Enabling Tools on the IVDr Platform: Lipoprotein Subclass Analysis

Research Use Only

Lipoproteins are supramolecular assemblies that transport water-insoluble lipids in blood. Lipoproteins also carry apolipoproteins, which determine structure and function. Apolipoproteins, a monolayer of amphiphilic phospholipids and cholesterol embedded therein, constitute the surface of the lipoproteins that 'hide' the lipids from the aqueous surrounding. The inner core mainly consists of triglycerides and esterified cholesterol.

Lipoproteins are commonly classified into five main groups:

- Chylomicrons
- Very Low Density Lipoproteins (VLDL)
- Intermediate Density Lipoproteins (IDL)
- Low Density Lipoproteins (LDL)
- High Density Lipoproteins (HDL)

In the context of cardiovascular risk assessment, the lipoprotein class-specific concentrations of cholesterol and triglycerides are of interest beyond their total concentrations within the plasma. There is a strong indication that additional sub-classification of lipoproteins could improve cardiovascular risk prediction.

Lipoprotein Subclass Analysis with NMR

The Bruker B.I.-LISA lipoprotein panel offers significant benefits versus the current testing modality, e.g. the ultracentrifugation method, which typically takes 1 week per sample. Ultracentrifuges currently handle up to 16 samples in parallel; however, the procedure requires repeated, precise manual interaction. For VLDL subclass analysis, an ultracentrifuge with adjustable angles is required. Alternative approaches are therefore sought.

Bruker has led a project to establish lipoprotein subclass analysis based on ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy of blood plasma or serum samples, using regression analysis of ultracentrifugation results.

The objective was to provide an analytical method that:

- Determines cholesterol, phospholipids, triglycerides, apolipoproteins A1, A2, B and LDL particle numbers for lipoprotein main and subclasses for plasma and serum.
- Works at high throughput (up to 150 samples per day in high quality regular screening) at low cost per sample, under full automation, simple sample preparation and automatic report generation
- Provides sufficient accuracy at highest reproducibility and complete transferability from instrument to instrument.

The method of choice is based on the analysis of signals in the ^{1}H-NMR spectrum that are related to the lipoproteins. Differences in lipoprotein composition, size and density translate into respective signal line shape differences, which can be used to extract information on lipoprotein main and subclasses (see figure 1).

A regression model had to be developed, using a training data set which consists of:

- Lipoprotein analytes from total plasma and ultra-centrifugation-based main and subclasses
- ^{1}H-NMR spectra of the same sample set

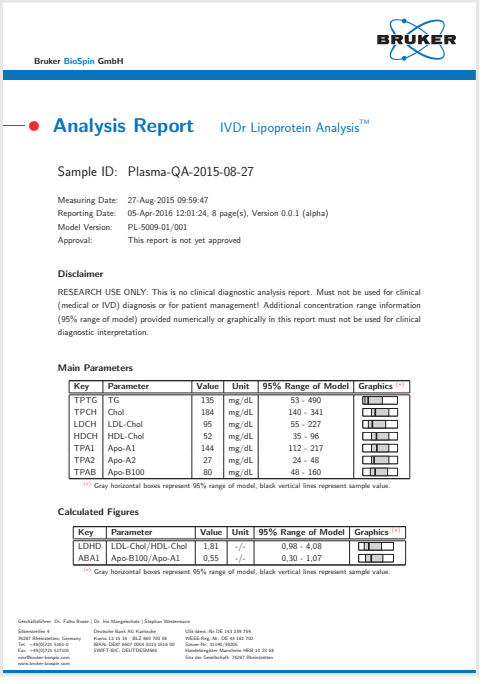
Once the regression model was established, a prediction algorithm calculates the lipoprotein analytes directly from the ^{1}H-NMR spectra of new plasma or serum samples, without further need for ultra-centrifugation.

