Metabo Test INFAI®

NMR based screening for inborn errors of metabolism

Inborn errors of metabolism:
A worlwide problem for children

Headquarters in Cologne (RTZ)

Factory in Hagen

INFAI is at the leading edge in the transfer of advanced analytical technology into medical diagnostics and the development of innovative pharmaceutical products. The company has pioneered the use of stable isotopes and NMR in gastroenterology, metabolic diseases, and oncology. INFAI's laboratories in Cologne, Germany are equipped with the most advanced NMR and NMR imaging instrumentation. These facilities are used for in-house research and product development and are also available for collaborative and contract research.

In the last years we have developed a range of non-invasive and highly effective stable isotope breath tests. One of these tests is already licensed and available for the routine diagnosis of Helicobacter pylori infection. Other tests to determine gastric emptying rate and pancreatic insufficiency will be soon available.

NMR spectroscopy and NMR imaging are used at INFAI to investigate a range of metabolic disorders and malignant conditions. The non-invasive characteristics of these techniques make them particularly suitable for pediatric use. INFAI conducted a clinical trial for newborn screening with 12 clinical centers in Turkey in cooperation with Bruker. The Metabo Test was developed and validated for inborn errors of metabolism.

INFAI is affiliated with a range of companies throughout Europe.

MTI Booklet-EN-20191216

Urine screening for metabolic diseases in neonates

Approximately 1 in 400 neonates in Turkey, and 1 in 500 neonates in EU countries are affected by congenital metabolic diseases. If undetected and untreated, these diseases can lead to irreversible organ failures, invalidity or death. Currently, neonates are routinely screened for only 12 metabolic diseases. However, there are many more known metabolic diseases. Nowadays, tandem-MS (mass spectrometry) and GC (gas chromatography) / MS are the most frequently used diagnostic methods for the diagnosis of metabolic diseases. Nevertheless, tandem-MS can only investigate a total of approximately 40 metabolites with one (group of) metabolite(s) at a time. Analysis of large molecules poses additional technical problems, leading to increased costs and analysis time. As sample preparation is also time consuming, these methods are not suitable for screening. Only investigations of suspected diseases are feasible. GC-MS also needs derivatization, with the risk of changing the sample and the outcome of the measurement.

Magnetic resonance spectroscopy (NMR) of body fluids

The NMR method was developed by Felix Bloch and Edward Purcell, who were awarded with the Nobel Prize in 1952 for this work. This method has been further developed and is frequently used in various fields. Its main application used to be the structural analysis of unknown molecules and their characterization and quantification in organic chemistry. Medical utilizations, especially imaging were developed by Richard Ernst, who received the Nobel Prize in 1991. In the past decade, other medical applications of NMR spectroscopy have been investigated, so that potential diseases can be detected in investigations of body fluids (urine, serum or cerebrospinal fluid).

For the diagnosis of congenital metabolic diseases, NMR spectroscopy has many advantages over other methods. It shows the majority of proton-containing compounds and therefore provides an overall view of metabolism. It is a non-invasive investigation

method, fast, and easy to perform. The NMR spectroscopy of body fluids may be considered as an alternative analytical approach for diagnosing known, but also unknown, inborn errors of metabolism, through targeted and non-targeted analysis of metabolites.

NMR spectrum of urine

The ^{1}H-NMR spectrum of a body fluid provides a characteristic ‚fingerprint' of almost all hydrogen nuclei in a given metabolite. In the NMR spectrum of urine, more than 1000 metabolites can be observed. The intensity of the observed resonance is proportional to the number of hydrogen nuclei in the sample. In this way, the concentration of each metabolite can be determined.
NMR spectroscopy can detect and analyze all metabolites in urine in a single measurement that only takes minutes, thus allowing fast and cost effective diagnostic screening. Besides buffering, no preprocessing of the urine samples is necessary for the measurement. Figure 1 below shows an NMR spectrum of urine with some assigned peaks.

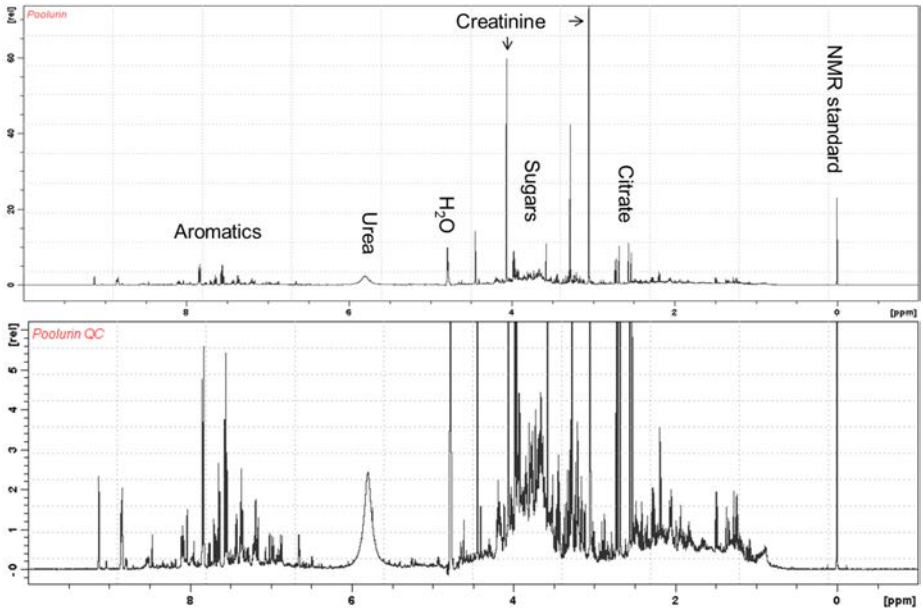


Figure 1: Urine spectrum with peak assignment; above: overall view; below: zoomed-in view.

