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

I Background Information:

A 510(k) Number

K210801

B Applicant

numares AG

C Proprietary and Established Names

AXINON® LDL-p Test System

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MRR	Class I, subject to limitations of exemptions per 21 CFR 862.9(c)(4)	21 CFR 862.1475 - Lipoprotein Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Low density lipoprotein particle (LDL-p)

C Type of Test:

Quantitative, Nuclear Magnetic Resonance (NMR) spectroscopy assay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The AXINON® LDL-p Test System is intended to measure lipoprotein particles to quantify LDL particle number (LDL-p) using nuclear magnetic resonance (NMR) spectroscopy that measures the 600 MHz proton nuclear magnetic resonance (NMR) spectrum of a human serum sample. LDL-p concentration values 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 system is for professional use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use with the AXINON® Analyzer

IV Device/System Characteristics:

E Device Description:

The test system included the following components:

AXINON® Analyzer
AXINON® Analyzer is a 600 MHz nuclear magnetic resonance (NMR) spectrometer equipped with an automated sample handler with barcode scanner for sample identification software (AXINON® software) to analyze digitized spectral data. In addition, AXINON® Analyzer comes with the optional software utility AXINON® Sample Wizard that supports the user during sample preparation procedures.

AXINON® LDL-p Test Software
The final test result is generated using the AXINON® LDL-p Test-Software which is integrated into the AXINON® Software to calculate LDL particle concentration.

AXINON® serum kit 2.0
AXINON® LDL-p test is intended for use with the AXINON® serum kit 2.0 (available in two configurations, for 100 tests and 1000 tests). The AXINON® Serum Kit 2.0 contains the AXINON® Serum Calibrator, the AXINON® Serum Control, and the AXINON® Serum Additives solution.

NMR tubes and caps, NMR Racks
Samples are measured in NMR tubes (glass vials) which are closed with NMR caps that carry a barcode for sample identification. The NMR tubes and caps are available in two different package sizes, sufficient for 100 or 1,000 tests.

AXINON® serum kit 2.0, NMR tubes and NMR racks are required but not provided.

A Principle of Operation:

The AXINON® LDL-p test involves measurement of the 600 MHz proton NMR spectrum of a serum sample, deconvolution of the composite signal at approximately 0.85 ppm to produce signal amplitudes of lipoprotein subclass proportions that contribute to the composite serum signal, and conversion of these subclass signal amplitudes to lipoprotein subclass concentrations. The 0.85 ppm serum NMR signal arises mainly from the methyl group protons of the lipids carried in LDL subclasses of varying diameters. The NMR signals from the various lipids within the lipoprotein subclasses have unique and distinctive shapes and frequencies, uncovered by the granular decomposition of the composite serum signal. Each of these lipid signal representatives 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 are summed to give the reported total LDL particle concentration (LDL-P), in units of nanomoles of particles per liter (nmol/L).

B Instrument Description Information:

1. Instrument Name:

   AXINON® Analyzer

2. Specimen Identification:

   Samples are measured in NMR tubes which are closed with NMR caps that carry a 2D barcode for sample identification.

3. Specimen Sampling and Handling:

   The intended specimen type is human serum. Blood specimens are collected in plain red-top serum collection tubes and allowed to clot prior to centrifugation. AXINON® serum additives solution 2.0 and serum are mixed 1:10 in a separate container (e.g., 70 µL additives solution + 630 µL serum), and then 600 µL of the mixture is transferred into an NMR tubes.

4. Calibration:

   The rack for measurements using the AXINON® LDL-p test requires a specific configuration: the first position (A1) carries an NMR tube containing AXINON® Serum Calibrator; the second (A2) and last position (H12) carry NMR tubes containing AXINON® Serum Control; in between, the clinical samples are positioned.

The AXINON® Serum Calibrator (containing Maleic Acid as Sodium salt), is used as a calibrator once per measured rack during measurement startup to establish current normalization factors in each analytical run. It also serves as a quality assessment tool to ensure quality NMR spectra are produced by the NMR analyzer.

5. Quality Control:

Internal Quality Control:

The AXINON® Serum Control (comprising Acetic Acid as Sodium salt) is used as the NMR control for the AXINON® Analyzer. AXINON® Serum Control is used routinely as a quality control material once per measured rack during measurement startup and termination to verify current normalization factors in each analytical run. It also serves as second quality assessment tool to ensure quality NMR spectra are produced by the NMR analyzer.

External Quality Control:

Bio-Rad control materials (LIQUID ASSAYED MULTIQUAL), level 1 (low), level 2 (normal) and level 3 (abnormal, high) can be used as external quality control materials. It is recommended that two levels of quality control materials are tested in the same manner as patient samples:

- After calibration
- According to federal, state or local regulations or at least once every day when patient testing is being performed.

