LMWMscale: A New Bioinformatics Tool for Low Molecular Weight Metabolites (LMWM) Quantification based on 1H-NMR spectroscopy

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Background

The use of "omic" approaches to unravel the disease complexity accelerates its understanding, allowing untargeted approaches necessaries when limited disease knowledge is available.
Nuclear Magnetic Resonace (NMR) metabolomics for biomarker discovery has repeatedly succeed describing different in nature aetiologies and pathogenesis [1]. However, the current metabolomic approaches need higher degree of automation and standardization, mandatory attributes for clinical applications.
The current study presents *LMWMscale® Test*, an automatic bioinformatics tool for high-throughput quantitative metabolic profiling based on NMR spectroscopy, increasing NMR positioning for high-throughput bioscreening applications [2].

Results

The algorithm read and processed ^{1}H-NMR spectra in order to optimize, phase and baseline correct. The LMWM-associated regions were selectively batched in order to isolate, align and deconvolute each signal automatically.

The deconvolution approach used *Voigt* analytical functions (a mixture between lorentzian and gaussian functions) to reproduce the experimental curve minimizing the fitting error, to quantify the area of each signal proportional to the metabolite concentration. The deconvolution approach allowed the quantification of several LWMW signals with complex coupling patterns, even in highly overlapped spectral regions.

The resulting areas were transformed to concentration units by applying specific conversion factors. Consistency between standard techniques were evaluated, the correlation coefficients were R^2>0.9 (for glucose and creatinine).

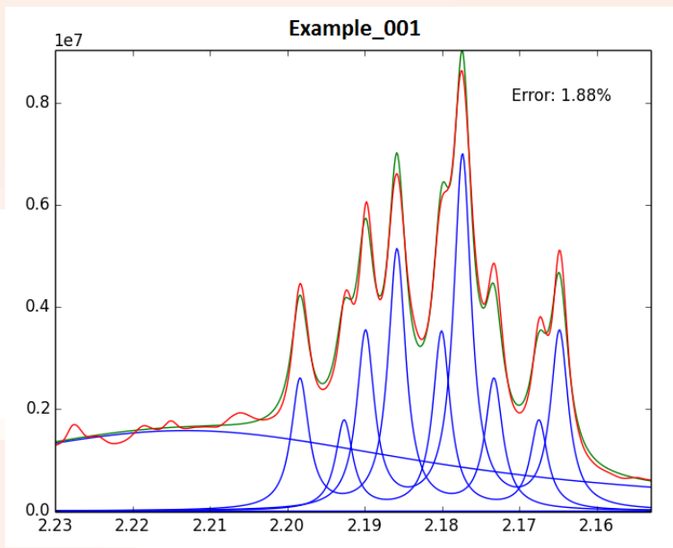


Figure 1. Image obtained from a urine sample showing the deconvolution of the specific region of azelate, pimelate and suberate.
The red line is the raw spectrum, the blue ones represent each individual analytical functions associated with each metabolite and the green one the resulting deconvolution.

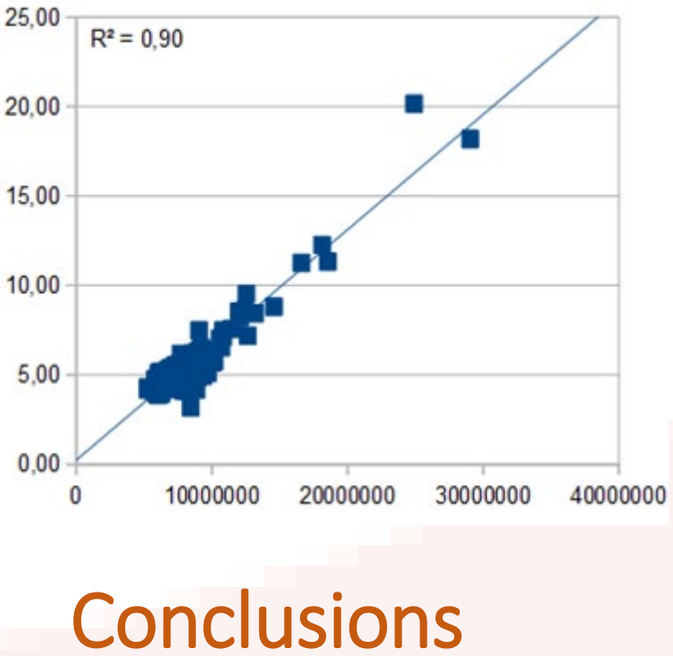
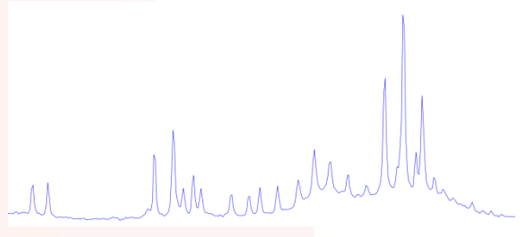