Using this ^{1}H-NMR approach, information could be extracted about lipoprotein related information on:

- Plasma and serum
- The main VLDL, IDL, LDL and HDL classes
- Six VLDL subclasses VLDL-1 to VLDL-6 (sorted according to increasing density and decreasing size, respectively)
- Six LDL sub-classes LDL-1 to LDL-6
- Four HDL-subclasses HDL-1 to HDL-4

Information consists of concentrations of lipids, i.e. cholesterol, free cholesterol, phospholipids, triglycerides, concentrations of apolipoproteins Apo-A1, ApoA2 and Apo-B and the LDL particle numbers. Table 1 shows all parameters calculated by the ^{1}H-NMR lipoprotein analysis. Figure 1 shows the front page of the automatic report (B.I.-LISA).

Figure 1



Bruker BioSpin GmbH

Analysis Report IVDr Lipoprotein Analysis™

Sample ID: Plasma-QA-2015-08-27

Measuring Date: 27-Aug-2015 09:59:47
Reporting Date: 05-Apr-2016 12:01:24, 8 page(s), Version 0.0.1 (alpha)
Model Version: PL-5009-01/001
Approval: This report is not yet approved

Disclaimer

RESEARCH USE ONLY: This is no clinical diagnostic analysis report. Must not be used for clinical (medical or IVD) diagnosis or for patient management! Additional concentration range information (95% range of model) provided numerically or graphically in this report must not be used for clinical diagnostic interpretation.

Main Parameters

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
TPTG	TG	135	mg/dL	53 - 490	
TPCH	Chol	184	mg/dL	140 - 341	
LDCH	LDL-Chol	95	mg/dL	55 - 227	
HDCH	HDL-Chol	52	mg/dL	35 - 96	
TPA1	Apo-A1	144	mg/dL	112 - 217	
TPA2	Apo-A2	27	mg/dL	24 - 48	
TPAB	Apo-B100	80	mg/dL	48 - 160	

(*) Gray horizontal boxes represent 95% range of model, black vertical lines represent sample value.

Calculated Figures

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
LDHD	LDL-Chol/HDL-Chol	1,81	-/-	0,98 - 4,08	
ABA1	Apo-B100/Apo-A1	0,55	-/-	0,30 - 1,07	

(*) Gray horizontal boxes represent 95% range of model, black vertical lines represent sample value.

Page 1 of a B.I.-LISA report covering the main fraction information.

It is important to note that due to the nature of the regression approach, the analytes listed in Table 1 are partially correlated and cannot be assumed to be independent analytes. The relation between the analytes depends on just a few latent factors that are determined by both lipid metabolism and the limits of the training data set from ultracentrifugation used for training the regression model. The regression model has been developed for 600 MHz spectra only.

	Triglyceride	Cholesterol	Free Cholesterol	Phospho-lipids	Apo-A1	Apo-A2	Apo-B	LDL-particle
PLASMA	✓	✓	✓	-	✓	✓	✓	✓
VLDL	✓	✓	✓	✓			✓	✓
VLDL-1 to -6	✓	✓	✓	✓	-	-	✓	-
IDL	✓	✓	✓	✓	-	-	✓	✓
LDL	✓	✓	✓	✓	-	-	✓	✓
LDL-1 to -6	✓	✓	✓	✓	-	-	✓	✓
HDL	✓	✓	✓	✓	✓	✓	-	-
HDL-1 to -4	✓	✓	✓	✓	✓	✓	-	-

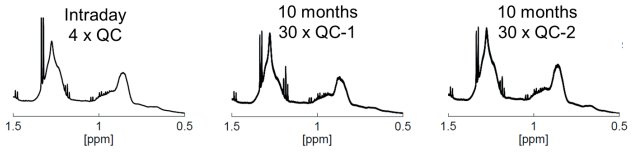
Table 1: Listing of all parameters generated by the ^{1}H-NMR lipoprotein subclass analysis.

