Supplementary Materials

Abbreviations and Acronyms.

ANGPTL-3	Angiopoietin-like Protein 3
ANGPTL-4	Angiopoietin-like Protein 4
ApoB	Apolipoprotein B
ApoCII	Apolipoprotein C-II
ApoCIII	Apolipoprotein C-III
GPIHBP-1	Glycosylphosphatidylinositol-anchored High Density Lipoprotein-binding Protein-1
HDL	High Density Lipoprotein particles
LDL	Low Density Lipoprotein
Lp(a)	Lipoprotein a
PCSK9	Proprotein Convertase Subtilisin/Kexin type 9
VLDL	Very Low Density Lipoprotein

Table S1.

Title: Study population and alirocumab dosage.

Definition	Population (N)	Alirocumab 75 mg (%)	Alirocumab 150 mg (%)
Recruitment	24	19 (79.2)	5 (20.8)
Completion	19	16 (84.2)	3 (15.8)

Legend: Trial completion population (n=19) includes patients attending their baseline plus week 10 visit and injected alirocumab as planned. Three patients could not conduct their scheduled week 10 visit due to COVID-19 countermeasures. Two patients injected alirocumab only twice at baseline and at week 2 but not beyond.

Table S2

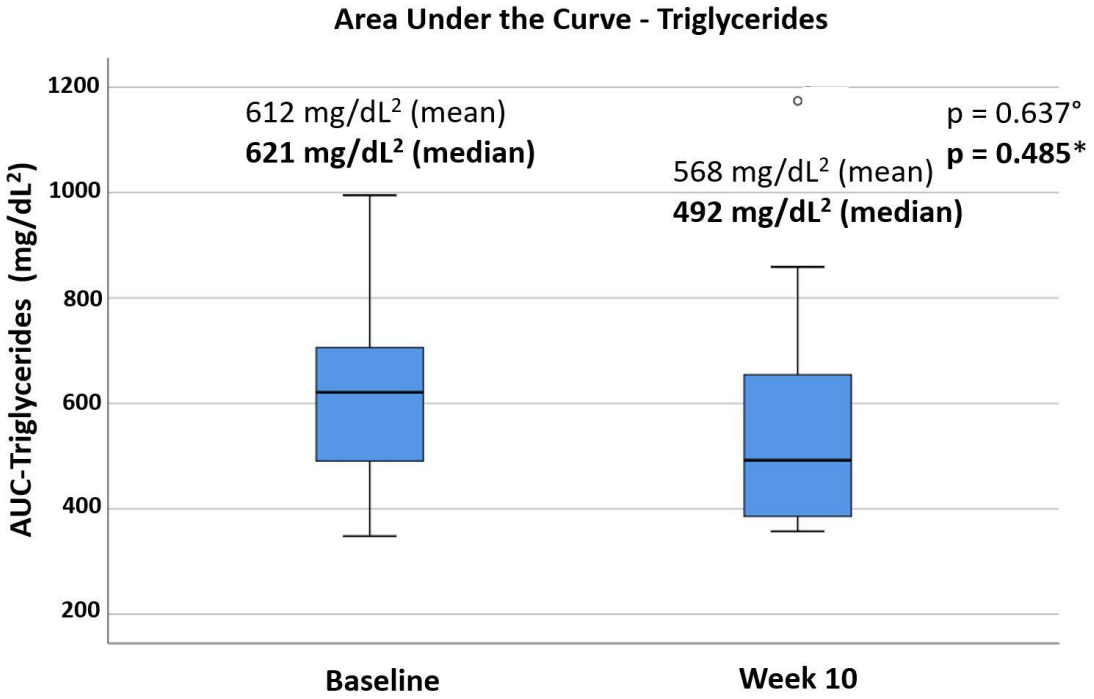
Title: Characteristics of all recruited trial participants.