Figure 2. Linear regression of glucose concentration.
Scatter plot of the plasmatic glucose concentration of 184 subjects comparing enzymatic method and ^{1}H-NMR.

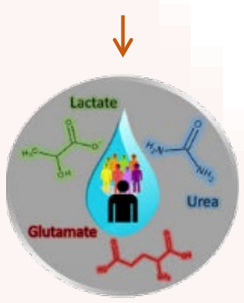
Materials and Methods

Study population

LMWMscale Test



^{1}H-NMR spectra from different biological matrixes including 4.800 sera, 107 fecal extracts, 443 urines, 21 cell cultures and 21 culture medium samples.



Our algorithm quantitatively profiled at least 10 metabolites (in 95 % of similar samples) for each biological matrix

Analytical validation

Analytical validation of the NMR metabolite concentration was evaluated by linear regressions and Pearson's correlation coefficient (r) with analogous measurements obtained by using enzymatic methods.

Table 1. List of some of the most relevant metabolites profiled by LMWMscale.

Metabolite	Biological matrixes				
	Serum	Fecal extract	Urine	Cell culture	Culture medium
3-Hydroxybutyrat	✔				
Acetate	✔	✔	✔		
Acetone	✔				
Alanine	✔	✔	✔		✔
Creatine	✔	✔	✔		
Creatinine	✔	✔	✔		
Formate	✔	✔	✔		✔
Glucose	✔	✔	✔		✔
Glutamate	✔	✔			
Glutamine	✔	✔	✔		✔
Glycine	✔	✔	✔	✔	✔
Lactate	✔	✔	✔		✔
Methylhistidine	✔	✔	✔		✔
Tyrosine	✔	✔	✔		✔
Valine	✔	✔	✔	✔	✔
Isoleucine	✔	✔	✔	✔	✔
Leucine	✔	✔	✔	✔	✔
Choline		✔	✔	✔	✔
ATP				✔	
Lysine		✔	✔	✔	✔
Phenylalanine		✔	✔		✔
Pyruvate					✔
Butyrate		✔			
Malonate		✔			
Methanol		✔			
Propionate		✔			
Succinate		✔	✔		
Threonine		✔	✔		
Trimethylamine		✔	✔		

Conclusions

The *LMWMscale® Test* provides automatic quantitative screening of LMWMs present in several biological matrixes from its ^{1}H-NMR spectra. Our algorithm can be coupled to our NMR lipoprotein and glycoprotein profile methodology (*the glycoscale and the Liposcale test* **-IVD-CE**) in a high-throughput mode for global molecular screening compatible with clinical and epidemiological requirements.

References

[1] C. B. Clish, "Metabolomics: an emerging but powerful tool for precision medicine," *Mol. Case Stud.*, vol. 1, no. 1, p. a000588, Oct. 2015.

[2] A. H. Emwas *et al.*, "NMR spectroscopy for metabolomics research," *Metabolites*, vol. 9, no. 7. MDPI AG, 01-Jul-2019.

Who are we?

