In Vitro Diagnostic Research Solutions

- IVDr by NMR, the Platform for Metabolic Profiling (for research use only)

Addressing Major Clinical Challenges

The accurate determination of even small changes in profiles related to a disease, therapeutic intervention, genetic modification or environmental variation is central to all metabolomics studies. At Bruker BioSpin we aspire to find NMR solutions that support metabolic profiling, be it on a personal or population wide basis.

IVDr Metabolic Profiling - Providing Key Information

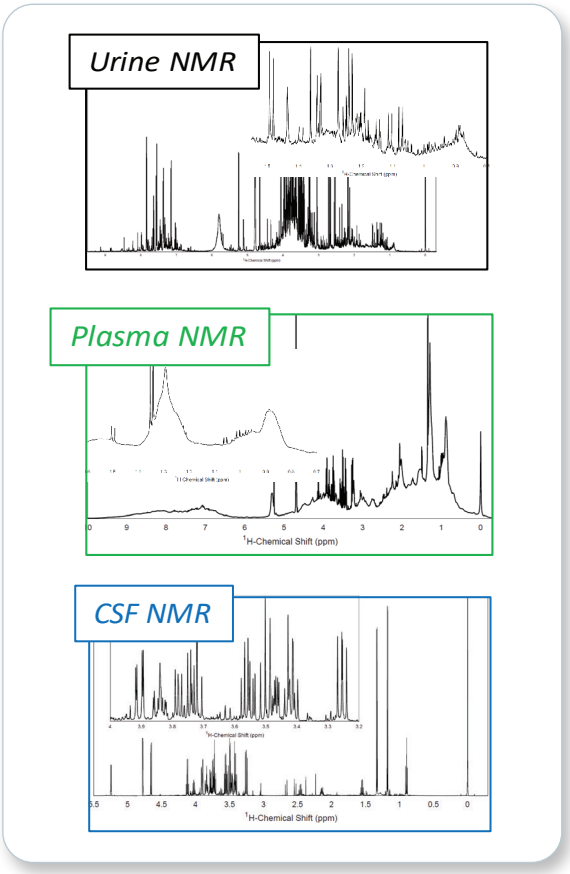
- Combination of targeted and non-targeted analysis throughout the human lifespan from neonates to elderly population.
- Helps to better understand disease from early detection, grading, treatment and even prevention.
- Develops concepts for personalized medicine and nutrition
- Especially suited for large scale epidemiology and clinical trials combining multiple site analysis data.

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

What IVDr by NMR can Bring to your Lab

NMR spectroscopy has been established as a reliable tool for targeted analysis (quantification of metabolites) as well as untargeted analysis (multivariate statistics on complete spectroscopic profiles) in human body fluids (plasma, serum, urine and CSF).

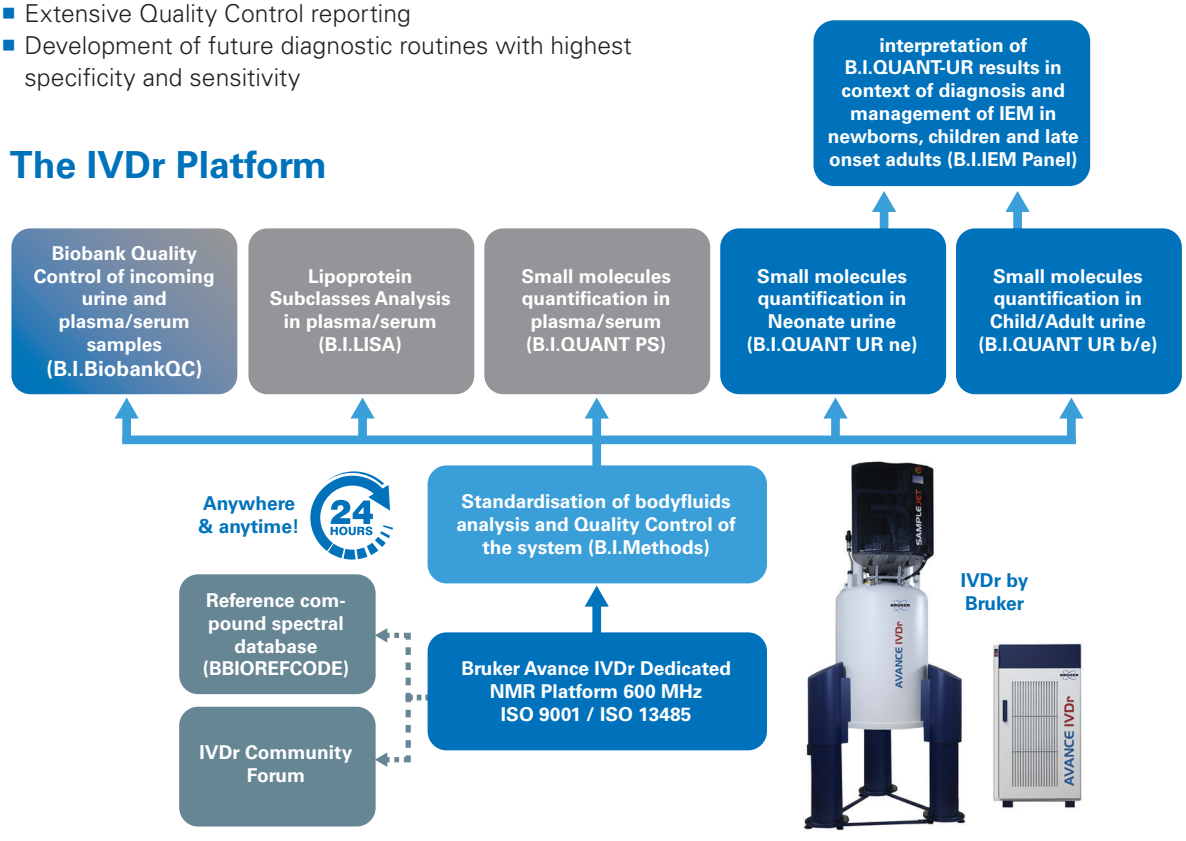
NMR is a nondestructive technique and allows for measurement of a sample as is. Thousands of metabolites contribute to the spectral fingerprint of a body fluid sample which consist of more than a thousand individual signals. As a result, a body fluid NMR spectrum contains information on a multitude of co-existing factors, relating to a diversity of physiological conditions, phenotype and genome. This makes NMR a unique platform for investigating human body fluid composition and spectral fingerprints in relation to health and disease.