Clinical study in Turkey

In a large, multi-center clinical study conducted by INFAI in cooperation with Bruker BioSpin and ten clinical centers in Turkey, urine samples from 950 newborn babies were collected and investigated by using high-throughput Bruker ADVANCE III 500 MHz and 600 MHz NMR spectroscopy, in order to establish whether pathological metabolites are observed in healthy newborns and to determine their concentration ranges. A standard model for newborn screening was developed. Currently, more than 600 metabolites can be detected and quantified with this NMR method. A statistical method of analysis (PCA) is used in order to achieve automatic separation of normal and abnormal samples. Conspicuous samples will be further investigated with more complex NMR techniques (such as 2D NMR). In this way, samples of healthy and diseased neonates will be separated.

Statistical analysis

The statistical analysis and quantification of the metabolite concentrations will be based on the combination of a 1D- and fast 2D-J-resolved spectra. This 2D-spectrum supports the reliable identification of a metabolite, through the deconvolution of the urine spectra, leading to identification of line, position and shape of the peak. The evaluation of suspicious urine samples is performed by comparing its spectrum with the spectra of all other healthy urine samples, using principal component analysis (PCA). Figure 2 gives an example of this type of analysis.

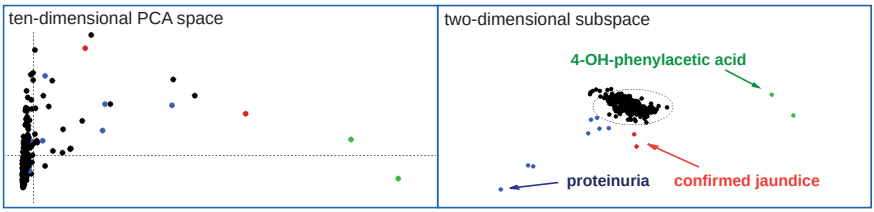


Figure 2: Example of untargeted analysis by PCA.

This statistical analysis was carried out with all of the 950 spectra collected in the clinical study in Turkey. PCA generates a representation in which 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, and shown in different colors (Figure 2). Further spectral investigations detect high concentrations of different fingerprint metabolites. Two of the subjects were confirmed to have suffered from jaundice, and one of them had to be

treated in an intensive care unit. For ten subjects, high concentrations of macromolecules were observed. An external laboratory identified albumin, which indicates manifest nephropathy. Several subjects were observed with 4-hydroxyphenylacetic acid in high concentrations, but no unusual findings were reported in clinical data.
However, all current NMR-signatures of diseases with their specific metabolites in our database are derived from patients with metabolic diseases. To serve as a screening test, calibration of this statistical method is first established by comparing all normal urine spectra of Turkish neonates. This especially concerns the metabolites that reveal immaturity and which are more variable than in older patients. That means that some disease-specific metabolites may physiologically still be present to some extent in healthy neonates. They naturally posses high variances, as seen in Figure 3.

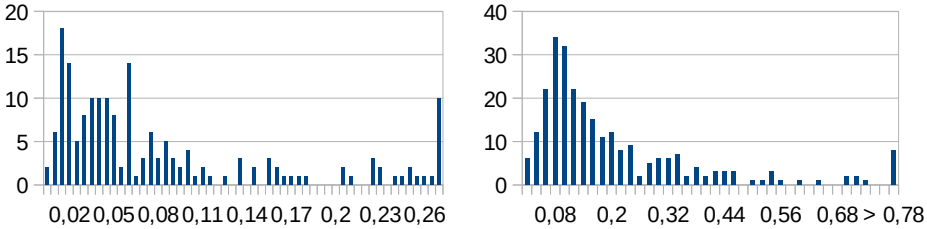


Figure 3: Assigned concentration profiles of 4-hydroxyphenylacetic (left) and D-galactose (right).

A thorough statistical analysis and classification requires collection of (urine) samples from various populations of patients with metabolic diseases, as well as from healthy control populations. The goal of the statistical model is to explore the range of variation (concentration and chemical shift) of specific metabolites in the urine of neonates without being clinically relevant. This is necessary for the identification of pathological thresholds for these specific metabolites, in comparison to the healthy neonates, and subsequent development of a normal model for urine spectra of Turkish neonates. Further statistical modules developed at INFAI and Bruker BioSpin GmbH use such a normal model for untargeted screening and allow the detection of yet unknown diseases in Turkish neonates.

Quantification

The peak area or signal intensity of a signal in a ^{1}H-NMR spectrum is proportional to the number of protons contributing to the signal. It is therefore also proportional to the concentration of the molecule concerned, thus allowing NMR spectroscopy to be used for metabolite quantification. The sensitivity of the technique is in the low micromolar range for most metabolites. After the identification of metabolites in the NMR spectrum, simple integration of some selected signals from each metabolite of interest gives full quantitative information on its concentration. Figure 4 below gives an example: the concentrations of 4-hydroxyphenylacetic acid (246 mmol / mol creatinine) and D-galactose (1274 mmol / mol creatinine) are given, where the level of D-galactose is double of what is described as pathological (631 mmol / mol creatinine).