V Substantial Equivalence Information:

A Predicate Device Name(s):

NMR Profiler and NMR LipoProfile® Test

B Predicate 510(k) Number(s):

K063841

C Comparison with Predicate(s):

Device & Predicate Device(s):	K210801	K063841
Device Trade Name	AXINON® LDL-p Test System	NMR Profiler and NMR LipoProfile® Test
General Device Characteristic Similarities		
Intended Use/Indications for Use	Intended to measure lipoprotein particles to quantify LDL particle number (LDL-p) as an aid in the management of	Same

Device & Predicate Device(s):	K210801	K063841
	lipoprotein disorders associated with cardiovascular disease	
Technology	Nuclear Magnetic Resonance	Same
General Device Characteristic Differences		
Instrument Required to Run the Test	AXINON® Analyzer	NMR Profiler
Detection Method	600 MHz proton NMR spectrum	400 MHz proton NMR spectrum
Specimen	Human serum	Human serum and plasma

VI Standards/Guidance Documents Referenced:

- Clinical Laboratory Standards Institute (CLSI) EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline–Third Edition
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline–Second Edition
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline–Third Edition
- CLSI EP39, A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of In Vitro Medical Laboratory Tests – First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision studies were conducted following the recommendations in the CLSI guideline EP05-A3.

a) Within-Laboratory Precision

Repeatability (within-run imprecision) and intermediate precision (between-run and between-day imprecision) were determined using five native serum sample pools, created by pooling patient samples, with different LDL-p concentrations (1-5) and one commercial control sample (6). Samples were measured in duplicated in two runs each day over a period of 20 days. The study was conducted on one AXINON® analyzer, with three lots of reagents. Repeatability, between-run, between-day, and within-laboratory precision were evaluated.

Results are summarized in the table below:

Sample	Lot	Mean Value (nmol/L)	N	Repeatability		Between-Run		Between-Day		Within-Laboratory	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	1	653.8	80	14.88	2.28	25.07	3.83	0	0	29.16	4.46
	2	674.2	80	21.51	3.19	29.19	4.33	0	0	36.26	5.38
	3	656.2	80	21.33	3.25	26.76	4.08	0	0	34.22	5.22
2	1	1006.8	80	20.34	2.02	22.35	2.22	13.09	1.30	32.93	3.27
	2	1026.3	80	22.95	2.24	9.63	0.94	19.67	1.92	31.72	3.09
	3	1001.2	80	21.25	2.12	32.20	3.21	11.06	1.10	40.14	4.01
3	1	1069.0	80	22.54	2.11	25.71	2.41	13.47	1.26	36.75	3.44
	2	1081.6	80	23.16	2.14	21.72	2.01	21.28	1.97	38.22	3.53
	3	1065.9	80	20.92	1.96	20.84	1.95	13.05	1.22	32.28	3.03
4	1	1098.0	80	16.95	1.54	23.98	2.18	15.85	1.44	33.37	3.04
	2	1117.0	80	22.07	1.98	16.70	1.50	20.13	1.80	34.22	3.06
	3	1105.2	80	24.04	2.18	23.42	2.12	22.47	2.03	40.39	3.65
5	1	1424.9	80	24.28	1.70	22.35	1.57	17.12	1.20	37.17	2.61
	2	1474.5	80	23.41	1.59	24.86	1.69	20.19	1.37	39.67	2.69
	3	1447.8	80	23.93	1.65	25.72	1.78	28.78	1.99	45.41	3.14
6	1	2857.3	80	35.76	1.25	33.29	1.17	105.38	3.69	116.15	4.07
	2	2909.5	80	33.02	1.13	47.33	1.63	87.28	3.00	104.63	3.60
	3	2875.4	80	28.61	0.99	55.11	1.92	93.96	3.27	112.62	3.92

b) Reproducibility

Reproducibility was determined using five native serum sample pools with different LDL-p concentrations (1-5) and one commercial control sample (6). Samples were measured at three sites, over 5 days, with one run per day and 5 replicates per run. The study was conducted on one AXINON® analyzer per site, with one lot of reagents.