The IVDr platform offers :

- Completely standardized and fully automated solutions for clinical and translational research
- Highest reproducibility of results across platforms suitable for large scale phenomics and clinical metablomics studies
- Complete transferability of solutions offered on the IVDr platform on a worldwide basis
- Output of a large number of parameters for plasma/serum and urine within one experiment
- Extensive Quality Control reporting
- Development of future diagnostic routines with highest specificity and sensitivity

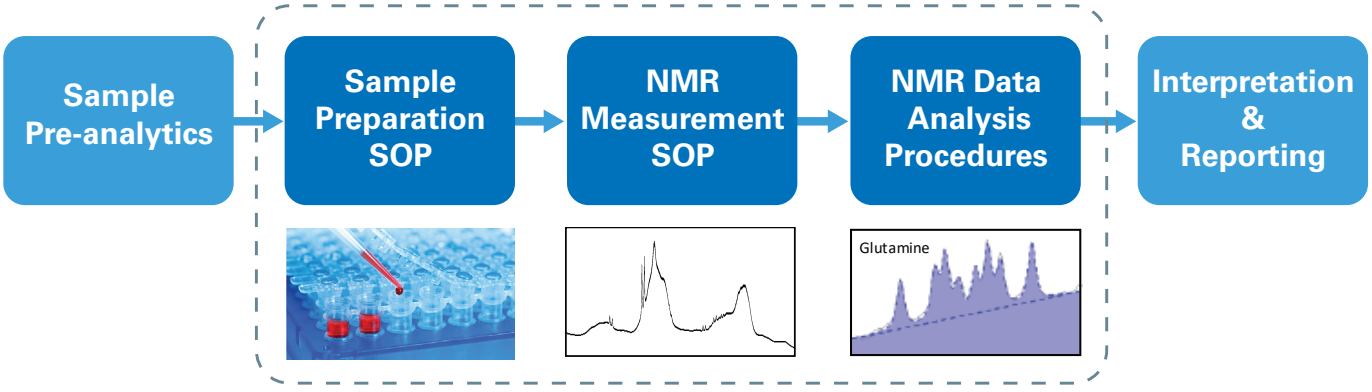
The IVDr Platform

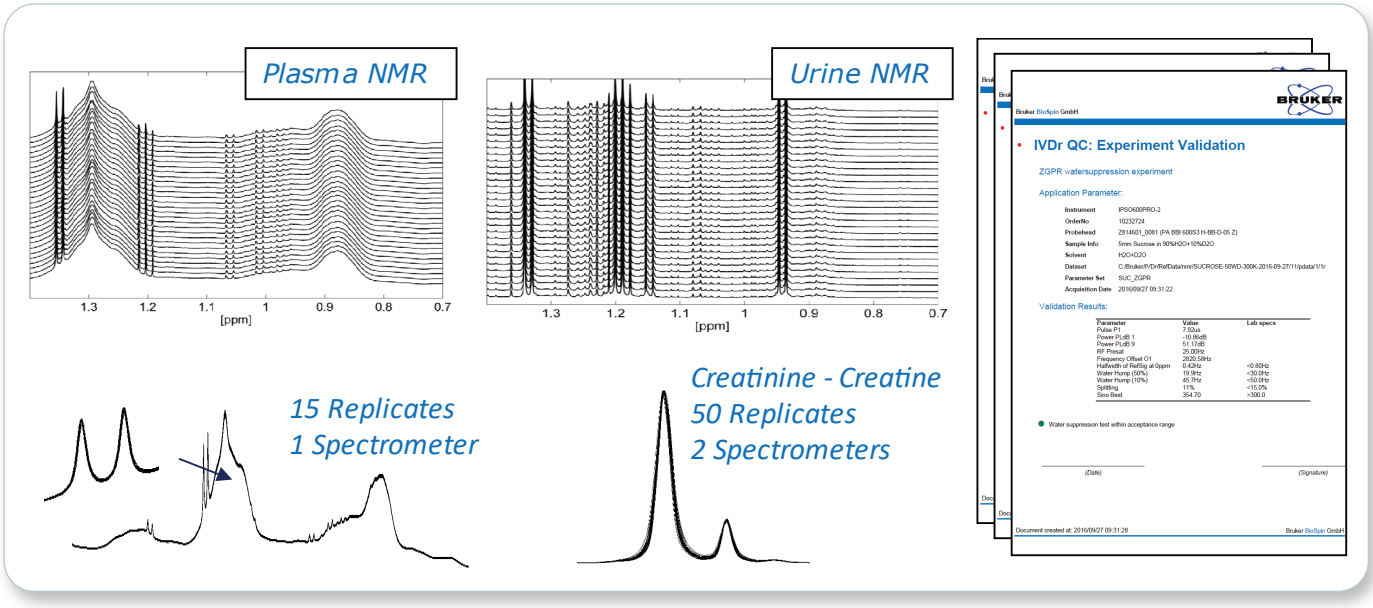


Highly Standardized and Automated Methods for Bodyfluid Analysis

New Bruker IVDr Methods (B.I.Methods 2.0)

