

## Metabo Test **INFAI**<sup>®</sup>

Metabo Test INFAI is a newly developed test that builds on INFAI's long-standing experience in investigating body fluids by nuclear magnetic resonance spectroscopy (NMR). Body fluid samples (urine, plasma, cerebrospinal fluid) of newborn children are used to screen for congenital metabolic disease.

### Congenital Metabolic Disease

Approximately 1:1000 neonates are affected by congenital metabolic diseases in central Europe and 1:500 in Turkey. Undetected and untreated these diseases can lead to irreversible organ failures, invalidity or death. Fully automated NMR spectroscopy of body fluids is used as an analytical approach for diagnosis of known, but also as yet unknown inborn errors of metabolism. Current screening programs only test for a limited number of metabolic diseases, although the number of known metabolic diseases is far higher. For example, newborn children in Germany are screened for only 12 diseases, in Turkey for 5 diseases.

Tandem-mass spectrometry (Tandem-MS) and gas chromatography combined with mass spectrometry (GC-MS) are currently the diagnostic methods of choice for the diagnosis of metabolic disease. However, Tandem-MS can only investigate a total of approximately 30 metabolites with one (group of) metabolite(s) at a time. Analysis of large molecules poses additional technical problems and leads to increasing costs. Due to the lengthy preparation of samples these methods are not suitable for screening. Only investigations in suspected disease are feasible. For GC-MS, samples need to be derivatized with the risk of changing sample properties.

### Magnetic resonance spectroscopy (NMR) of body fluids

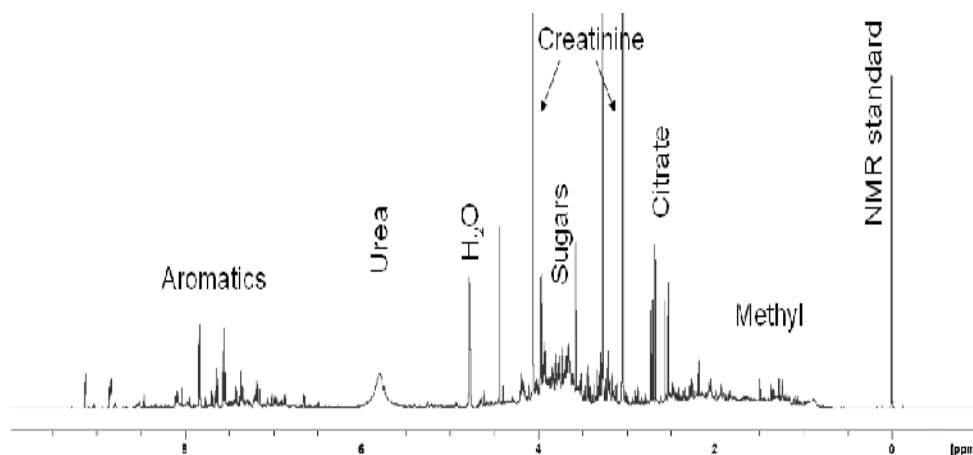
Body fluids can be investigated by nuclear magnetic resonance spectroscopy (NMR) to gain complementary information for the diagnosis of metabolic disease (1-9). <sup>1</sup>H-NMR of body fluids shows the majority of hydrogen-containing compounds and provides an overall 'snapshot' view of metabolism. For the diagnosis of inherited metabolic diseases, this is a great advantage when compared with other techniques. NMR of body fluids is a promising alternative analytical approach to the diagnosis of known inborn errors of metabolism, as well as errors that are not yet known. NMR can be applied to all relevant body fluids, i.e. urine, serum, heparinized plasma, and cerebrospinal fluid.

A strong magnet is the heart of any NMR system. The picture (right) shows the magnet used at INFAI for the Metabo Test. It uses a superconducting coil and operates at a field of 11.7 Tesla.