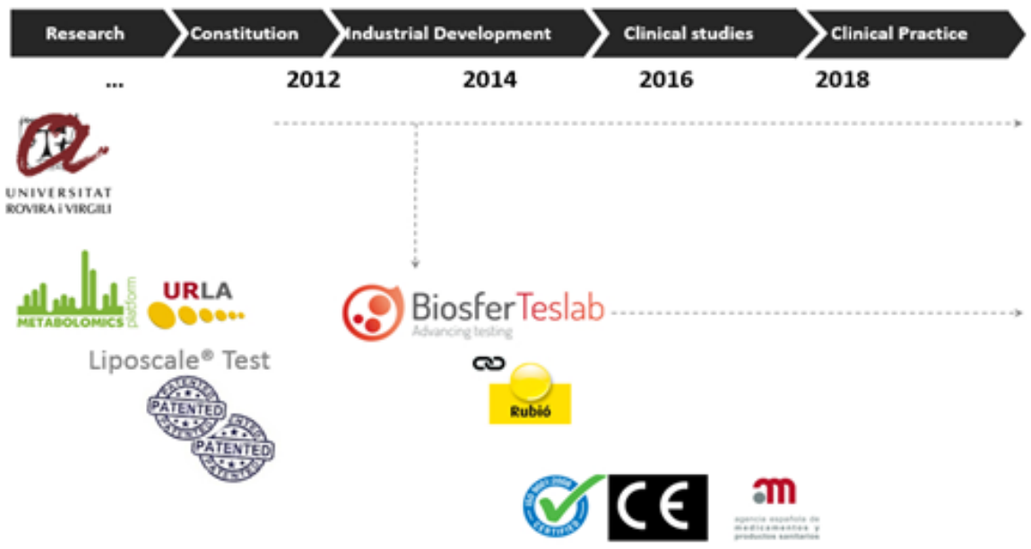
Company: Biosfer Teslab is a spin-off from Rovira i Virgili University (URV) and Pere Virgili Health Research Institute (IISPV), established in 2013 (Reus, Spain).

Philosophy:

Mission: To provide services for the analysis of biological fluids and tissues by Nuclear Magnetic Resonance (NMR) and to develop medical software, to help and facilitate health professionals and the scientific community to study, diagnose and treat metabolic alterations in order to advance knowledge and improve the health of people.

Vision: To be a reference biotechnology company, well connected with the main actors that form the international biomedical research network and to be committed to innovation and continuous improvement.

Values: Transparency, Collaboration, Professionality, Territoriality and Multidisciplinarity.



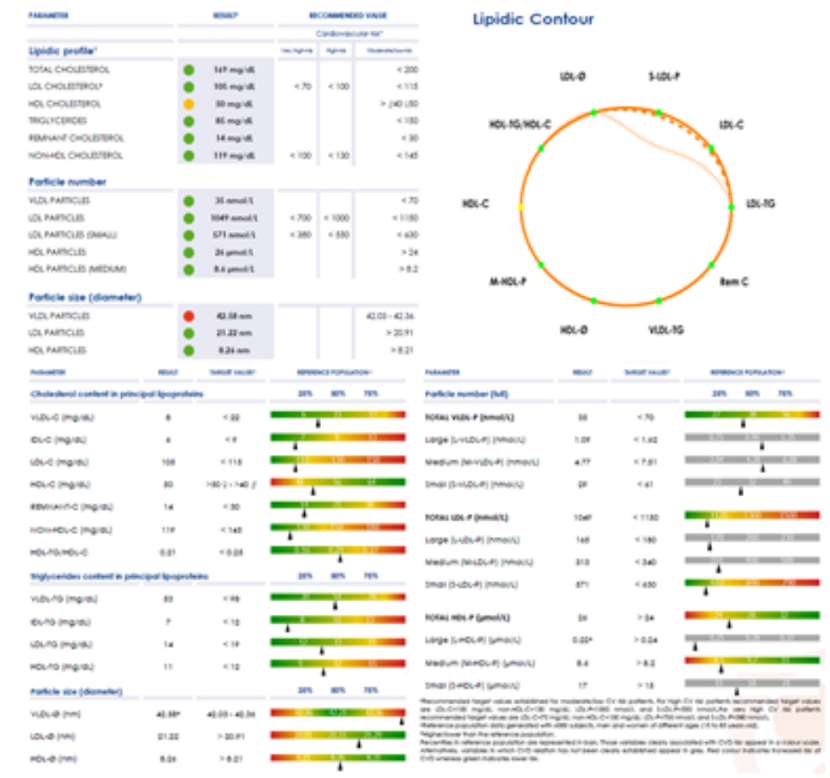
What do we offer?

SERUM & PLASMA — Lipoprotein Profile (Liposcale Test ®)

The Liposcale Test® is an advanced lipoprotein test, based on NMR spectroscopy, a high throughput methodology, which allows a more accurate stratification and evaluation of cardiovascular risk (CVR) beyond classical parameters[1].