- Simple and rapid sample preparation for urine, plasma/serum, CSF and Methanol extract of bodyfluids, cell cultures and tissues
- No complex sample identification required
- Ease of use – no need for operator expertise in NMR
- Extensive Quality Control from sample preparation to through measurement, documented in a QC report
- For Methanol extract samples, fully automated and highly reproducible multiple suppression of the methanol and satellites peaks. This multiple solvent suppression is extremly valuable to enhance the Signal to Noise Ratio in intact biofluid samples.
- Highest reproducibility and data quality under full automation – anytime & anywhere
- Handling of large data sets with simple retrieval of information
- Rapid transfer from spectra to relevant information
- Retrospective analysis
- Integration of spectra and results from different groups





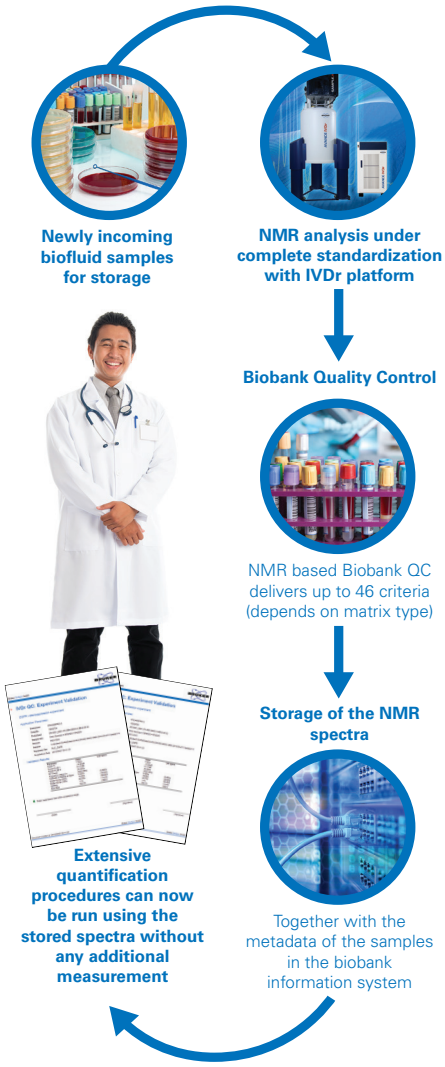
Stringent Quality Control and Characterization Under Full Automation

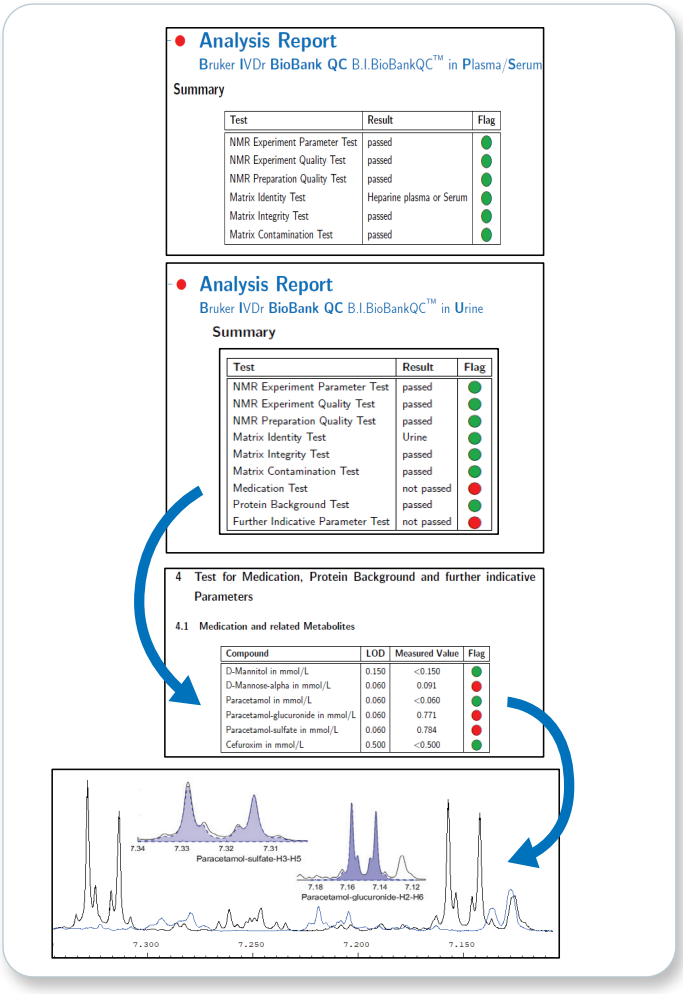
Bruker IVDr BioBankQC (B.I.BioBankQC)

- Delivers comprehensive number of quality parameters starting from pre-analytics to degradation state , check for fasting conditions or unreported drugs
- Retrospective analysis of previously recorded data
- Standardized and transferable procedure
- Worldwide exchangeability of NMR data among biobanks
- Extensive quantification procedures can be run with the stored spectra without any new measurement, delivering:
  - 150 compound quantification in urine
  - > 150 parameters in plasma/serum on lipoprotein subclass analysis and small molecule quantification.

