentrations of HDL-C and triglycerides are used in conjunction with other lipid measurements and clinical evaluation to aid in the management of lipoprotein disorders associated with cardiovascular disease.	Same
Specimen type	Serum and plasma	Same
Reference range study	5^{th} through 95^{th} percentile values for LDL-P obtained for samples from the Multi-Ethnic Study of Athlerosclerosis	Same (Ranges from predicate transferred to new device via CLSI Guideline C28-A2; see section M5 below)

Differences		
Item	Predicate device (k063841)	Proposed device
Software version that includes algorithm used to curve fit to the NMR peak and calculate the LDL-P results and software architecture	LP2	Gen2.1
Software architecture	Calculation of all three analytes (LDL-P, HDL concentration and triglycerides) performed by one algorithm in one software module. Changes to the algorithm result in changes in calculations for all analytes.	Modular (separate software modules for each analyte that allows for modules to change and new modules to be added with no impact to the signal generation or to other unmodified analytes)

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

- CLSI Guideline EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods*
- CLSI Guideline EP 17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*
- CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*
- CLSI Guideline EP9-A2*: Method Comparison and Bias Estimation Using Patient Samples*
- CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry*
- CLSI Guideline C28-A2*: How to Define and Determine Reference Intervals in the Clinical Laboratory*

L. Test Principle:

The *NMR LipoProfile*® test and NMR Profiler involves measurement of the 400 MHz proton NMR spectrum of a plasma/serum sample, deconvolution of the composite signal at approximately 0.8 ppm to produce signal amplitudes of the lipoprotein subclasses that contribute to the composite plasma/serum 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 LDL, HDL and VLDL subclasses of varying diameters. The NMR signals from the various lipoprotein subclasses have unique and distinctive frequencies and line shapes, each of which is accounted for in the deconvolution analysis model. Each subclass signal amplitude is proportional to the number of subclass particles emitting the signal, which enables subclass particle concentrations to be calculated from the subclass signal amplitudes derived from the spectral deconvolution analysis. 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 assuming 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:

   a. Precision/Reproducibility:

   Within-run and within-laboratory precision were evaluated in accordance with the methods defined in the CLSI Guideline EP5-A, "Evaluation of Precision Performance of Clinical Chemistry Devices."

   Three serum pools with different analyte concentrations were prepared and tested in multiple runs over multiple days. For the Within-Lab, multiple instruments, operators and reagent lots were incorporated into the testing and a variance component analysis was conducted to estimate the individual sources of the total system variability.

   Within-run precision - A single run of 20 replicates for the low and the medium pool was conducted on one instrument and a single run of 20 replicates for the high pool was conducted on a second NMR instrument. A single operator conducted all three runs.

LDL-P (nmol/L)

Pool	N	Mean	SD	SD 95%CI	CV
Low	20	908	45.4	34.5 – 66.3	5.0%
Medium	20	1493	64.8	49.3 – 94.7	4.3%
High	19*	1967	72.8	55.0 – 107.6	3.7%

*One replicate of the high pool produced no data due to instrument failure (sample delivery failure) and therefore only 19 replicates were analyzed.

HDL-C (mg/dL)

Pool	N	Mean	SD	SD 95%CI	CV
Low	20	23.7	0.5	0.4 - 0.7	2.0%
Medium	19*	54.9	1.0	0.8 - 1.5	1.9%
High	20	95.1	0.9	0.6 - 1.2	0.9%

Triglycerides (mg/dL)

Pool	N	Mean	SD	SD 95%CI	CV
Low	20	81.0	2.1	1.6 - 3.1	2.6%
Medium	19*	140.6	2.5	1.9 - 3.7	1.8%
High	20	649.5	8.7	6.6 - 12.7	1.3%

* One replicate of the medium pool produced no data due to an instrument failure (sample delivery failure) and therefore, only has 19 replicates analyzed.

Within-laboratory precision – Two runs per day with two replicates per run for three pools were tested on three instruments for a total of 20 testing days. Five operators conducted the study over the 20 testing days. Additionally, six lots of NMR Diluent 1 were used over the 20 days of testing.