**Figure 1:** NMR spectrometer with automation equipment.

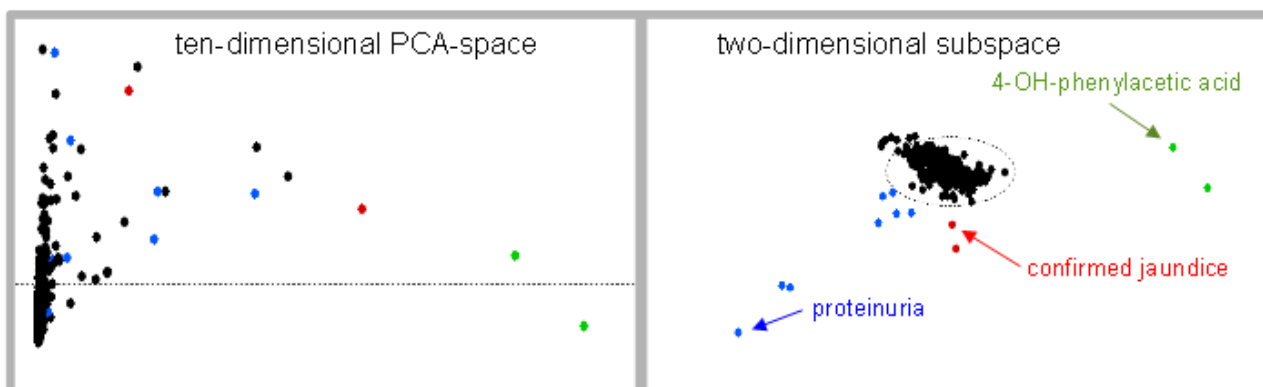
**Typical NMR spectrum:** The  $^1\text{H}$ -NMR spectrum of a body fluid provides a characteristic 'fingerprint' of almost all hydrogen nuclei in a given metabolite. Figure 2 shows 20 main metabolites and in low concentration more than 1000 metabolites can be observed. Each resonance peak corresponds to a hydrogen nucleus in a metabolite. The resonance frequency is characteristic of each hydrogen in each metabolite, and gives their unambiguous assignment. The intensity of the observed resonance lines is proportional to the number of the respective hydrogen nuclei in the sample. This way, the concentration of each metabolite can be determined.



**Figure 2:** Typical  $^1\text{H}$ -NMR spectrum of healthy subject.

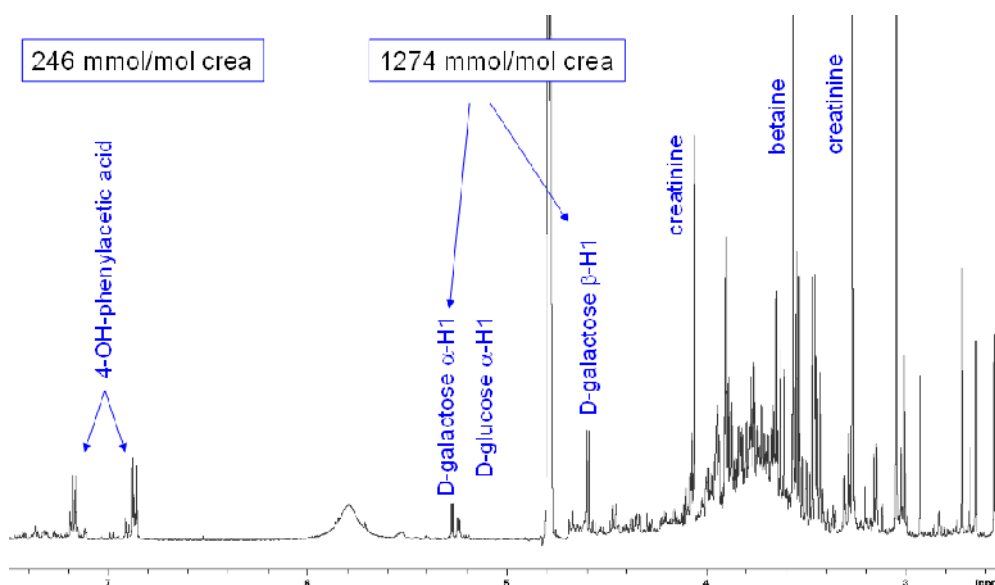
**Statistical analysis:** The statistical analysis and quantification of metabolite concentrations will be based on a combination of 1D-spectra and fast 2D-J-resolved spectra. Here, the 2D-spectra support the secure identification and deconvolution based quantification of metabolites identifying line position and multiplet structure as obtained from a J-resolved 2D-spectrum. Identification of suspicious urine samples is done by statistical methods. Principal component analysis (PCA) is used to compare the spectra of urine samples.

Statistical analysis of spectra was used to identify suspicious samples. Figure 3 gives an example of this type of analysis. Principal component analysis (PCA) generates a representation where each sample is shown as an individual point. Suspicious samples become evident as outlier points. In the example, three types of outlier samples are observed, shown in different colors. Spectral analysis shows high concentration of a fingerprint metabolite. Two of the subjects were confirmed to have suffered from jaundice, one had to be treated in an intensive care unit. For ten subjects high concentrations of macromolecules have been observed in the aliphatic region. An external laboratory identified albumin, which indicates manifest nephropathy. Several subjects were observed with 4-hydroxyphenylacetic acid in high concentration.



