IVDr Research by NMR

- An Innovative Analytical Solution for Biobanking (for research use only)

NMR

Innovation with Integrity

NMR in the World of BioBanks

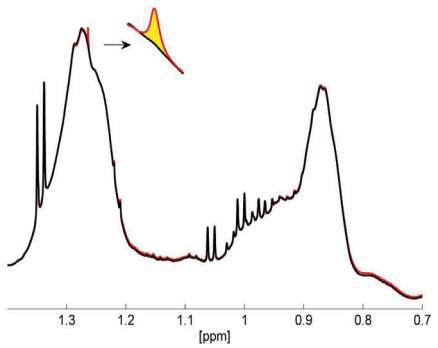
High Resolution NMR has rapidly developed into a leading tool for the analysis of body fluids and tissues. It is used in high-throughput push button automation to generate spectra, which are the input for automatic analysis to quantify markers or to be compared with statistical models of health or disease states. It also allows large scale epidemiological studies due to its outstanding instrumental reproducibility and transferability from lab to lab. Combining this with Standard Operation Procedures and a standardized instrumental platform allows to integrate data generated all over the world with data from a biobank network.

How Does NMR Relate to Biobanks and their Daily Work?

NMR can be used as QC for input of samples into the biobank and at the same time with the same measurement create additional value in terms of analytical information:

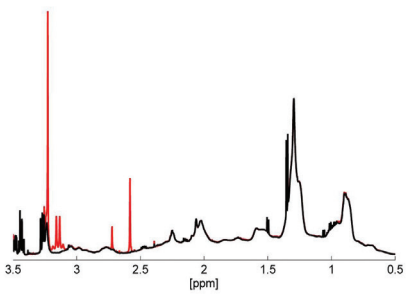
- Validate the type of sample e.g. EDTA Plasma (see Figure 1), Citrate Plasma and Quality Control to check for contamination and impurities (see Figure 2). Results are summarized in a Qualtity Control report (B.I.BioBankQC , see Figure 3)
- Produce NMR spectra for input into the biobank information for each sample, NMR spectra are produced under standard conditions and can be used for integrated spectral bases from different biobanks worldwide
- Generate a lipoprotein subclass panel with 114 parameters and with the same experiment generate a list of 41 quantified small molecules in plasma/serum
- Generate a list of 150 quantified parameters in urine including concentration ranges

Figure 2



Identification of contamination with disinfection material, see yellow labeled peak

Figure 1



Identification of EDTA Plasma - see signals in red

Figure 3

Analysis Report
Bruker IVDr **BioBank QC** B.I.BioBankQC™ in **U**rine

Test	Result	Flag
NMR Experiment Parameter Test	passed	
NMR Experiment Quality Test	passed	
NMR Preparation Quality Test	passed	
Matrix Identity Test	Urine	
Matrix Integrity Test	passed	
Matrix Contamination Test	passed	
Medication Test	not passed	
Protein Background Test	passed	
Further Indicative Parameter Test	not passed	

Analysis Report
Bruker IVDr **BioBank QC** B.I.BioBankQC™ in **P**lasma/**S**erum

Test	Result	Flag
NMR Experiment Parameter Test	passed	
NMR Experiment Quality Test	passed	
NMR Preparation Quality Test	passed	
Matrix Identity Test	Citrate plasma	
Matrix Integrity Test	not passed	
Matrix Contamination Test	not passed	

Figure 3 : Extract of the summary page of the B.I.BioBankQC-UR and B.I.BioBankQC-UR analysis report

The methods and solutions described here are for research use only and not for use in clinical diagnostic procedures.

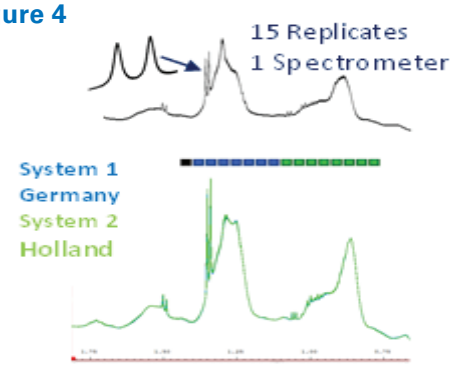
Suitability of NMR for Spectral Database Usage and Data Exchange

Reproducibility and transferability of the NMR analysis under identical SOPs and using a standardized NMR platform is shown in Figure 4. Aliquots of Serum samples have been prepared and measured, showing in the upper part the overlay of spectra of 15 replicates prepared and measured individually one instrument, while the lower spectrum shows the overlap of 8 spectra each measured in 2 locations on identical machines under the same SOPs using replicate samples. As can be seen there is a perfect overlap of all spectra. This is the proof, that spectra from different biobanks can be used for integrated statistical analysis. For new studies therefore spectra can be used instead new aliquots to be taken from the biobank.