Characteristic	All Recruited (N = 24)
Age - yr	66 (9)
Female sex - n (%)	9 (37.5)
Male sex - n (%)	15 (62.5)
Smoker[a] - n (%)	12 (50)
Current Smoker[b] - n (%)	5 (20.8)
Concomitant Diseases - n (%)	
Cardiovascular Disease	24 (100)
a. Coronary Heart Disease	23 (95.8)
Coronary Intervention or Surgery	19 (79.2)
Documentation of Coronary Stenosis[c]	4 (16.7)
b. Peripheral Artery Disease	3 (12.5)
c. Cerebral Artery Disease	8 (33.3)
Chronic Kidney Disease	5 (20.8)
Familial Hypercholesterolaemia[d]	4 (16.7)
Adiposity	4 (16.7)
Type-2 Diabetes Mellitus	4 (16.7)
Type-1 Diabetes Mellitus	0 (0)
Hypertension	19 (79.2)
Number of prior Cardiovascular Events[e] - n (%)	
Three	2 (8.3)
Two	6 (25)
One	12 (50)
Zero	4 (16.7)
Concomitant Lipid Medication – n (%)	
High-Intensity Statins[f]	5 (20.8)
Statins	7 (29.2)
Ezetimibe	15 (62.5)
Dietary Supplements[g]	6 (25)
Statin Intolerance[h]	19 (79.2)

Legend: Values are numbers (percentages) or means (standard deviations) for categorical and continuous variables, respectively. [a]Current or former smoker. [b]Documented as current smoker or no stop date documented. [c]Confirmed by cardiac computed tomography but without documentation of prior cardiovascular event (e.g., stroke, myocardial infarction, or percutaneous intervention). [d]According to medical records. [e]Documented as stent, balloon, coronary artery bypass graft, myocardial infarction, or percutaneous intervention, prior strokes/transient ischemic attacks. [f]Documented as ≥ 40 mg of atorvastatin or ≥ 20 mg of rosuvastatin. [g]Exclusively red yeast rice combination products (monacolin K). [h]Patients that did not receive high-intensity statins at baseline (includes partial or complete intolerance).

Figure S1

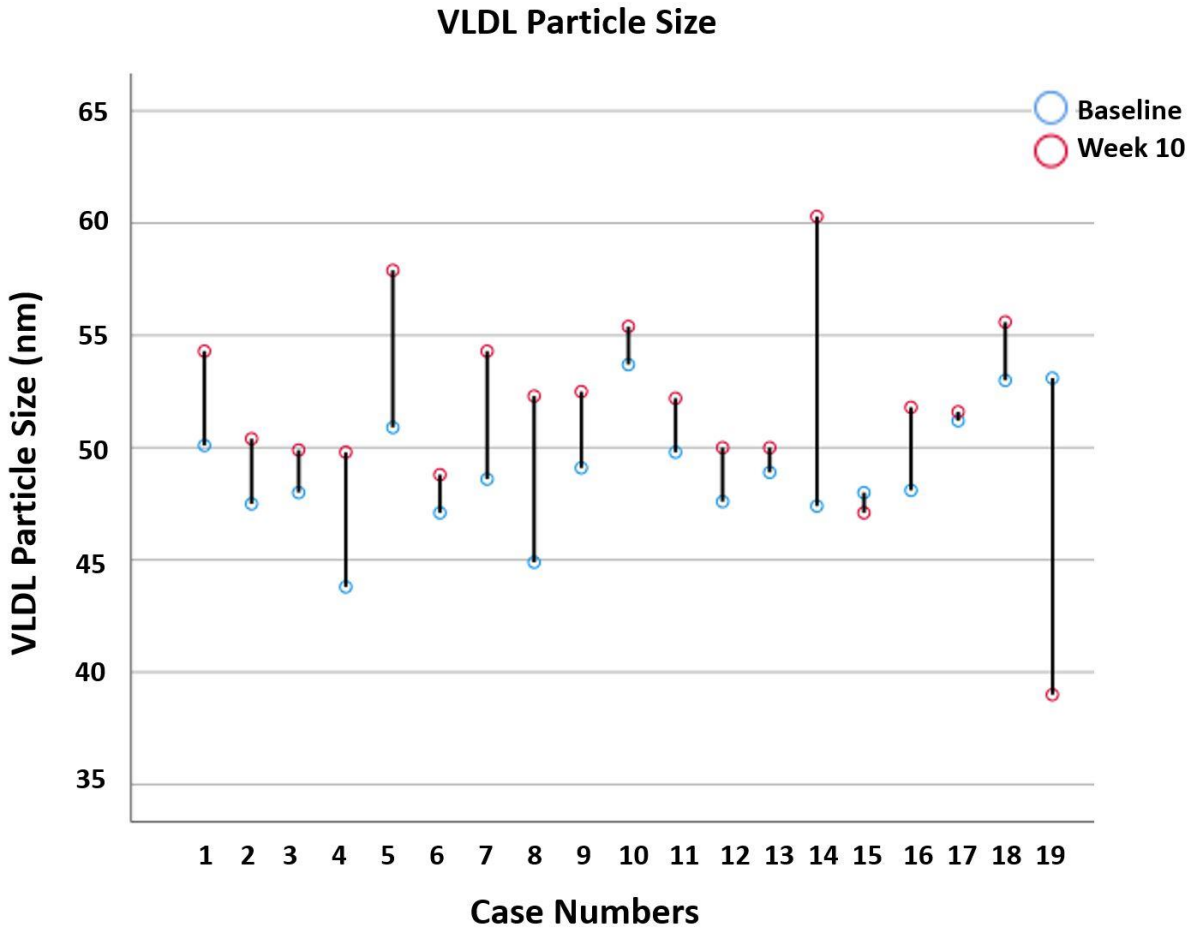
Title: Area under the curve (AUC) of triglycerides during fat-tolerance testing (t=0-4 hours) at baseline visit and after 10 weeks of alirocumab.