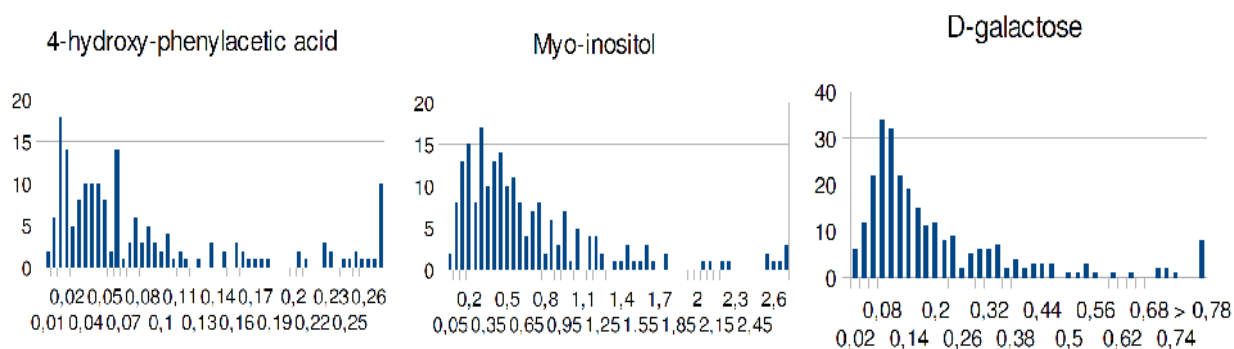
**Figure 3:** Example of untargeted analysis by PCA.

**Quantification:** After identification of metabolites in NMR spectra, simple integration of selected metabolites of interest yields fully quantitative information on metabolite concentration. The Figure below gives an example. The concentrations of D-galactose (246 mmol/mol creatinine) and 4-hydroxy phenylacetic acid (1274 mmol/mol), double of what has been described as pathological (631 mmol/mol) [10].



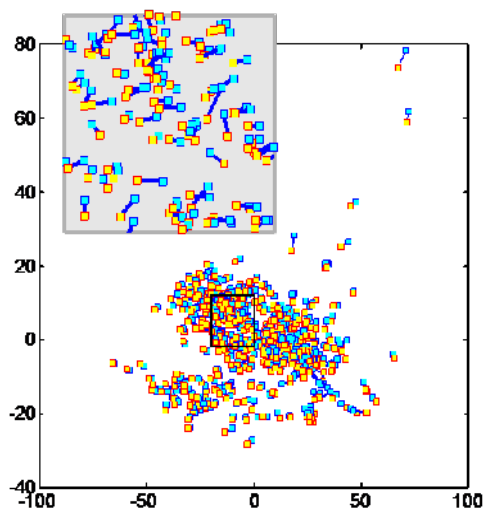
**Figure 4:** Quantification demonstrated on a subject where both 4-OH-phenylacetic acid and D-galactose are found in pathological concentrations.

**Fully automatic quantification:** Further metabolites were observed in high concentration: 3-hydroxybutyric acid (child had symptoms of ketosis), myoinositol (found associated with aminoaciduria), and D-galactose were detected. Concentrations were determined fully automatically for all 690 samples; a histogram of the distribution of concentrations is given in Figure 5.



**Figure 5:** Concentration plots of three metabolites detected in high concentration.

**Reproducibility between laboratories:** All samples were measured in duplicate, in INFAL's lab in Cologne and in Bruker's lab in Karlsruhe. In Figure 6, the results of a PCA analysis applied on the combined data set (both laboratories, Lab-1: yellow, Lab-2: blue) are shown. Each pair of samples is represented by a pair of markers connected by a blue line. The small insert plot shows a zoom into the black rectangle. It is found that the two aliquots of each initial sample measured independently in the two labs are always represented by points which are in close proximity demonstrating excellent reproducibility.



**Figure 6:** Comparison of PCA analysis for data obtained in two laboratories (Laboratory 1: Bruker, Karlsruhe, Laboratory 2: INFAl, Cologne)

### We offer:

- Establish automatic quantification and 'normal ranges' for common metabolites
- Identify pathological metabolites that can be used as disease markers
- Integrate into a comprehensive screening test for newborn health
- INFAl maintains a growing database of currently more than 600 metabolites that are characterized in one- and two-dimensional NMR spectra at different pH values
- The statistical analysis and quantification of these metabolites allow developing a normal model in a specific population and also a general assessment of neonate health state

**For more information and contact details, visit**

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### References

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