In addition to quality control, biobanks can now support epidemiological studies or clinical trials by using the metadata of selected NMR spectra or quantification results. This will reduce the need for new aliquots and substantially reducing the cost of clinical trials or research studies.

Further savings can be gained by sharing the spectral data. New NMR-based diagnostic tests can be validated on a worldwide basis and on multiple phenotypes without exploding the cost of the trial. Data generated from an 11 IVDr platform Inter-Laboratory Trial proves this unique benefit of NMR[1].



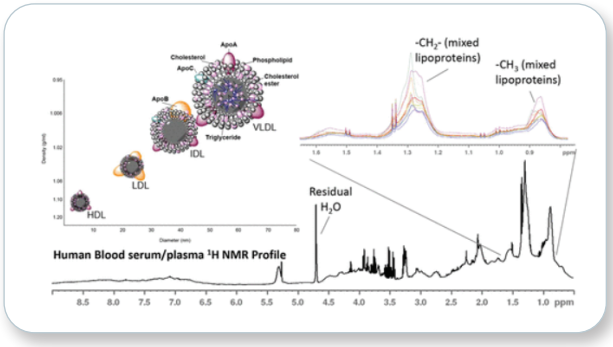


Lipoprotein Composition

Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA)

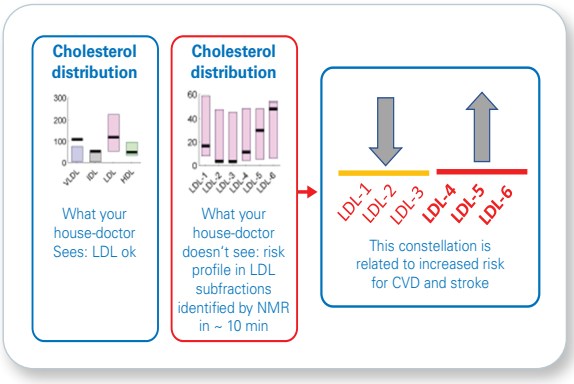
Lipoprotein composition plays a major role in differentiating health and disease state as well as treatment monitoring. The output of B.I.LISA consists of 112 parameters describing the lipoprotein composition in plasma/serum in detail. Such analysis supports research and produces invaluable input in:

- Epidemiology
- Frequent health problems like cardiovascular diseases, diabetes and cancer
- Information about personalized profile in plasma/serum
- Food and environmental influence to health
- Ability to monitor and optimize treatment

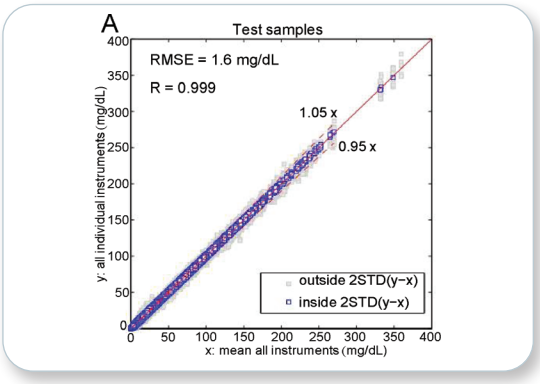


	Total Plasma	VLDL	IDL	LDL	HDL	VLDL 1-5	LDL 1-6	HDL 1-4
Triglycerides	✓	✓	✓	✓	✓	✓	✓	✓
Cholesterol	✓	✓	✓	✓	✓	✓	✓	✓
Free Cholesterol	✓	✓	✓	✓	✓	✓	✓	✓
Phospholipids	-	✓	✓	✓	✓	✓	✓	✓
Apo-A1	✓	-	-	-	✓	-	-	✓
Apo-A2	✓	-	-	-	✓	-	-	✓
Apo-B	✓	✓	✓	✓	-	-	✓	-
Particle number	✓	✓	✓	✓	-	-	✓	-

Listing of all parameters generated by B.I.LISA



Lipoprotein Subclass analysis sees more within a turnover of 10 min. Example for future diagnostics



Interlaboratory reproducibility. Analysis of commercially sourced samples (74 measures in total for 11 instruments)[1]

Quantification of Small Molecules in Plasma/Serum

Bruker Quantification in plasma/serum (B.I.QUANT-PS 2.0)

Having 2 different angles on a disease allows advanced analysis and future diagnosis. With the same experiment as B.I.LISA, it is possible to quantify small molecules. The output of B.I.QUANT-PS 2.0 consists of 41 parameters describing the small molecule composition in plasma/serum in detail. Such analysis supports research and produces invaluable input in:

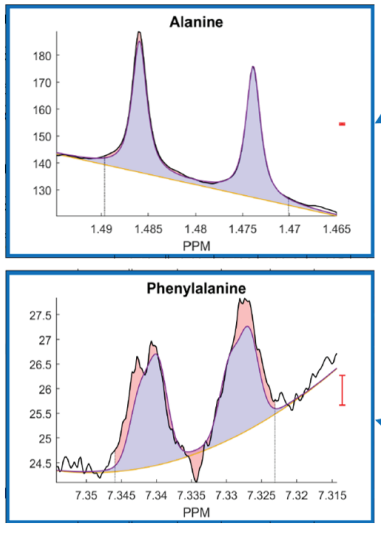
- High blood pressure
- Fatty Liver disease
- Alzheimer
- Inflammatory diseases
- Cancer (e.g. via blood triglycerides)
- Cerebrovascular diseases
- Inborn Errors of Metabolism
- Stroke
- Diabetes type 2
- Obesity
- Metabolic syndrome
- General Cardiovascular diseases (in prevention, early detection and treatment)

The automated quantification in plasma/serum is based on in house developed algorithms involving fitting predefined ^{1}H signals as it is already the case for urine samples (B.I.QUANT-UR™). By looking at the pdf report, it is possible to judge interactively on the fit reliability for each metabolite.