Legend: Among the trial-completion population, eleven patients had completed all measurements for AUC calculation (n = 11). Paired samples t-test with two-sided p-value. °Paired samples t-test; *Related samples Wilcoxon signed rank test. Calculations according to the trapezoid model.

Figure S2

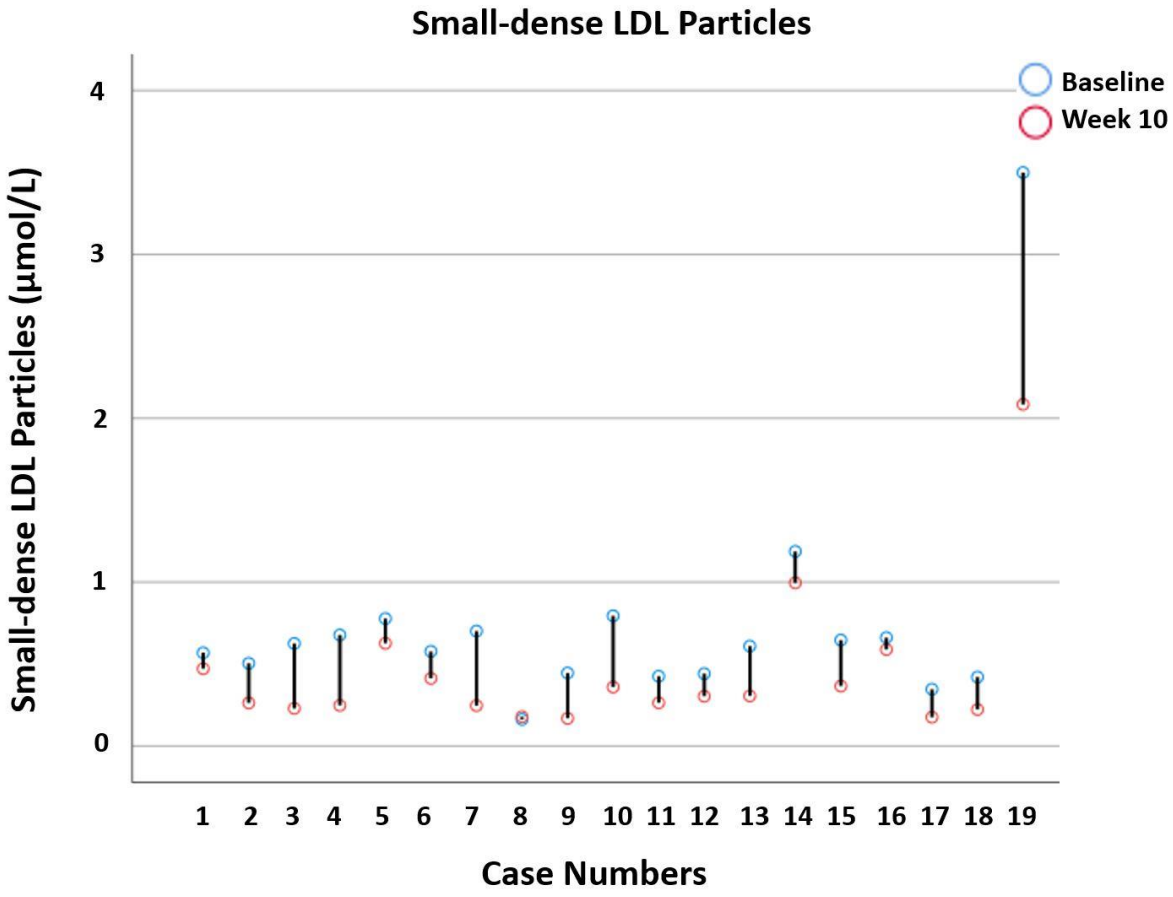
Title: Distribution of VLDL particle size and changes by alirocumab treatment.