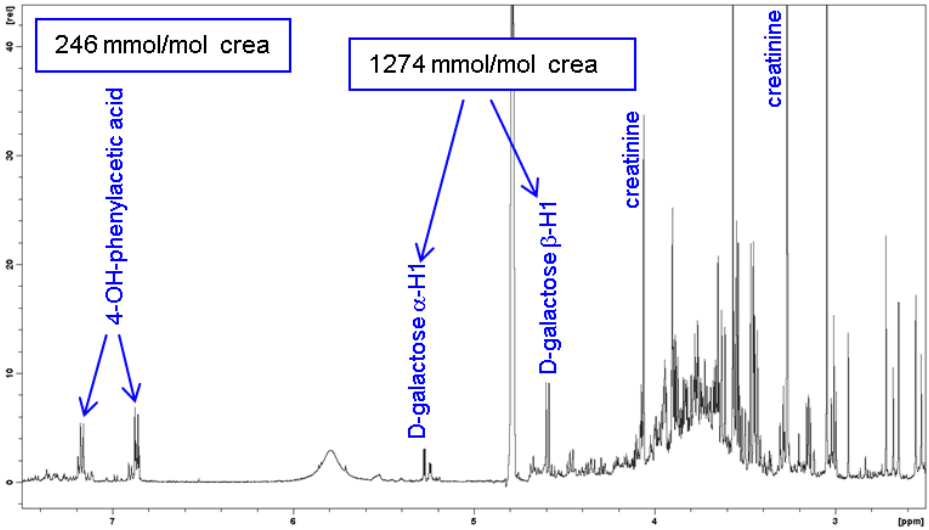


Figure 4: Quantification demonstrated on a subject where both 4-OH-phenylacetic acid and D-galactose are found in pathological concentrations.

Diseases and metabolites in urine spectra

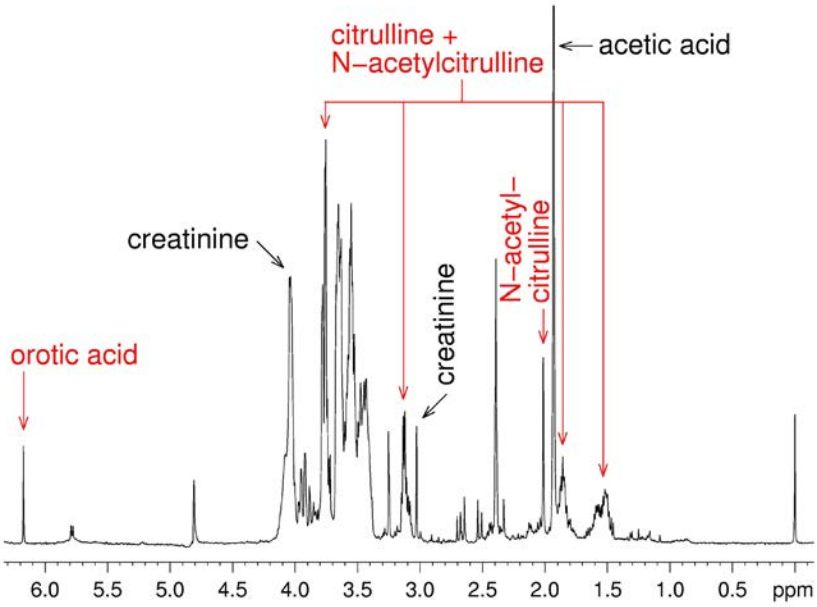
INFAI provides 20 years of experience in NMR-based investigations of body fluids. Over the years, a comprehensive library of spectral information on numerous diseases and isolated metabolites has been accumulated. Some examples of urine spectra of children with diagnosed diseases will now be presented. For further information, the OMIM-numbers of the diseases are given (omim.org).
Online Mendelian Inheritance in Man (OMIM®) is a continuously updated catalog of human genes, genetic disorders and traits, with particular focus on the molecular relationship between genetic variation and phenotypic expression. It is thus considered to be a phenotypic companion to the Human Genome Project.

- Hereditary urea cycle abnormality
The hereditary urea cycle abnormality is an inherited condition that can cause several problems with the removal of waste from the body in the urine. The urea cycle is a process in which waste (ammonia) is removed from the body. When you eat proteins, the body breaks them down into amino acids, which are converted to ammonia, and which has to be removed from the body.
The liver produces several chemicals (enzymes) that convert ammonia into urea, which the body can remove in the urine. If this process is disturbed, ammonia levels begin to rise. Several inherited conditions can cause problems with this waste removal process. People with an urea cycle disorder are missing a gene that makes the enzymes needed to break down ammonia in the body.

These diseases include:
- Argininosuccinic aciduria
- Arginase deficiency
- Carbamyl phosphate synthetase (CPS) deficiency
- **Citrullinemia**
- N-Acetyl glutamate synthase deficiency (NAGS)
- Ornithine transcarbamylase deficiency (OTC)

As a group, these disorders occur in 1 in 30,000 newborns. Ornithine transcarbamylase deficiency is the most common of these disorders.