Benefits of B.I.QUANT-PS 2.0

- Ease of use, simple and rapid sample preparation without protein denaturation
- Fully automated and simultaneously quantification of 41 metabolites from different analytes classes in a single run
- Absolute concentration and additional quantification result assessment information for each metabolite are given due to the calibration with only one Quantification Reference Sample (B.I.Methods2.0™)
- Interactive pdf report allowing visual fit ambiguity assessement
- Validation of all LODs has been done following ISO 17025 guidelines for wet spiking
- Rapid analysis: on a daily basis up to 160 samples can be prepared, measured and analyzed
- Retrospective analysis possible if the sample was prepared and measured using B.I.Methods module

By positing the mouse cursor on a metabolite name, the corresponding graphical fit display pops up in PDF report



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	0.000	0 ○	0.411	⩽ 0.10	
Alanine	0.19	0.02	0.186	100 ●	0.005	0.29 - 0.64	
Asparagine	< 0.05	0.05	0.018	77 ○	0.008	⩽ 0.08	
Creatine	< 0.01	0.01	0.005	99 ●	0.001	⩽ 0.07	
Creatinine	0.05	0.01	0.049	99 ●	0.001	0.06 - 0.14	
Glutamic acid	< 0.05	0.05	0.025	23 ○	0.033	⩽ 0.24	
Glutamine	< 0.02	0.02	0.000	0 ○	1.428	0.30 - 0.83	
Glycine	0.15	0.01	0.153	100 ●	0.004	0.17 - 0.44	
Histidine	0.06	0.02	0.063	99 ●	0.001	0.07 - 0.16	
Isoleucine	0.04	0.03	0.035	98 ●	0.004	0.03 - 0.11	
Leucine	0.07	0.01	0.072	99 ●	0.004	0.07 - 0.20	
Lysine	0.12	0.04	0.120	53 ○	0.046	⩽ 0.29	
Methionine	< 0.05	0.05	0.020	88 ○	0.003	0.05 - 0.13	
N,N-Dimethylglycine	< 0.01	0.01	0.000	95 ●	0.000	⩽ 0.01	
Ornithine	< 0.02	0.02	0.018	91 ○	0.010	⩽ 0.16	
Phenylalanine	< 0.03	0.03	0.028	94 ○	0.003	⩽ 0.07	
Proline	0.15	0.05	0.155	64 ○	0.103	⩽ 0.59	
Sarcosine	< 0.01	0.01	0.004	100 ●	0.000	⩽ 0.01	
Threonine	< 0.04	0.04	0.000	0 ○	1.113	⩽ 0.24	
Tyrosine	< 0.03	0.03	0.028	99 ●	0.001	⩽ 0.08	
Valine	0.13	0.03	0.129	100 ●	0.003	0.15 - 0.35	

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

Extract from B.I.Quant-PS 2.0 report: Taking the example of Alanine, we are in an ideal situation, where the fit corresponds fully to the metabolite signal well above LOD, the raw concentration (r) is similar to the final result concentration and the correlation (ρ) is > 95% and the residue (Δ) is close to zero mmol/L. Concerning the phenylalanine signal, the raw concentration is below LOD. Using the graphical figure displayed, the fit signal can be clearly discriminated from the rest of the spectrum and the correlation is 95%. The raw concentration can be used as approximative concentration estimate.

Automated and Robust Quantification in Urine

Bruker IVDr Quantification in Urine (B.I.QUANT-UR)

Urine is an important and often used bodyfluid in metabolomics because it is easy to obtain and there is mostly no interference with metabolite binding proteins. Furthermore, urine is an extremely information-rich but very complex bodyfluid that contains metabolic breakdown products from a wide range of nutrients, drugs, environmental contaminants, endogenous metabolites and bacterial by-products. The automatic simultaneous quantification of a multitude of metabolites from different classes is very challenging but possible with B.I.QUANT-UR enabling tool.
The urine quantification B.I.QUANT-UR offers 3 versions:

- B.I.QUANT-UR b: basic version, 50 compounds with concentration ranges, occurring in most human urines
- B.I.QUANT-UR e: extended version, 150 compounds with concentration ranges, age 6 month and up, also including IEM and other disease markers
- B.I.QUANT-UR ne: neonates extended version , 150 compounds with concentration ranges, also including disease markers and non-targeted classification against healthy newborn model

B.I.QUANT-UR b is Used in the Following Cases

- Epidemiology
- Frequent diseases like kidney damage, diabetes, metabolic syndrome, obesity and cancer
- Ability to monitor and optimize treatment
- Microbiom related health problems
- Food and environmental influence to health
- Monitor compound concentrations in personalized urinary metabolic profile

The Extended Versions are Used in the Same Cases as B.I.QUANT-UR b plus:

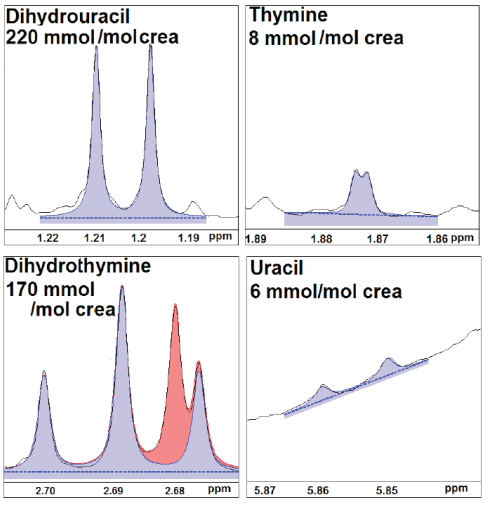
- Pediatrics
- Drug efficiency and treatment monitoring for IEM patients
- Functional food efficiency and dosage/composition
- Selective screening
- Neonatal health

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.

Except report B.I.QUANT-UR b



Visualization of quantification results

Selective Screening by NMR for Newborns

Bruker IVDr for Newborn Screening (B.I.QUANT-UR ne)

Urine selective screening by NMR combines targeted analysis of metabolites including Inborn Error of Metabolism and other disease markers and non-targeted classification against healthy newborn models. Non-targeted screening enables the detection of all NMR-visible deviations from normal references, whether known or unknown. Figure 1 illustrates the identification of deviations from normality. All NMR spectra included in the reference model are combined in a so-called quantile plot, shown as a color band over the NMR spectrum. A spectrum from a new sample can be overlaid and tested for consistency with the model, i.e. all resonances will fit into the envelope defined by the model. This testing can be performed automatically in a uni- or multivariate fashion. The sample spectrum represents a canavan disease case. In the expansion of the overall NMR spectrum, signals of N-Acetylaspartic acid are clearly visible. As part of the B.I.QUANT-UR panel, N-Acetylaspartic acid can be identified and quantified automatically; however statistics would also reveal the existence of previously unseen deviations with the same certainty.

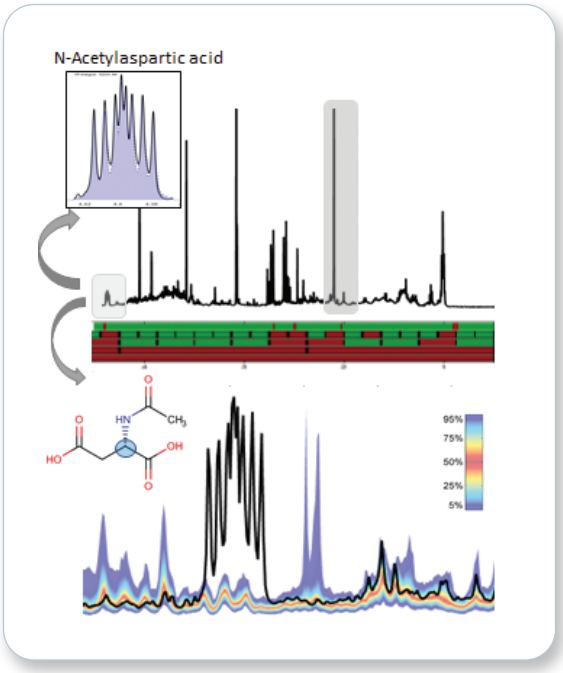


Figure 1 : Projection of the urine spectrum of a inborn error sample into the German normal model.

Influence of Metadata

Beside the use of normal models, it is also possible to investigate the influence of metadata on the NMR spectra. The influence of the day of life after birth on the NMR spectra has been especially investigated and the evolution of Metabolic Profile during the first days of life can be shown (figure 2). When new samples are projected onto this trajectory, it is possible to determine whether the babies' development follows the model pattern. It is clear that sample B corresponds to a baby who has developed further than usual. Sample A is interesting because it comes from day 5 but falls into the group of day 1 and 2. This could indicate a delay in development and therefore advise further testing, as the delay could be the first sign of an inborn error not yet manifested by the usual biomarkers.

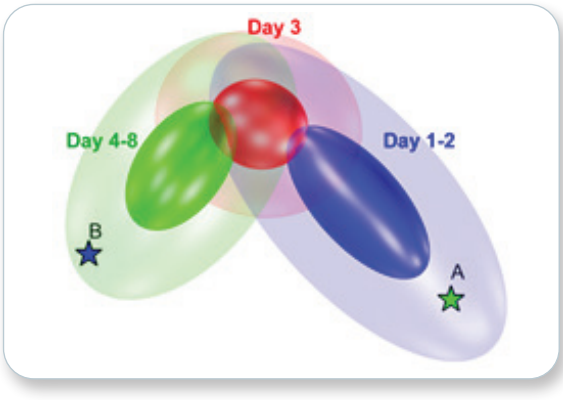
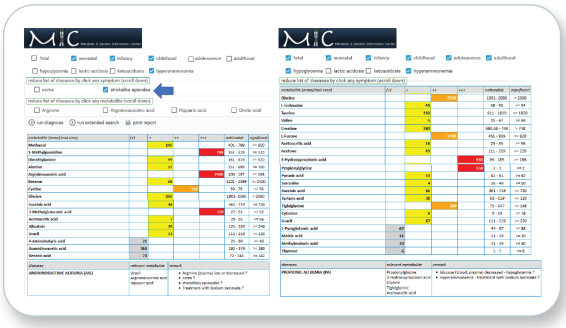


Figure 2 :Trajectory of the first days of life as obtained from the NMR analysis of urine, containing 2 new samples projected onto the trajectory. The color of the stars represents the age group in which the babies should be.