Add Value to the Biobank with Analytical Data

For every Biobank, the value also lies in the metadata associated to the samples stored. This can be patient data, lifestyle information, but also analytical data, like lipoprotein subclasses analysis (B.I.LISA) and concentration of small molecules (B.I.QUANT-PS 2.0) in plasma/serum and concentration of endogenous and disease related metabolites (B.I.QUANT-UR b,e,ne) in urine. Using directly the spectra acquired for quality control, these analytical results can be generated automatically including concentration distributions calculated from all biobank samples analyzed. Since many samples are analyzed in a biobank it is possible to define normal concentrations in the cohort investigated. Figure 5 shows an excerpt of the lipoprotein subclass analysis obtained from 2 samples, one from a healthy subject and one from a person who had a stroke 3 days after sample collection, clearly showing the deviations, which are indicative of a risk profile. Figure 6 shows an excerpt from the quantification of endogenous metabolites including the cohort concentration distribution with the actual sample shown in the profile as a stick.

Figure 4



^{1}H NMR in Serum at 600 MHz

Figure 5

Lipoprotein subclass analysis comparing healthy (left) and risk profile (right)

Figure 6

2 Amines and derivatives

Compound	Conc. mmol/L	Conc. mmol/mol Crea	LOD mmol/mol Crea	95% Range mmol/mol Crea	Graphics (*)
Dimethylamine	0.25	57	31	≤ 54	
Trimethylamine	0.03	6	2	≤ 3	

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

3 Amino acids and derivatives

Compound	Conc. mmol/L	Conc. mmol/mol Crea	LOD mmol/mol Crea	95% Range mmol/mol Crea	Graphics (*)
1-Methylhistidine	< 0.07	< 15	15	≤ 15	
2-Furoylglycine	< 0.17	< 39	39	≤ 40	
4-Aminobutyric acid	< 0.09	< 20	20	≤ 20	
Alanine	0.34	77	10	11 - 72	
Arginine	< 3.3	< 750	750	≤ 750	
Betaine	0.34	76	7	9 - 78	
Creatine	1.9	440	50	≤ 280	
Glycine	1.3	300	34	38 - 440	
Guanidinoacetic acid	0.52	120	100	≤ 140	
Methionine	< 0.08	< 18	18	≤ 18	
N,N-Dimethylglycine	0.08	19	5	≤ 15	
Sarcosine	0.01	3	2	≤ 7	
Taurine	0.76	170	140	≤ 170	
Valine	0.02	4	2	≤ 7	

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

Excerpt of the quantification of endogenous metabolites in urine (B.I.QUANT-UR b)

Figure 7 shows an excerpt from the quantification of small molecules in plasma/serum including the cohort concentration distribution with the actual sample shown in the profile as a stick. Further analytical analysis is available on request.

Figure 7

3 Amino acids and derivatives

Compound	Conc. mmol/L	LOD mmol/L	r mmol/L	ρ %	Δ mmol/L	95% Range mmol/L	Graphics (*)
2-Aminobutyric acid	< 0.05	0.05	0.031	8 ○	0.087	⩽ 0.10	
Alanine	0.35	0.02	0.351	100 ●	0.010	0.29 - 0.64	
Asparagine	< 0.05	0.05	0.000	0 ○	5.082	⩽ 0.08	
Creatine	0.01	0.01	0.014	100 ●	0.002	⩽ 0.07	
Creatinine	0.09	0.01	0.091	100 ●	0.002	0.06 - 0.14	
Glutamic acid	< 0.05	0.05	0.047	53 ○	0.031	⩽ 0.24	
Glutamine	0.83	0.02	0.832	99 ●	0.023	0.30 - 0.83	
Glycine	0.26	0.01	0.257	100 ●	0.006	0.17 - 0.44	
Histidine	0.13	0.02	0.127	100 ●	0.002	0.07 - 0.16	
Isoleucine	0.04	0.03	0.043	93 ○	0.010	0.03 - 0.11	
Leucine	0.09	0.01	0.090	95 ●	0.013	0.07 - 0.20	
Lysine	0.19	0.04	0.194	66 ○	0.073	⩽ 0.29	
Methionine	0.09	0.05	0.093	97 ●	0.009	0.05 - 0.13	
N,N-Dimethylglycine	< 0.01	0.01	0.001	88 ○	0.000	⩽ 0.01	
Ornithine	< 0.02	0.02	0.000	0 ○	2.497	⩽ 0.16	
Phenylalanine	< 0.03	0.03	0.028	97 ●	0.003	⩽ 0.07	
Proline	0.29	0.05	0.293	24 ○	3.267	⩽ 0.59	
Sarcosine	< 0.01	0.01	0.004	80 ○	0.001	⩽ 0.01	
Threonine	< 0.04	0.04	0.000	0 ○	3.053	⩽ 0.24	
Tyrosine	0.04	0.03	0.038	97 ●	0.003	⩽ 0.08	
Valine	0.23	0.03	0.233	100 ●	0.005	0.15 - 0.35	

Excerpt of the quantification of amino acids and derivatives metabolites in plasma/serum (B.I.QUANT-PS 2.0)

Bruker BioSpin

Providing NMR Solutions for Metabolomics

NMR is an advantageous technique for metabolomic research, providing high reproducibility, simple sample preparation and the ability to measure different small molecule metabolites simultaneously. Today, new advances in software and hardware platforms have made NMR more effective, easier to use and more cost efficient. Discover for yourself how NMR can help illuminate metabolic networks.

Bruker BioSpin

info@bruker.com
www.bruker.com