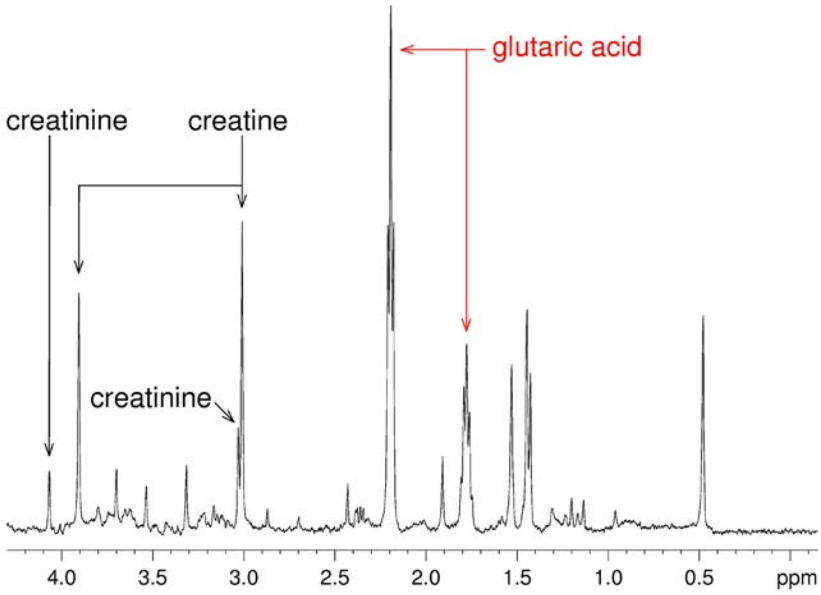
- Citrullinemia



OMIM #215700

Citrullinemia is an inherited disorder that causes ammonia and other toxic substances to accumulate in the blood. Two forms of citrullinemia have been described. They have different signs and symptoms and are caused by mutations in different genes.
Type I citrullinemia is the most common form of this disorder and usually becomes evident in the first few days of life.
Type II citrullinemia chiefly affects the nervous system, causing confusion, restlessness, memory loss, abnormal behaviors, seizures, and coma. In some cases, the signs and symptoms of this disorder appear during adulthood (adult-onset).
Increased levels of citrulline and N-acetylcitrulline can be observed in the urine spectra.

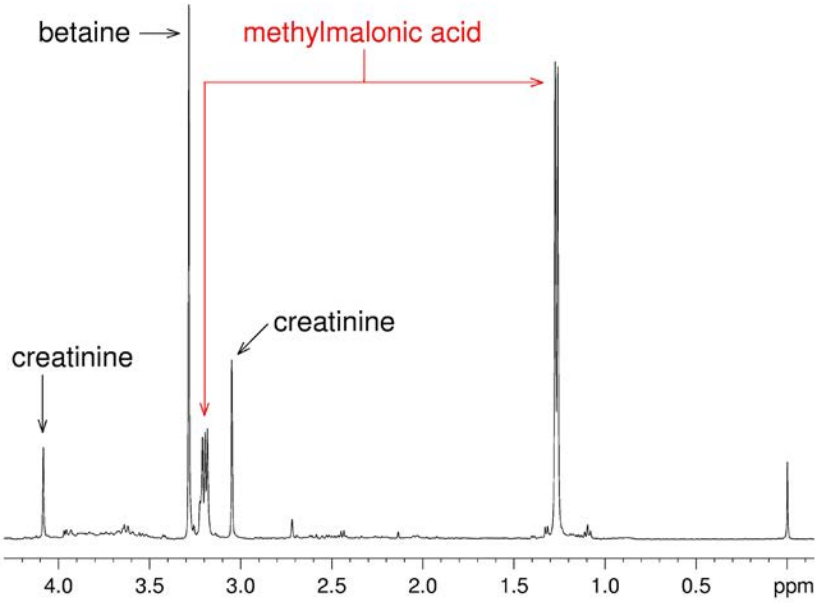
- Glutaric aciduria type I



OMIM #231670

Glutaric aciduria type I is an inherited disorder in which the body is unable to process certain proteins properly. People with this disorder have inadequate levels of an enzyme that helps break down the amino acids lysine, hydroxylysine and tryptophan, which are building blocks of proteins.
The severity of glutaric aciduria type I vary widely; some individuals are only mildly affected, while others have severe problems. In most cases, signs and symptoms first occur in infancy or early childhood. Glutaric aciduria type I occurs in approximately 1 of every 30,000 to 40,000 individuals.
High concentrations of glutaric acid were observed in all body fluids.

- Methylmalonic aciduria



OMIM #251000

Methylmalonic aciduria is an inherited disorder in which the body is unable to process certain proteins and fats (lipids) properly. The effects of methylmalonic aciduria, which usually appear in early infancy, vary from mild to life-threatening. Affected infants can experience vomiting, dehydration, weak muscle tone (hypotonia), developmental delay, excessive tiredness (lethargy), an enlarged liver (hepatomegaly) or failure to gain weight and grow at the expected rate (failure to thrive). Long-term complications can include feeding problems, intellectual disability, chronic kidney disease and inflammation of the pancreas (pancreatitis). This disease occurs in an estimated frequency of 1 in 50,000 to 100,000 people.

D- and L-form of 2-hydroxyglutaric aciduria

D- and L-2-hydroxyglutaric acidurias are rare, clinically variable, and neurological forms are characterized biochemically in urine (Figure 5), plasma, and cerebrospinal fluid. The different enantiomeric forms of 2 hydroxyglutaric acid are related to different diseases: L-2-hydroxyglutaric aciduria (L-2-HGA) is related to the L-enantiomer of 2-hydroxyglutaric acid, while the less common D-2-hydroxyglutaric aciduria (D-2-HGA) is related to the D-enantiomer. The prevalence of this disorder is not known; only 80 cases worldwide have been reported to date.