LDL-P (nmol/L):

Pool	N	Mean	SD	SD 95%CI	CV
Low	240	932.6	64.6	58.8 - 71.7	6.9%
Medium	240	1539.3	86.9	77.3 – 99.3	5.6%
High	240	2046.1	111.8	99.3 – 127.9	5.5%

HDL-C (mg/dL):

Pool	N	Mean	SD	SD 95%CI	CV
Low	240	23.5	0.9	0.8 – 1.0	3.9%
Medium	240	56.5	1.3	1.2-1.6	2.4%
High	240	96.2	2.5	2.1 – 2.9	2.6%

Triglycerides (mg/dL):

Pool	N	Mean	SD	SD 95%CI	CV
Low	240	78.0	2.9	2.6 – 3.3	3.7%
Medium	240	146.0	4.2	3.6 – 4.9	2.8%
High	240	627.5	20.7	17.7 – 24.8	3.3%

b. Linearity/assay reportable range:

The claimed measuring ranges of this device are:

LDL-P: 300 - 3500 nmol/L

HDL-C: 7 - 140 mg/dL

Triglycerides: 5 - 1100 mg/dL

LDL-P:

The dilution series consisted of eleven samples that were prepared by combining selected de-identified, residual specimens from LipoScience's commercial laboratory. The samples covered the range from 184 nmol/L to 3868 nmol/L. All the pools were run on the same day in 4 replicates. The study was conducted in a single run on one instrument. Significance of the terms for both the linear and polynomial regression was examined.

R=0.9987, R^2=0.9974			
Linear Term	Coefficient	SE	p
Intercept	-11.9944	19.4903	0.5424
X	0.9843	0.0086	< 0.0001
R=0.9990, R^2=0.9980			
Non-Linear Term	Coefficient	SE	p
Intercept	39.4779	23.4440	0.1016
X	0.8929	0.0293	< 0.0001
X^2	0.0000	0.0000	0.0027

In the polynomial regression, the X^2 term was significant (p=0.0027). Given the significance of the nonlinear term, acceptance criteria for allowable nonlinearity were evaluated. Samples 5-11 met the sponsor's pre-determined allowable nonlinearity criteria (10%). The low concentration samples 2-3, compared to the absolute nonlinearity criteria of 52.1 nmol/L, meet the sponsor's allowable nonlinearity criteria. Therefore the linearity observed for LDL-P in this study supports the claimed measuring range of 300 to 3500 nmol/L.

HDL-C and triglycerides: Twelve concentration levels were prepared across the range of the assays by serial dilutions of the source pools and tested. The study was conducted in a single run on one instrument and each level was tested in replicates of four. For HDL-C, the samples covered the range of 6.3 – 205 mg/dL; for triglycerides the samples covered the range 5.4 – 1880 mg/dL. Linear regression analysis yielded the following results:

HDL-C:
R^2 = 0.9998
y = 1.004x - 0.5956

Triglycerides:
R^2 = 0.9998
y = 1.006 + 2.057

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

Reagents, calibrators and controls are unchanged from the previously cleared

device (k063841).

d. Detection limit:

The lower ends of the measuring ranges for LDL-P, HDL-C and triglycerides were determined by linearity, above in 2b, and unchanged from the predicate (k063841):

LDL-P: 300 mmol/L

HDL-C: 7 mg/dL

Triglycerides: 5 mg/dL

e. Analytical specificity:

A new interference study was performed to evaluate whether substances could be potential interferents of the device and its modified software. In addition, two new therapeutics for the intended use population became available and were also evaluated in the new study.

In order to evaluate potential interferents with the new version of the LDL-P software Gen2.1, LipoScience employed the following study design: first, identify potentially interfering substances that show resonance(s) in the vicinity of lipoprotein resonances. Each potential interferent was diluted in the appropriate solvent and analyzed on the NMR Profiler to determine if it demonstrated peak(s) in the 0.7 – 1.0 ppm region. Five endogenous substances and 22 drugs were screened in this step using concentrations in accordance to CLSI EP7-A2.

Second, once a potentially interfering substance was identified, a spiking study was completed where the substance was added to sample pools containing two different levels of LDL-P, HDL-C and triglycerides for a paired difference test.