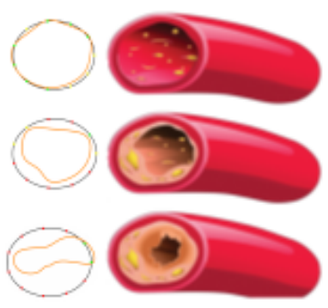
The Liposcale Test® determines 23 variables (see figure below), including: cholesterol and triglycerides concentration in each lipoprotein fraction (VLDL, IDL, LDL and HDL), as well as the particle concentration of 9 lipoprotein subfractions (small, medium and large VLDL, LDL and HDL) and the VLDL, LDL and HDL particle size[1].

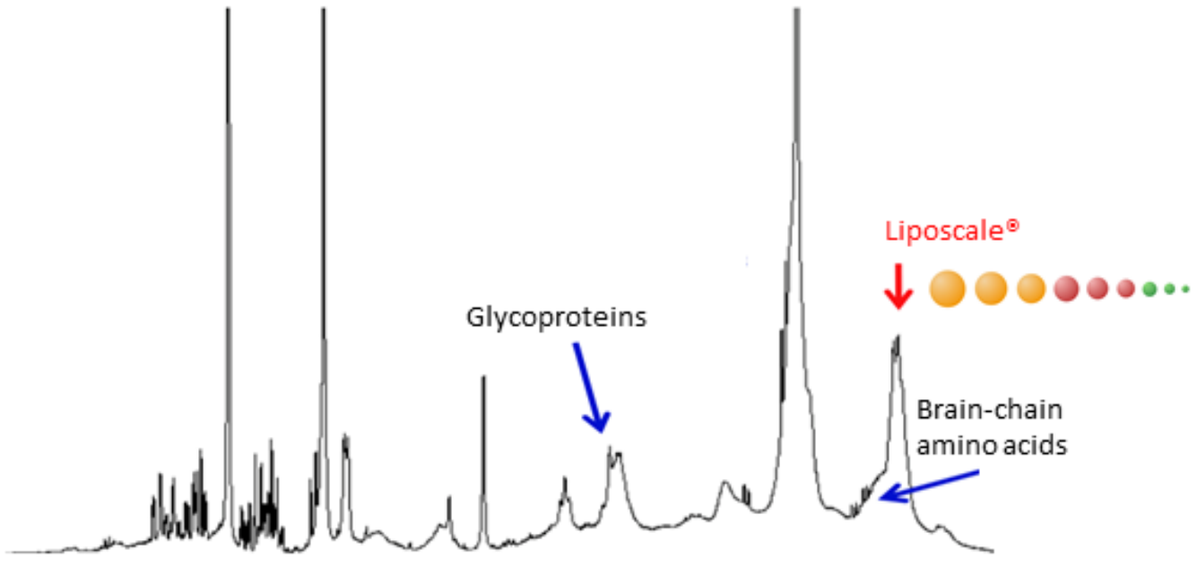
Example of The Liposcale Test report. First part includes the classical lipid profile and a graphic model which summarizes patient's lipoprotein profile.



The lipid contour is a graphical model which simulates an arterial transversal section (see figure below) providing a comprehensive and rapid evaluation of patients' lipoprotein metabolism beyond classical parameters.

Example of three different lipid contours: When lipoprotein variables are altered and associated with a higher risk for atherosclerosis, the patient's orange silhouette defines a reduced area.



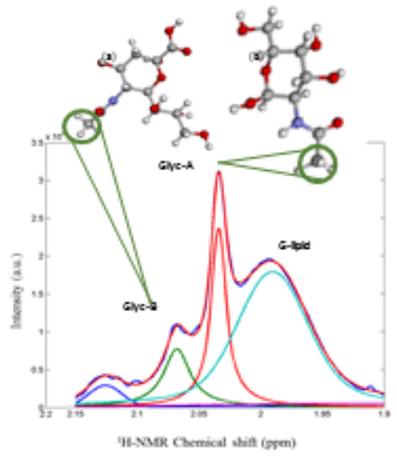


^{1}H-NMR Longitudinal Edit Current Delay (LED) spectrum of a serum sample.

SERUM & PLASMA — The Glycoprotein Profile

Glycosylation is the most common post-translational protein modification, being a key biological process involved in different situations including cell adhesion, molecular transport, signal transduction, modulation of the immune system and **inflammation**.
Their role in protein structural changes makes them suitable as potential early biomarkers of disease.