Validation of the Lipoprotein Subclass Analysis

To test the validity of the results, the following measures were taken and continuously followed:

- Test reproducibility of measurement and analysis (Figure 2)
- Test transferability of measurement and analysis
- Laboratory comparison tests
- Traceability tests on main parameters using certified reference samples

Figure 2



		TG	CH	LDL-C	HDL-C
target: imprecision (*)		< 5%	< 3%	< 4%	< 4%
average imprecision (intraday)	QC sample-1	0.3 %	0.5 %	0.8 %	1.0 %
	QC sample-2	0.6 %	0.5 %	0.7 %	0.8 %
imprecision (long term) over 10 months	QC sample-1	1.7 %	1.4 %	1.9 %	2.4 %
	QC sample-2	1.4 %	1.5 %	2.3 %	2.2 %

(*) *NCEP Criteria for Lipid and Lipoprotein Testing*

Short and longterm imprecision of lipoprotein measurements by NMR (NCEP National Cholesterol Education Program, United States).

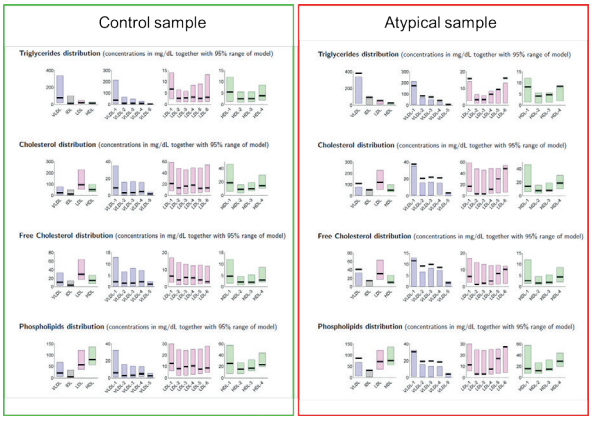
Benefits of Bruker B.I.-LISA IVDr Lipoprotein Analysis

- Lower cost than ultracentrifugation
- Much faster turnaround time – minutes versus days
- Completely automated and simple to run (e.g. by an MTA)
- Equal sensitivity and specificity as ultracentrifugation
- Small sample volume (500 microliter) – better patient compliance
- Straightforward sample preparation
- No direct contact between sample and device
- Spectra can be used for multiple types of analysis

Use of Lipoprotein Subclass Analysis in Clinical and Translational Research Applications

1. Atherosclerosis
2. Cardiovascular diseases (research on prevention, early detection, grading and treatment)
3. Type 2 diabetes, obesity, metabolic syndrome
4. Fatty liver disease
5. Thrombosis
6. Stroke
7. Cerebrovascular diseases
8. Inflammatory diseases
9. Cancer
10. Influence of food on health
11. Biobanks, quality control of plasma/serum, fasting state, concentration panel with spectra
12. Epidemiological studies

Figure 3



Two examples for lipid distributions calculated from Bruker IVDr Lipoprotein Subclass Analysis B.I.-LISA.

Requirements for Lipoprotein Subclass Analysis

- IVDr platform at 600 MHz
- Use of Bruker SOPs for plasma/serum
- Absolute temperature, solvent suppression and quantification reference sample must be checked regularly (preferably daily)
- Access to Bruker Data Analysis server for fully automated remote analysis (transfer of spectra after measurement to Bruker server via private ftp, back-transfer of result report)

Disclaimer

The Lipoprotein Subclass Analysis tool is for research use only. It must not be used for diagnosis and patient management. Concentration ranges given for the parameters listed represent the distribution in the model and must not be used for diagnostic purposes.

Purchase Options

The lipoprotein subclass analysis is an optional item of the IVDr platform. It can be ordered as a flatrate for 3 years (for pricing ask your sales person). A service contract is strongly recommend to ensure the performance of the spectrometer and the accuracy of the analysis.

IVD-CE Lipoprotein Subclass Analysis

On the IVDr platform an IVD-CE method is also available for those needing results for diagnostic purposes. This method is supplied by Numares. Contact can be made through your sales person.

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