Legend: Shows nuclear magnetic resonance results of individual VLDL size and changes in response to alirocumab from baseline to week 10. Trial-completion population (n = 19). Case number 19 was a patient with mixed familial hyperlipidaemia and with baseline LDL-cholesterol of > 300 mg/dL, baseline small-dense LDL particles of 3500 nmol/L and with baseline triglyceride-rich large VLDL particles of 24 nmol/L.

Figure S3

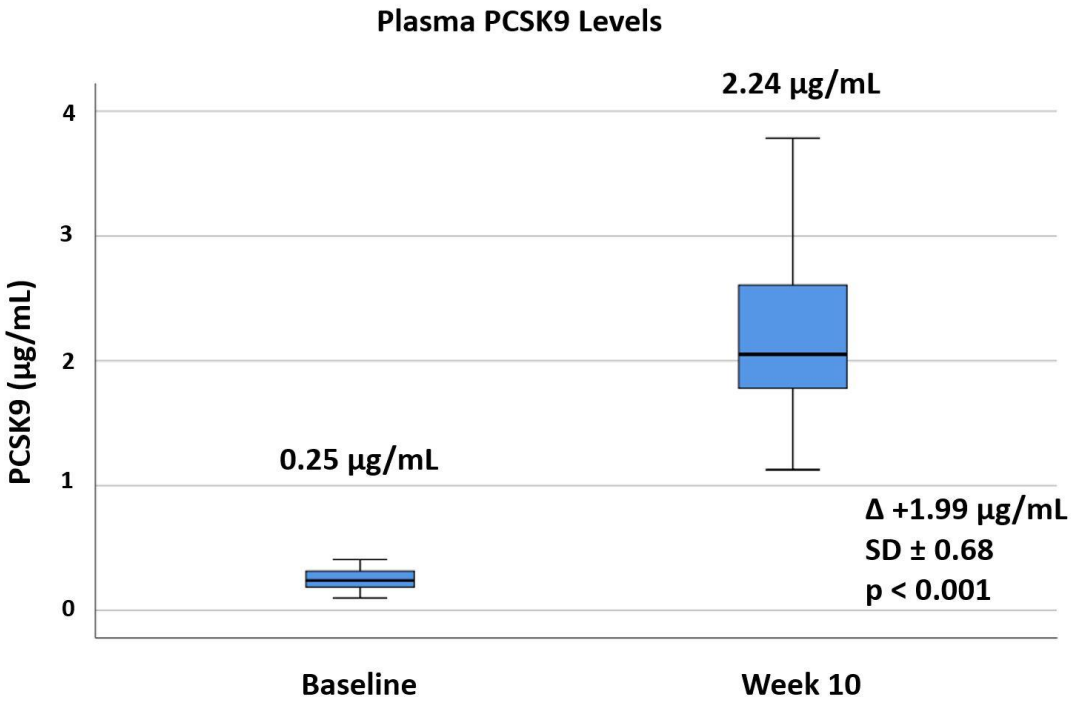
Title: Distribution of small-dense LDL particles and changes by alirocumab treatment.



Legend: Shows nuclear magnetic resonance results of individual small-dense LDL particles and changes in response to alirocumab from baseline to week 10. Trial-completion population (n = 19). Case number 19 was a patient with mixed familial hyperlipidaemia and with baseline LDL-cholesterol of > 300 mg/dL, baseline small-dense LDL particles of 3500 nmol/L and with baseline triglyceride-rich large VLDL particles of 24 nmol/L.

Figure S4

Title: Plasma PCSK9 levels at baseline and after 10 weeks of alirocumab treatment.



Legend: Figure shows trial-completion analysis (n = 19); Paired t-test with two-sided p-value; SD: Standard deviation.

Table S3

Title: LDL cholesterol and PCSK9 plasma levels per patient.