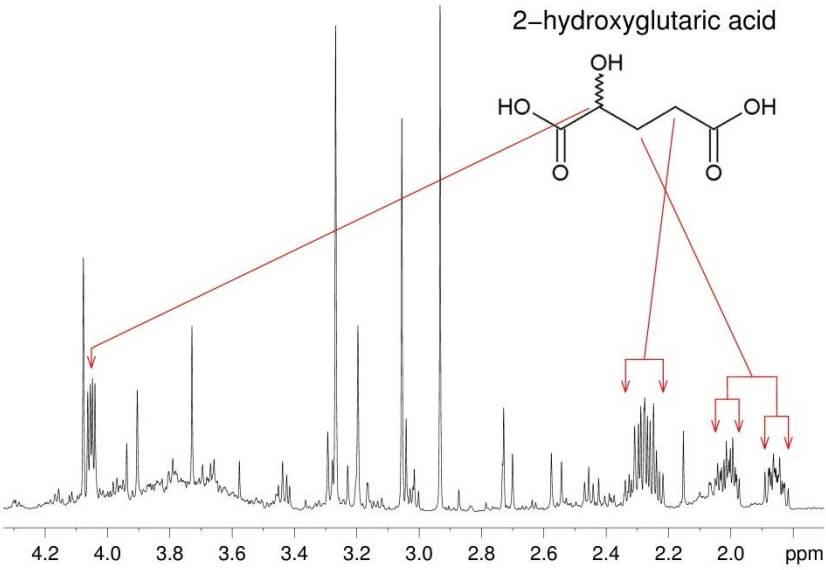


Figure 5: NMR spectra of urine with 2-hydroxyglutaric aciduria.

The distinction between the L- and D-forms is not possible using ordinary analytical techniques (Tandem-MS, GC-MS). For a definite diagnosis, a genetic analysis of the affected genes is usually necessary. The affected genes are L2HGDH (in L 2-HGA) or either D2HGDH or IDH2 (in D-2-HGA). A lanthanide shift reagent (Figure 6) allows distinction between L- and D-2-hydroxyglutaric acid in the NMR spectra of urine. When this samarium complex is added as a chemical shift reagent, the NMR resonances of L- and D-2-hydroxyglutaric acid are shifted in different directions (shown in Figure 7) and can be clearly distinguished.

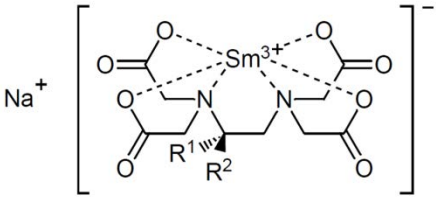


Figure 6: Lanthanide-Shift-Reagent.

This method was applied to urine samples from three different patients of age 1, 5, and 28 years treated by Prof. Turgay Coskun and Prof. Ali Dursun, Hacettepe University, Ankara, Turkey. In all three cases, the L-enantiomer was found, confirming the diagnosis of L-2-HGA.

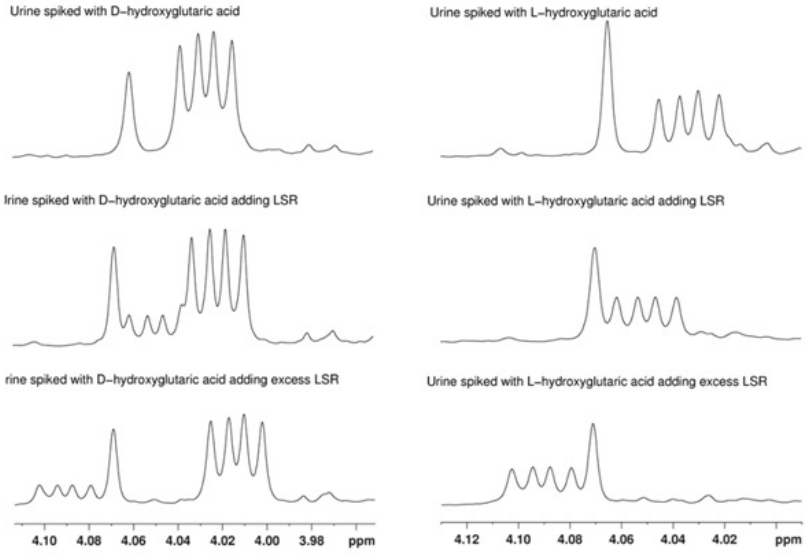


Figure 7: Distinction of L- and D-2-hydroxyglutaric acid by different chemical shifts.

Problems in Newborn Screening

Problem of false positive and false negative results should be avoided by classifying outlier groups. Unknown metabolites need additional investigations and different techniques.

In type II tyrosinemia, 4 metabolites: 4-hydroxyphenylacetic acid, 4-hydroxyphenyllactic acid and 4-hydroxyphenylpyruvic acid (Tomoeda et al. 2000) were found in urine, and the amino acid tyrosine must be detectable in plasma. Figure 5 shows the results from a neonate with possible type II tyrosinemia.

In hawkinsinuria, the first three above mentioned metabolites also appear in urine, but additionally 5-oxoproline and 4-hydroxycyclohexylacetate must be present. However, in this case these metabolites were not found. Therefore, this neonate may suffer from type II tyrosinemia. Unfortunately, a conclusive diagnosis would only have been possible with a plasma sample, which was not available. Moreover, we have no information on the neonate's subsequent clinical course.