Endogenous		*Exogenous (OTC drugs, etc.)*	
Potential Interferent	***Test Concentration***	***Potential Interferent***	***Test Concentration***
Hemoglobin	2 mg/mL	Simvastatin	48 µg/mL
Bilirubin, unconj.	200 µg/mL	Fenofibrate	45 µg/mL
Creatinine	50 µg/mL	Nicotinic Acid Sodium salt	1.2 mg/mL
Urea	2.6 mg/mL	Acetylsalicylic acid	652 µg/mL
Uric acid	235 µg/ml	Acetaminophen	200 µg/mL
		Naproxen Sodium	547 µg/mL
		Ibuprofen Sodium salt	210[1], 70[2] µg/mL
		Piroxicam	60 µg/mL
[1]		Hydrochlorothiazide	6.0 µg/mL
		Triamterene	8.9 µg/mL
		Pravastatin	48 µg/mL
		Furosemide	60 µg/mL
		Metoprolol tartrate	6.4 µg/mL
		Nifedipine	400 µg/mL
		Enalaprilat Dihydrate	0.3 µg/mL
		Hydralazine hydrochloride	180 µg/mL
		Isosorbide dinitrate (lactose mixture 2:3)	375 µg/mL
		Clopidogrel hydrogensulfate	360 µg/mL
		Glipizide	2.0 µg/mL
		Metformin Hydrochloride	600 µg/mL
		Pioglitazone hydrochloride	27 µg/mL
		Atorvastatin	48 µg/mL

[1] For LDL-P and HDL concentration
[2] test results for triglycerides were ~4 to 6% lower at the 140 and 210 µg/mL concentrations of ibuprofen

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

LDL-P: The comparison was conducted in agreement with CLSI EP9-A2: *Method Comparison and Bias Estimation Using Patient Samples*. The study involved testing singlicates followed by statistical analysis of 1600 freshly-collected clinical de-identified specimens across the range of the LDL-P test. Bias was calculated for LDL-P from the linear regression line at the medical decision limits of 1000, 1300 and 1600 nmol/L as well. 1555 samples are included in the final analysis (44 samples were outside of the claimed

measuring range; one sample failed QC check due to an instrument shim error and could not be repeated due to insufficient sample volume). The range of the samples was 315 to 3497 nmol/L.

Linear regression/least squares analysis:

r = 0.970

Slope = 0.98 95% CI: 0.97 – 0.99

Intercept = 46.42 95% CI: 22.44 – 70.40

Deming Regression analysis:

Slope = 1.01 95% CI: 1.00 - 1.03

Intercept = -7.97 95% CI: -31.45 – 15.50

In addition to conducting a method comparison for the overall data set, the data were classified into the three following segments based on LDL-P values; (1) results below the medical decision limits of 1000 nmol/L, (2) results between 1000 and 1600 nmol/L and (3) results above 1600 nmol/L. For each segment the bias was determined. The determined % Bias for each segment was less than the acceptable allowable bias (< 5% for each level).

Observed LDL-P Bias per linear regression at Medical Decision Limits

	Linear regression		Deming regression	
LDL-P (nmol/L)	**Absolute Bias**	**% Bias**	**Absolute Bias**	**% Bias**
1000	28.0	2.8%	3.9	0.4%
1300	22.6	1.7%	7.5	0.6%
1600	17.0	1.1%	11.1	0.7%

HDL-C and triglycerides:

A total of 1600 freshly-collected clinical de-identified specimens from the LipoScience commercial lab were evaluated. The performance of these samples run on the proposed device was compared to results given by the predicate (k063841).

HDL-C:
y = 1.00x + 0.03
R = 0.999
Range = 12.0 – 140.0 mg/dL

Triglycerides:
n = 1597 (two samples outside the measuring range; one sample failed as indicated above under LDL-P method comparison)
y = 1.00x + 1.17
r = 1.000
Range = 20 – 925 mg/dL

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 plain serum collection tubes; or plasma drawn into EDTA or heparin collection tubes. A study was conducted to validate the equivalence of NMR LipoProfile results using these serum and plasma specimens. Blood was collected in the specified collection tubes and immediately tested. The study comparing samples collected in serum tubes (NMR LipoTube, reference) to plain serum (red-top), EDTA plasma (purple top) or Na Heparin plasma (green top) tubes tested individual specimens from 30 volunteers. Samples tested spanned most of the assay range. Average biases observed ranged between 2.6 to 6.1% for LDL-P. No concentration-dependent trends were observed.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

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

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The sponsor used procedures in CLSI Guideline C28-A2 to bridge the transference of a reference range established with the predicate method in k063841 to the proposed device. For the predicate devices, 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. Results were presented as percentiles.

In the sponsor's bridging study, the plasma samples from the MESA study were re-tested. Statistical analysis demonstrated that the percentiles determined for the predicate are also valid for the proposed device.

	All (n=5,362)	**Men** (n=2,529)	**Women** (n=2,833)
Percentile	**LDL-P** (nmol/L)	**LDL-P** (nmol/L)	**LDL-P** (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 concentration and triglycerides: The proposed device has similar reference values as the predicate. The 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	
Low	*High*
<40	≥60

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

N. Proposed Labeling:

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

O. Conclusion:

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