Method	Lipoprotein-Electrophoresis			Lipoprotein Analysis (Combined)			ELISA		
Patient-ID	LDL-C Baseline	LDL-C Week 10	LDL-C Change (%)	LDL-C Baseline	LDL-C Week 10	LDL-C Change (%)	PCSK9 Baseline	PCSK9 Week 10	X-Fold Increase
1	147	155	+5	145	176	+21	0.23	0.37	<2
2	90	48	-47	119	74	-38	0.31	2.53	8
3	128	41	-68	142	53	-63	0.41	3.70	9
4	81	27	-67	85	46	-46	0.31	1.83	6
5	242	34	-86	278	63	-77	0.19	2.86	15
6	161	67	-58	168	58	-65	0.36	2.84	8
7	154	78	-49	160	94	-41	0.17	1.77	11
8	193	57	-70	197	61	-69	0.21	2.22	10
9	125	60	-52	148	87	-41	0.22	2.59	12
10	59	27	-54	86	49	-43	0.31	1.13	4
11	126	46	-63	121	47	-61	0.10	1.32	13
12	90	11	-88	99	52	-47	0.14	1.65	11
13	116	76	-34	119	78	-34	0.18	1.45	8
14	160	92	-43	181	114	-37	0.24	2.03	8
15	206	91	-56	215	82	-62	0.23	1.98	9
16	165	68	-59	160	68	-58	0.25	1.79	7
17	173	140	-19	172	134	-22	0.27	2.63	10
18	100	35	-65	110	43	-61	0.14	2.05	15
19	79	80	+1	122	94	-23	0.16	0.25	<2
20	117	51	-56	132	64	-52	0.35	2.31	7
21	446	431	-3	319	433	+36	0.32	3.78	12

Legend: The table shows mean LDL-cholesterol (mg/dL) and mean PCSK9 (µg/mL) values per patient with week 10 assessments (n=21), as well as the relative impact of alirocumab treatment on these two parameters. Patients with the number 1 and 19 were non-adherent, thus did not inject alirocumab beyond week 2. Among participants with self-reported adherence, patient number 17 and 21 reflect low/non-response to alirocumab treatment but show high PCSK9 increase. Green: Lipoprotein-Electrophoresis; Blue: Lipoprotein analysis using the combined ultracentrifugation precipitation method; Purple: Enzyme-linked immunosorbent assay (ELISA). LDL-C: LDL-cholesterol.

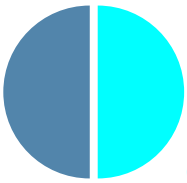
AXINON® lipoFIT®*

Advanced Lipoprotein Testing for Cardiovascular Risk Assessment

AXINON® lipoFIT® – NMR Lipoprotein Analysis for CVD Risk Management

- Determination of lipoprotein particle numbers & sizes for advanced cardiovascular risk assessment in a single measurement
- Standardized on-site NMR system
- Easy usability and automated platform with QC standards for reliable, high-throughput results

Cardiovascular disease (CVD) is the number one cause of death worldwide [1]. Clinical CV risk factors include hypertension, diabetes mellitus, chronic kidney disease, obesity, cigarette smoking and family history. The most important biomarkers for CV risk determination are lipids including total cholesterol, triglycerides, LDL and HDL cholesterol. However, about 50% of patients hospitalized for coronary artery disease (CAD) have LDL cholesterol (LDL-C) levels within the normal range [2].



50% of patients hospitalized for coronary artery disease (CAD) do not have elevated LDL-C.[2]

Particle Number and Size Matter

Considerable discordance between LDL-C and the number of LDL particles (LDL-p) has been observed, especially in those individuals with other comorbid conditions such as diabetes mellitus [3-5]. Despite having the same level of LDL-C, patients can have different concentrations of LDL-p (Fig. 1). Small, dense LDL particles are described to be more atherogenic than large ones [6-9].

Multiple studies have shown that CV risk is more closely associated with LDL particle concentration rather than cholesterol content [4, 5, 10-12]. Recent studies demonstrated the cost-effectiveness of LDL-p guided therapy [13-16].

Furthermore, lower concentrations of high-density lipoprotein particles (HDL-p) are associated with increased risk of CVD [17], while large HDL particles (LHDL-p) seem to have a protective effect [18, 19]. Thus, the determination of lipoprotein parameters beyond the conventional lipid panel can offer a more accurate risk assessment.