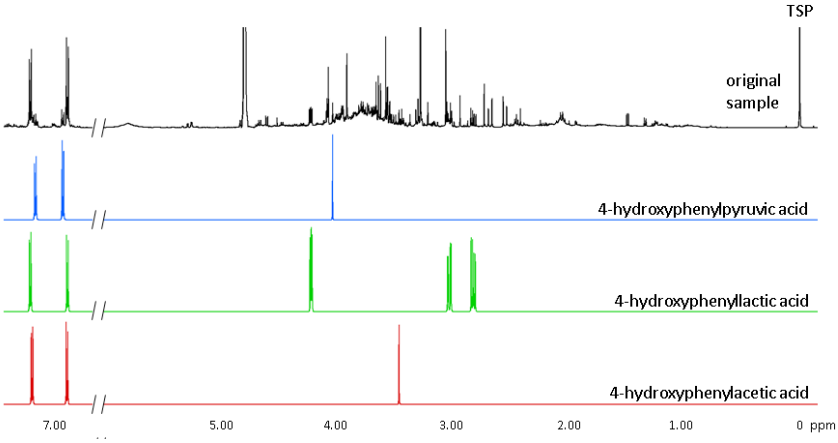


Figure 8: Matching of urine with database spectra of diagnostic metabolites.

Reproducibility between laboratories

All samples were measured in duplicate, in INFAI's lab in Cologne and in Bruker's lab in Karlsruhe. In Figure 9, 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, thus demonstrating excellent reproducibility.

	Total Number of NMR Samples	NMR Samples with clinical metadata
INFAI	953	953
Bruker	892	890
Both labs	885	885
Only at INFAI	68	68
Only at Bruker	7	5

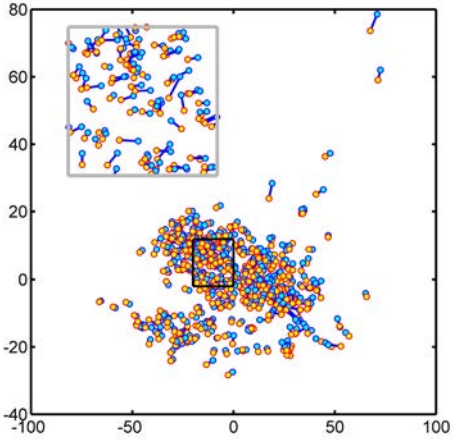


Figure 9: PCA analysis of NMR-spectra measured and analyzed in two different laboratories.

Current list of automatically quantified metabolites

- Marker for Inborn Errors

Markers for IEM Diseases			
Nr.	Name	Nr.	Name
1	Orotic Acid	25	2-Hydroxyphenylacetic Aid
2	Methylmalonic Acid	26	2-Phenyllactic Acid
3	2-Hydroxyisovaleric Acid	27	3-Phenyllactic Acid
4	3-Hydroxyisovaleric Acid	28	4-Hydroxyphenyllactic Acid
5	Ethylmalonic Acid	29	3-Methyl-2-Oxovaleric Acid
6	*N*-Acetylaspartic Acid	30	2-Hydroxy-4-Methylvaleric Acid
7	Glutaric Acid	31	D-Galactonic Acid
8	Xanthine	32	3-Methylcrotonylglycine
9	Uridine	33	Uracil
10	Acetone	34	Galactitol
11	3-Hydroxybutyric Acid	35	Isovaleroylglycine
12	Acetoacetic Acid	36	5-Aminolevulinic Acid
13	Propionic Acid	37	2-Oxoisocaproic Acid
14	L-Isoleucine	38	Propionylglycine
15	Allo-Isoleucine	39	4-Hydroxyphenylacetic Acid
16	Leucine	40	3-Hydroxyvaleric Acid
17	Valine	41	D-Sorbitol
18	Citrulline	42	3-Hydroxyglutaric Acid
19	3-Hydroxypropionic Acid	43	E-Glutaconic Acid
20	Phenylalanine	44	2-Oxoisovaleric Acid
21	Phenylpyruvic Acid	45	L-pyroglutamic Acid
22	*N*-Acetylphenylalanine	46	Tiglylglycine
23	Neopterin	47	Suberic Acid
24	Phenylacetic Acid	48	Sebacid Acid

- Endogenous Compounds, Impurities, Food- and Drug related

Metabolites always present in urine			
Nr.	Name	Nr.	Name
1	Creatinine	15	Fumaric Acid
2	Creatine	16	Formic Acid
3	D-Glucose-beta	17	1-Methylnicotinamide
4	D-Galactose-alpha	18	*N*,*N*-Dimethylglycine
5	D-Lactose	19	*Myo*-inositol
6	Alanine	20	Taurine
7	Lactic Acid	21	Trimethylamine-*N*-oxide (TMAO)
8	Acetic Acid	22	Hippuric Acid
9	Succinic Acid	23	4-Aminobutyric Acid GABA
10	Citric Acid	24	Trigonelline
11	Dimethylamine	25	Methanol
12	Trimethylamine	26	Ethanol
13	Betaine	27	Benzoic Acid
14	Glycine		