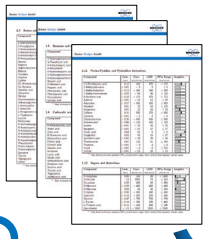
Support for Interpretation in Context of IEM

Bruker IVDr Inborn Error of Metabolism Panel (B.I.IEM Panel)

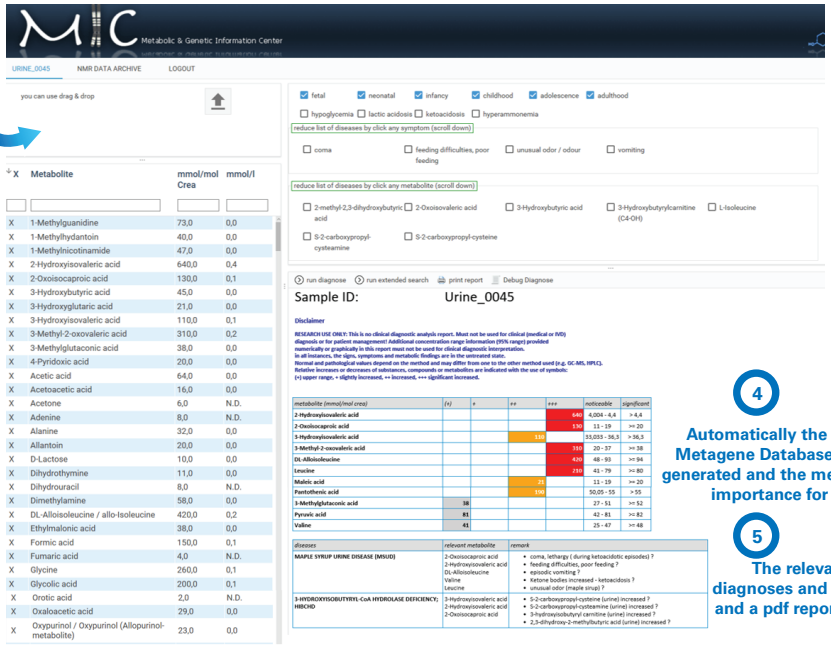
The Bruker IVDr Inborn Errors of Metabolism Panel (B.I.IEM Panel), developed for research use only (RUO), automatically produces valuable support for the diagnostic interpretation of B.I.QUANT-UR results by using the database Metagene. Healthcare professionals can use the information in the context of diagnosis and management of inborn errors of metabolism in newborns, children and adults. The NMR metabolic findings of B.I.QUANT-UR can be compared against a dedicated Bruker version of Metagene's existing database which contains almost 1000 diseases and differential diagnoses, providing clinicians with a wealth of data to support diagnoses. Interactively, it is possible to add clinical relevant information which could help for the ranking of potential diagnoses. This panel approach not only supports diagnostics of inborn errors of metabolism but also research in the Pediatrics field.



Example of 2 reports automatically generated from the B.I.IEM Panel. Additional information has been interactively added by the user.



1 Automated upload of the xml analysis report B.I.QUANT-UR to the dedicated Bruker-Metagene website via drag and drop.



2 All metabolites from B.I.QUANT-UR are listed on this table and labeled with a "X" when the concentration is > LOD. Those metabolites are then used for the comparison with the database.

3 Additional information as age, clinical symptoms or other metabolites not yet quantified by NMR but relevant for the disease can be added interactively to the quantitative results to improve the quality of the ranking of potential diseases.

4 Automatically the metabolites are compared with the Metagene Database. The overview table is automatically generated and the metabolites are classified according their importance for suggesting potential diagnoses.

5 The relevant urinary metabolites for potential diagnoses and clinical symptoms are listed in a ranking and a pdf report with all information can be generated.

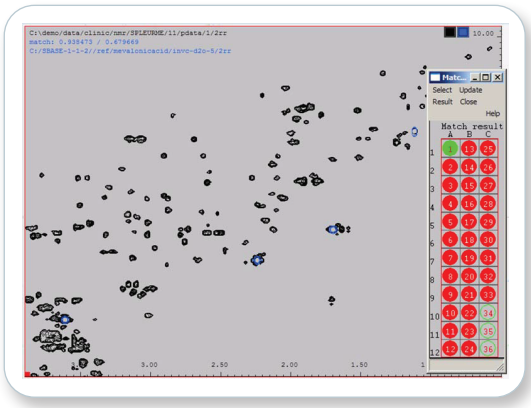
Get More Information on Additional Metabolites

BBIOREFCODE

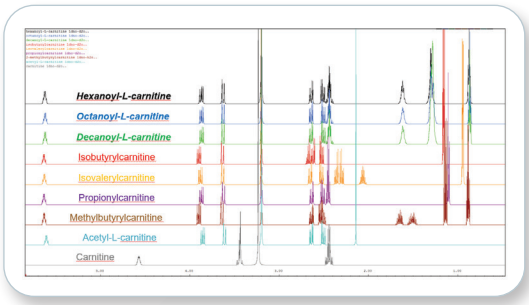
Bruker's Biofluid Reference Compound spectral database (BBIOREFCODE) has been developed for bodyfluids but can be also used for tissues or cell extracts in aqueous solutions. BBIOREFCODE contains information on the most common endogenous metabolites in bodyfluids, some known metabolites of several over-the-counter drugs and selected food components. BBIOREFCODE is of high interest for the non-NMR expert to get more information on further metabolites beyond the ones automatically quantified with B.I.QUANT-UR and B.I.QUANT-PS 2.0