Results for combined sites are summarized in the table below:

Sample	Mean Value (nmol/L)		Repeatability		Within-Laboratory		Between-Site		Reproducibility	
		N	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	583.08	75	16.49	2.83%	22.93	3.93%	22.53	3.86%	32.15	5.51%
2	1034.24	75	15.87	1.53%	24.34	2.35%	20.37	1.97%	31.74	3.07%
3	1318.83	75	25.94	1.97%	34.29	2.60%	12.37	0.94%	36.45	2.76%
4	1649.61	75	20.95	1.27%	32.31	1.96%	14.34	0.87%	35.35	2.14%
5	2108.25	75	25.02	1.19%	38.86	1.84%	26.92	1.28%	47.28	2.24%
6	2314.95	75	44.77	1.93%	48.55	2.10%	35.03	1.51%	59.87	2.59%

2. Linearity:

The linearity study was conducted following the recommendations in the CLSI guideline EP6-A2.

A dilution series was prepared by diluting a human serum pool ("high pool" with LDL-p concentration of 3284 nmol/L) with bovine serum albumin (BSA)-saline solution to produce 10 samples with a concentration range of 219-3285 nmol/L. The samples were analyzed in five replicates within one run, on one AXINON® Analyzer, and with one lot of reagents.

For all samples within the measuring range, the deviation from linearity observed in the study was less than 10%. The studies support the sponsor's claimed measuring range of 300 to 3,100 nmol/L.

3. Analytical Specificity/Interference:

Interference studies were conducted following the recommendations in the CLSI guideline EP07 3rd Edition.

Endogenous substances normally found in blood and exogenous substances (common and prescription drugs) were evaluated for potential interference with the AXINON® LDL-p test. Native human serum with two different concentrations of LDL-p (approximately 800 and 2000 nmol/L) were spiked with varied concentrations of potential interferents (test pool). No interference was defined by the sponsor as less than ± 10% deviation from the control pool. If a potentially interfering substance was suspected to have significant interference defined as difference from control greater than 10%, a dose-response experiment was conducted. Five replicates of test pools and control pools were measured in one run on one AXINON® analyzer. The following substances were tested and were found not to interfere at the concentration shown:

Substance	Concentration	SI
Acetaminophen		1.3 µmol/L
Acetylsalicylic acid		3.6 mmol/L
Albumin	6 g/dL	
Atorvastatin	600 µg/L	
Bilirubin, conjugated	28.8 mg/dL	341.4 µmol/L
Bilirubin, unconjugated		380 µmol/L
Clopidogrel hydrochloride		95.3 µmol/L
Creatinine	5 mg/dL	445 µmol/L
Enalaprilat dihydrate		0.9 µmol/L
Ethanol		130 mmol/L
Fenofibrate		125 µmol/L

Substance	Concentration	SI
Furosemide		2 mmol/L
Glipizide		44.8 µmol/L
Hemoglobin	1 g/dL	
Heparin		3000U/L
Hydralazine hydrochloride		918.2 µmol/L
Ibuprofen Sodium salt		2375 µmol/L
Isosorbide dinitrate		6.4 µmol/L
Menhaden oil	2.4 mg/mL	
Metformine hydrochloride		3.6 mmol/L
Metoprolol tartrate		18.7 µmol/L
Nicotinic acid sodium salt		8.3 mmol/L
Nifedipine		1559 nmol/L
Olive Oil	1000 mg/dL	
Pioglitazone hydrochloride		156.8 µmol/L
Piroxicam		215 µmol/L
Pravastatin		107.5 µmol/L
Salicylic acid		1.3 mmol/L
Simvastatin		114.5 µmol/L
Soy Oil	1000 mg/dL	
Triglycerides	500 mg/dL	
Urea		43 mmol/L
Uric acid	23.9 mg/dL	1.4 mmol/L
2- propanol	130 mmol/L	

1-Propanol and Naproxen (sodium) were identified as potentially interfering due to relative bias ≥ 10% in at least one pool or because results were not reported. For these substances dose-response experiments were carried out. For dose-response experiments, 5 evenly spaced intermixtures of each substance were prepared in a low (<1000 nmol/L) and high (>1600 nmol/L) LDL-p control pool. For 1-Propanol, negative bias (maximum of -6.55%) was observed at 1 mM in the low pool and below 2 mM in the high pool. Testing higher concentrations of propanol yielded no results, however this is expected, because the 1-Propanol signals overlap with the spectral region of interest for the lipoprotein signals and if too dominant interferes with LDL-p quantification. For Naproxen (sodium), negative bias

(maximum of -14.11%) was observed starting at 0.55 mM in the low pool and starting at 1.65 mM in the high pool.

The sponsor included the following limitations in the labeling:

"For patients under Naproxen treatment (at above 0.55 mmol/L) LDL-p values up to -14.11% lower than expected may be observed.".