List of Metabolites in the Inborn Error Reference Spectra Database

Nr.	compound	main function/disease, further diseases in most cases
1	1,2-propanediol	Artefacts-pharmaceutical products (additive)
2	1-methyl-1-cyclohexanecarboxylic acid	3-methylcrotonylglycinuria
3	2,3-butanediol	Artefacts-pharmaceutical products (from ethanol)
4	2,8-dihydroxyadenine	Adenine phosphoribosyltransferase deficiency
5	2-aminoadipic acid	2-Aminoadipic aciduria
	2-aminoadipic acid	2-Ketoadipic aciduria
6	2-aminoisobutyric acid	ß-Aminoisobutyric aciduria
7	2-butanone	ß-Ketothiolase deficiency
8	2-deoxyadenosine	Adenosine deaminase deficiency
	2-deoxyguanosine	Purine nucleoside phosphorylase deficiency
9	2-hydroxy-3-methylvaleric acid	Maple syrup urine disease
	2-hydroxy-4-methylvaleric acid	Maple syrup urine disease
10	2-hydroxyadipic acid	2-Ketoadipic aciduria
11	2-hydroxybutyric acid	Lactic Acidosis
12	2-hydroxyglutaric acid	Glutaric aciduria type II
13	2-hydroxyisocaproic acid	Maple syrup urine disease
14	2-hydroxyisovaleric acid	Maple syrup urine disease
15	2-hydroxyphenylacetic acid	Phenylketonuria
16	2-hydroxysebacic acid	Zellweger Syndrome
17	2-ketoadipic acid	2-Ketoadipic aciduria
18	2-methyl-3-hydroxybutyric acid	ß-Ketothiolase deficiency
19	2-methylacetoacetic acid	ß-Ketothiolase deficiency
20	2-methylbutyric acid	Glutaric aciduria type II
21	2-methylbutyrylcarnitine	Short/branched-chainacyl-CoA dehydrogenase deficiency (SBCADD)
22	2-methylbutyrylglycine	Glutaric aciduria type II
23	2-oxo-3-methylvaleric acid	Maple syrup urine disease
24	2-oxoadipic acid	2-Oxoadipic aciduria
25	2-oxobutyric acid	Methionine malabsorption
26	2-oxoglutaric acid	Dihydrolipoyl dehydrogenase (E3)
27	2-oxoisocaproic acid	Maple syrup urine disease
28	2-oxoisovaleric acid	Maple syrup urine disease

Nr.	compound	main function/disease, further diseases in most cases
29	3-aminoisobutyric acid	Hyper-ß-alaninemia
30	3-hydroxy-3-methylglutaric acid	3-Hydroxy-3-methylglutaryl-CoA lyase deficiency
31	3-hydroxyadipic acid	Long-chain 3-hydroxyacylcoenzyme A dehydrogenase
32	3-hydroxybutyric acid	Maple syrup urine disease
33	3-hydroxydodecanedioic acid	Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (SCHAD)
34	3-hydroxyglutaric acid	Glutaric aciduria type I
35	3-hydroxyheptanoic acid	Long-chain 3-hydroxyacylcoenzyme A dehydrogenase
36	3-hydroxyisovaleric acid	Biotinidase deficiency
37	3-hydroxykynurenine	Hydroxykynureninuria
38	3-hydroxypropionic acid	Methylmalonic aciduria
39	3-hydroxysebacic acid	Long-chain 3-hydroxyacylcoenzyme A dehydrogenase
40	3-hydroxyvaleric acid	Propionic Acidemia
41	3-methoxytyramine	Aromatic L-aminoacid decarboxylase deficiency
42	3-methoxytyrosine	Aromatic L-aminoacid decarboxylase deficiency
43	3-methyl-2-oxovaleric acid	Maple syrup urine disease
44	3-methylcrotonylglycine	Biotinidase deficiency
45	3-methylglutaconic acid	3-Methylglutaconic aciduria type 1
46	3-methylglutaric acid	3-Hydroxy-3-methylglutaryl-CoA lyase deficiency
47	3-oxoglutaric acid	Acidosis, gluconeogenesis
48	3-ureidoisobutyric acid	Ureidopropionase deficiency
49	3-ureidopropionic acid	Ureidopropionase deficiency
50	4-aminobutyric acid	GABA-transaminase deficiency
51	4-hydroxy-3-methoxymandelic acid	Asphyxia
52	4-hydroxybutyric acid	4-hydroxybutyric aciduria
53	4-hydroxybutyric acid lactone	4-hydroxybutyric aciduria
54	4-hydroxyphenylacetic acid	Tyrosinemia I
	4-hydroxyphenyllactic acid	Lactic Acidosis
55	4-hydroxyphenylpyruvic acid	Hawkinsinuria
56	5,6-dihydrothymine	Dihydropyrimidinase deficiency
57	5,6-dihydrouracil	Dihydropyrimidinase deficiency
58	5-aminolevulinic acid	Tyrosinemia I

Nr.	compound	main function/disease, further diseases in most cases
59	5-hydroxyhexanoic acid	Medium chain acyl-CoA dehydrogenase deficiency
60	5-hydroxyindole-3-acetic acid	Blue diaper syndrome
61	5-hydroxymethyluracil	Dihydropyrimidine dehydrogenase deficiency (DHPD)
62	5-hydroxytryptophan	Aromatic L-aminoacid decarboxylase deficiency
63	5-oxoproline	5-Oxoprolinuria (Gluthathione synthase deficiency)
64	5-phosphpmevalonic acid	Mevalonic aciduria
65	6-methyluracil	ß-Ketothiolase deficiency
66	acetoacetic acid	Propionic aciduria
67	acetone	Propionic aciduria
68	adipic acid	Glutaric aciduria type II
69	adrenaline	Dopamine beta-hydroxylase deficiency (DßH)
70	alanine	Hartnup disorder
71	alanine-proline	Prolidase deficiency
72	*allo*-isoleucine	Pyrimidine disorders
73	arabinose	Polyol disease with arabinose and arabinitol
74	arabitol	Polyol disease with arabinose and arabinitol
75	arginine	Lysinuric protein intolerance
76	argininosuccinic acid	Argininosuccinic aciduria
77	asparagine	Hartnup disorder
78	aspartic acid	Acidosis, gluconeogenesis
79	aspartylglucosamine	Aspartylglucosaminura
80	azelaic acid	Adrenoleukodystrophy, neonatal
81	biopterin	Biopterin synthesis deficiency
82	ß-alanine	Gaba transaminase deficiency
83	betaine	Tyrosinemia type I
84	biocytin	Biotinidase deficiency
85	butanon	ß-Ketothiolase deficiency
86	carnitine	3-Hydroxy-3-methylglutaryl-CoA lyase deficiency
87	carnosine	Carnosinemia
88	*cis*-4-hydroxyproline	Renal dysfunction
89	citric acid	Fumaric aciduria
90	citrulline	Citrullinemia
91	creatine	Guanidinoacetate methyltransferase (GAMT) deficiency