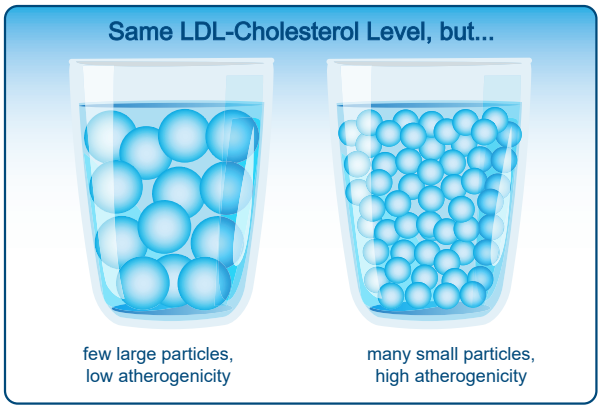


Fig. 1: Different LDL-p can sum up to the same LDL-C.

AXINON® lipoFIT® provides a detailed analysis of lipoprotein subclasses allowing further CV risk stratification. In addition to the standard lipid panel, the test measures concentration and size of lipoprotein particles.

Standard lipid panel +	Particle concentrations +	Particle sizes
Total cholesterol	LDL-p	VLDL-s
LDL cholesterol	HDL-p	LDL-s
HDL cholesterol	LVLDL-p	HDL-s
Triglycerides	SLDL-p	
	LHDL-p	

In particular, *AXINON® lipoFIT®* parameters include

■ **LDL-p: Concentration of LDL particles.**
Strong cardiovascular risk marker beyond LDL cholesterol [4,5].

■ **Small LDL-p: Concentration of small LDL particles.**
Elevated concentrations are associated with increased risk for coronary heart disease [11].

■ **HDL-p: Concentration of HDL particles.**
Reduced levels are strongly and independently linked to atherosclerotic risk [17].

** Available as a CE-labeled in vitro diagnostic product in the European Union and as Research-Use-Only product in the United States. numares´ products have not yet been approved or cleared by the U.S. Food and Drug Administration.*

Expert Panels Recommend LDL-P

AXINON® lipoFIT® can help to identify those at increased risk despite normal LDL-C levels. Several expert panels recommend use of LDL-p to optimize treatment of intermediate and high risk patients [20-25].

Year	Expert Panel
2020	**AACE/ACE Diabetes Management Algorithm** [20] American Association of Clinical Endocrinologists/ American College of Endocrinology' Comprehensive Diabetes Management Algorithm
2017	**AACE/ACE Guidelines** [21] American Association of Clinical Endocrinologists' and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease
2015	**NLA Recommendations** [22] National Lipid Association recommendations for patient-centered management of dyslipidemia
2013	**AACC Assessment** [23] Association of apolipoprotein B and nuclear magnetic resonance spectroscopy-derived LDL particle number with outcomes in 25 clinical studies: assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices
2011	**National Lipid Association (NLA)** [24] Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists

How to Use the Test

The combination of *Magnetic Group Signaling™* (*MGS®*) and the *AXINON®* System allows for spectral analysis with highly-reproducible results. The system is fast and efficient with automation capabilities allowing short hands-on-time, minimal operator interaction and high walk-away capability.

Test Features

Capacity: Five racks hold positions for up to 93 analytical samples each. Samples are processed in batches. The test system can be continuously loaded over just a few minutes.

Throughput: ~ 450 samples/24 h.

Walk-away operation: *AXINON®* supports the fully-automated measurement of up to 465 samples.

Hands-on Time: ~ 1 min. per sample (shorter when an automatic pipetting system is used)

Specimen collection, storage and transport

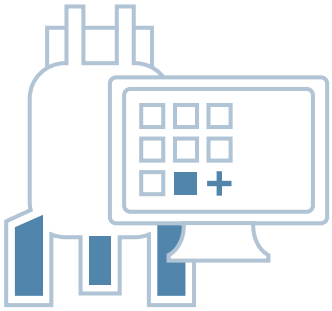
AXINON® lipoFIT® is performed on human serum samples collected according to standard techniques for laboratory testing. Appropriate tubes without anti-coagulation additives must be used. Specimens can be stored at 2-8°C for up to one week or can be frozen at -20°C or below.

Test Principle

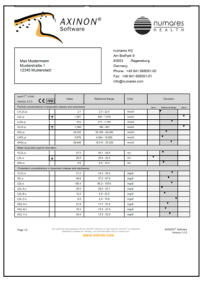
Samples are prepared with the *AXINON®* serum kit and measured using a qualified *AXINON®* 600 MHz NMR system. The high magnetic field strength of 600 MHz enhances signal resolution and sensitivity. The test parameters are calculated by fitting the broad methyl group signals of lipoproteins using mathematical functions.

AXINON® lipoFIT® Workflow

1) One-step sample preparation



2) Automated processing - 24/7



3) Output into LIS

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