"1-propanol at concentrations above 1 mmol/L may cause missing or falsely low results. Though rare, certain hand sanitizers may be contaminated with 1-propanol. While hand sanitizers are not recommended for skin disinfection during venipuncture, transfer of 1-propanol may occur (e.g., on personnel hands or gloves) at the venipuncture site. However, a concentration of 1mmol/L resulting in interference with the AXINON® LDL-p test is very unlikely, if adhering to the WHO recommended procedure for standard venipuncture procedures in blood collection."

4. Assay Reportable Range:

The analytical measurement range is 300 - 3,100 nmol/L.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Stability:

The sample stability studies support the following claims:

- Blood collection, serum preparation and specimen storage at room temperature must be completed within 6h.
- Separated serum may be stored at 2°C to 10°C for up to 2 days.
- Prepared samples may be stored on board for up to 5 days (at 4-8°C).

Traceability:

The AXINON® Serum Calibrator (containing Maleic Acid as Sodium salt), is used as the NMR calibrator for the candidate device. The calibrator is traceable to Maleic acid certified reference material (CRM) that is then traceable to a NIST standard (NIST SRM 84l (KHP).

6. Detection Limit:

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) studies were conducted following the recommendations in the CLSI guideline EP17-A2.

The LoB was determined based on confirmation of the LoB set to zero in advance. 30 blank samples prepared from commercially available lipoprotein deficient serum were tested using three lots of reagents. The claimed LoB is 0 mmol/L.

The LoD was determined using the Probit approach. Three sample pools were measured in eight dilution levels per pool in replicates of seven over three days, using two lots of

reagents. The maximum observed LoD across the two lots tested was 99.08 nmol/L. The claimed LoD is 99.1 nmol/L.

The LoQ was defined as the lowest investigated concentration with acceptable within-laboratory precision of less than 20% CV. Four sample pools with sufficiently low concentrations of LDL-p were measured in replicates of five over three days, using three lots of reagents. The maximum observed LoQ across the three lots tested was 139.67 nmol/L. The claimed LoQ is 139.7 nmol/L.

7. Assay Cut-Off:

   Not applicable.

8. Accuracy (Instrument):

   Not applicable.

9. Carry-Over:

   The instrument does not come into direct contact with the samples; therefore, carry-over studies are not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   102 native serum samples were tested in singlicate using the candidate device and the NMR LipoProfile® test on the Vantera Clinical Analyzer (K113830). Data for the AXINON® LDL-p test used with the AXINON® Analyzer was collected over six days on three instruments, with a single reagent lot (including calibrator), and six calibration cycles. Method comparison studies were conducted at three different sites, in three separate experiments (using 34 different samples at each site). The range of samples tested was 344-2526 nmol/L LDL-p.

   The results were analyzed using Passing-Bablok regression analysis. A summary of results is presented in the table below:

Site	N	Concentration range, comparator device	Regression equation	r
1	34	344-1819	y=1.12x-135.27	0.961
2	34	498-2526	y=0.99x+6.55	0.948
3	34	365-2241	y=1.06x-66.23	0.953
Combined	102	344-2526	y=1.07x-9.16	0.955

   The bias at the medical decision levels was similar across sites.

2. Matrix Comparison:

Not applicable. The only tube type intended for use with the device is a plain re-top serum collection tube without anti-coagulation additives. All studies were carried out with serum samples.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable.

2. Clinical Specificity:

Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

The sponsor referenced clinical information provided in the following studies:

a) El Harchaoui, K., et al., Value of low-density lipoprotein particle number and size as predictors of coronary artery disease in apparently healthy men and women: the EPIC Norfolk Prospective Population Study. J Am Coll Cardiol, 2007. 49(5): p. 547-53.
b) Mora, S., et al., Lipoprotein Particle Profiles by Nuclear Magnetic Resonance Compared with Standard Lipids and Apolipoproteins in Predicting Incident Cardiovascular Disease in Women. Circulation, 2009. 119(7): p. 931-939.

D Clinical Cut-Off:

See Section VII.C.3 above.

E Expected Values/Reference Range:

The sponsor conducted a transference study to evaluate the comparability of the reference intervals for the quantitative measurement of LDL-p concentrations in human serum using the AXINON® LDL-p Test System. The study was conducted using serum samples collected from 40 apparently healthy subjects (20 male, 20 female). The study followed the recommendations in CLSI EP28-A3c guideline

The sponsor provided adequate information to support the following reference range information in their labeling[1]:

The reference interval for LDL-p is
- 542 – 1986 nmol/L for women, and
- 528 – 2169 nmol/L for men,
being the central 90% of values (at the 5th and 95th percentiles).

The sponsor recommends that each laboratory investigate the transferability of the reference interval to its own patient population and if necessary, determine its own reference ranges.

1. Matyus et al., Clin.Biochem. 47 (2014), 203-210

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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