BBIOREFCODE contains actually 800 compounds with more than 23.500 spectra with:

- 1-dimensional spectra (noesygppr1d) at 11 pH values (from 3 to 8 in 0.5 pH steps)
- 2-dimensional spectra (HSQC,HMBC, COSY, TOCSY and JRES) at 2 pH values (3,5 and 7)
- 3D-molfile of all compounds
- Multiple compound names searchable
- All spectra in the database have been acquired on a 600 MHz NMR spectrometer, no data from literature are used.
- It represents the industry standard and all steps are traceable.
- This database together with the Bruker AMIX or ASSURE software allows visual inspection of bodyfluids against pure compound spectra in 1- or 2-dimensional experiments.
- Automatic matching of bodyfluid spectra against the BBIOREFCODE content is also possible.
- In the ongoing process of identifying additional body fluids metabolites, BBIOREFCODE is updated in packages of 50 compounds regularly.



^{1}H spectra of medium chain carnitine compounds (pH 7)



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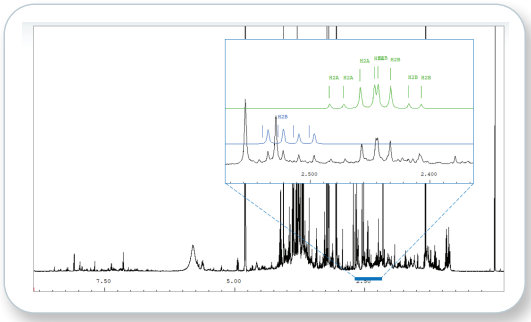
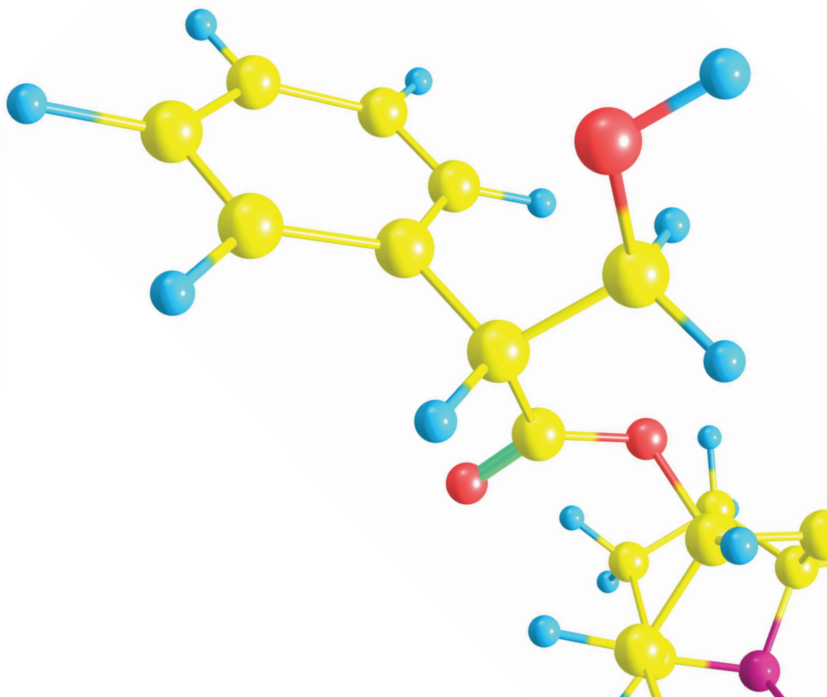


Identification of Acetyl-L-carnitine (blue) and carnitine (green) in the urine ^{1}H spectrum



Communication on a Global Scale

IVDr Forum for Worldwide Communication, Information and Data Exchange

The IVDr Forum is designed to facilitate contact between users of the IVDr and IVDr compatible platform, allowing researchers to easily share information, learn from each other and get access to beta versions of software, Matlab Scripts and, perhaps most importantly, a spectral database of bodyfluids.

Register Once and Gain Access to:

- Spectral database of bodyfluids: Bruker is building a database of spectra from different studies with integrated metadata, which can be downloaded for free and use for academic purposes. Members are encouraged to enter their spectra sets into the database as well.
- Literature, Bruker presentations and news: Relevant literature for the application fields of the IVDr platform is given either as link or directly if allowed. This literature list is regularly updated, when new information is coming in. Early information regarding the IVDr platform, new SOPs, new enabling tools and additions to BBIOREFCODE are communicated.

- Matlab Scripts: A set of Matlab scripts can be downloaded for experienced Matlab users. These have no product status and are delivered as is. Scripts included for read processed TopSpin spectra, plot spectra, Multi-Integrate, Bucketing for input into statistics under Matlab, Statistical correlation spectroscopy (STOCSY), Statistical Heterospectroscopy (SHY) and Quantile Plot.
- Conference information: Learn about the conference participation and activities of Bruker staff. In addition members can ad d information about talks or posters they present at conferences.

1 [Quantitative Lipoprotein Subclass and Low Molecular Weight Metabolite Analysis in Human Serum and Plasma by ^{1}H NMR Spectroscopy in a Multilaboratory Trial Anal Chem. 2018 Oct 16;90(20):11962-11971. doi: 10.1021/acs.analchem.8b02412. Epub 2018 Sep 27]

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