Glycosylated protein quantification in plasma is not included in the clinical routine, due to the lack of a high-performance, fast and sufficiently sensitive technology. ^{1}H-NMR spectroscopy appears as a suitable methodology for plasma glycoprotein profiling in a fast and reliable way[2].



The Glyc-A and Glyc-B NMR signals are associated with N-acetylglucosamine and N-acetylgalactosamine (Glyc-A) and N-acetylneuraminic acid (Glyc-B) bound protein concentration and their aggregation state.

SERUM, PLASMA & TISSUES — Low Molecular Weight Metabolite Profile — URINE, FECAL & CELLS

Metabolic profiling using NMR spectroscopy is a key application in modern metabolic research, used to provide detailed characterization for biofluid (urine, serum/plasma) and tissue samples that allows to monitor different groups of metabolites for biomarker discovery. The standard acquisition time per sample is 4-5 min, and both preparation and analysis can be automated to allow application to high-throughput screening for clinical diagnostic and toxicological studies, as well as molecular phenotyping and functional genomics.

Common low molecular weight aqueous metabolites profiled in human biofluids using NMR spectroscopy.

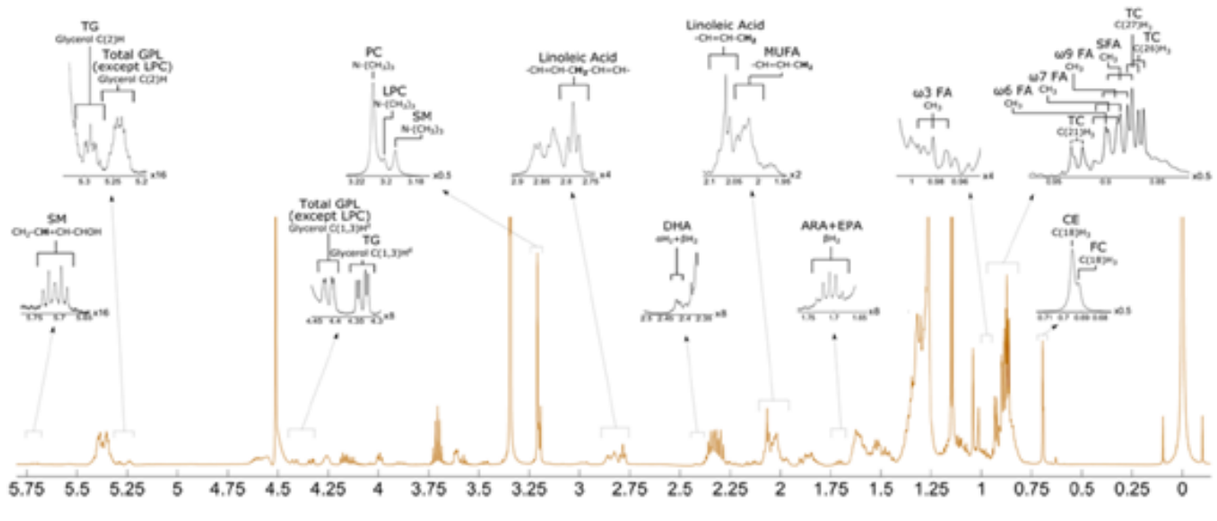
Leucine	Acetate	Isoleucine	Creatinine	Lysine	Hypoxantine
Valine	Acetone	Lactate	Myo-inositol	Dimethylamine	Xanthosine
Alanine	Glucose	Creatine	Succinate	Asparagine	Malonate
Glycine	Formate	Choline	Pyruvate	Oxoglutarate	Galactose
Tyrosine	3-Hydroxybutirate	Butyrate	NAD	Threonine	Xylose
Glutamine	Citrate		ATP	Cis-Aconitate	P-Cresol

SERUM & PLASMA — Lipid Profile — CELLS & TISSUES

Lipids are very heterogeneous molecules involved in most of the biological processes, and they can be very useful in the study of several metabolic disturbances [3].

Lipid profiling can be done from different biological sources, such as: plasma, serum, tissues and cell cultures, after a standard extraction protocol by using organic solvents.

Lipid species present in human serum determined by 1H-NMR spectroscopy [3]:



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