Nr.	compound	main function/disease, further diseases in most cases
92	creatinine	Cystinosis
93	cresol	Artefacts-bacterial contamination
94	cystathionine	Cystathionase deficiency
95	cysteine	γ-Glutamyltransferase deficiency
96	cystine	Cystinuria
97	D-2-hydroxyglutaric acid	D-2-Hydroxyglutaric aciduria
98	decanoyl-L-carnitine	3-Hydroxy-3-methylglutaryl-CoA lyase deficiency
99	decenedioic acid	Medium chain acyl-CoA dehydrogenase deficiency
100	deoxyinosine	Purine nucleoside phosphorylase deficiency
101	deoxyuridine	Mitochondrial NeuroGastroIntestinal Encephalopathy
102	dermatane sulfate	Mucopolysaccharidosis
103	D-galactonic acid	Galactosemia
104	D-galactose	Galactosemia
105	D-glucose	Glucose transporter defect (SGTL2)
106	D-Lactose	Malabsorption syndromes
107	D-mannitol	Dehydration
108	dopamine	Aromatic L-aminoacid decarboxylase deficiency
109	D-sorbitol	Galactosemia
110	D-xylose	Pentosuria
111	D-xylulose	Pentosuria
112	erythritol	Transaldolase deficiency
113	ethanolamine	Ethanolaminosis
114	ethylmalonic acid	Ethylmalonic encephalopathy (EPEMA)
115	fructose	Hereditary fructose intolerance
116	fumaric acid	Fumaric aciduria
117	galactitol	Galactosemia
118	glutaconic acid	Glutaric aciduria type I
119	glutamic acid	Acidosis, gluconeogenesis
120	glutamine	Hartnup disorder
121	glutaric acid	Glutaric aciduria type I
122	glutathione	Glutathionuria
123	glyceric acid	D-Glyceric aciduria
124	glycerol	Glycerol kinase deficiency

Nr.	compound	main function/disease, further diseases in most cases
125	glycine	Iminoglycinuria
126	glycine-proline	Prolidase deficiency
127	glycolic acid	Primary hyperoxaluria I, PH1
128	glyoxilic acid	Primary hyperoxaluria I, PH1
129	guanidinoacetic acid	Guanidinoacetate methyltransferase (GAMT) deficiency
130	guanosine	Purine nucleoside phosphorylase deficiency
131	hexanoic acid	MCAD
132	hexanoyl-D,L-carnitine	Multiple acyl-coenzyme A dehydrogenase deficiency
133	hexanoylglycine	Dehydrogenase deficiency
134	hippuric acid	Artefacts-bacterial contamination
135	histamine	Histidinemia
136	histidine	Histidinemia
137	homoarginine	Hyperlysinemia
138	homocarnosine	Homocarnosinosis
139	homocitrulline	Citrullinemia
140	homocysteine	Methylmalonic aciduria and homocystinuria, cblC type
141	homocystine	Homocystinuria
142	homogentisic acid	Alkaptonuria
143	homovanillic acid	Neuroblastoma
144	hydroxyproline	Glutaric aciduria type II
145	hypoxanthine	Molybdenum cofactor deficiency
146	imidazoleacetic acid	Histidinemia
147	imidazolepyruvic acid	Histidinemia
148	indican	Blue diaper syndrome
149	indole-3-aceticacid	Blue diaper syndrome
150	inosine	Purine nucleoside phosphorylase deficiency
151	isobutyric acid	Glutaric aciduria type II
152	isobutyryglycine	Glutaric aciduria type II
153	isovaleric acid	Glutaric aciduria type II
154	isovalerylglycine	Ethylmalonic encephalopathy (EPEMA)
155	kynurenine	Hydroxykynureninuria

Nr.	compound	main function/disease, further diseases in most cases
156	L-2-hydroxyglutaric acid	L-2-Hydroxyglutaric aciduria
157	lactic acid	Biotinidase deficiency
158	L-dopa	Aromatic L-aminoacid decarboxylase deficiency
159	leucine	Hartnup disorder
160	L-glyceric acid	Hyperoxaluria type II
161	L-isoleucine	Hartnup disorder
162	L-xylulose	Pentosuria
163	lysine	Glutaric aciduria type II
164	malic acid	Acidosis, gluconeogenesis
165	malonic acid	Malonic aciduria
166	methionine	Cystathionine beta-synthase deficiency
167	methionine sulfoxyde	Cystathionine beta-synthase deficiency
168	methylfumaric acid	Isovaleric Acidemia
169	methylmalonic acid	Methylmalonic aciduria
170	methylsuccinic acid	Methylmalonic aciduria
171	mevalonic acid	Mevalonic aciduria
172	mevalono lactone	Mevalonic aciduria
173	*myo*-inositol	Dehydration
174	*N*,*N*-dimethylglycine	Dimethylglycine dehydrogenase deficiency
175	*N*-acetyl-2-aminoadipic acid	Ketoadipic aciduria
176	*N*-acetylalanine	Aminoacylase I deficiency
177	*N*-acetylaspartic acid	Canavan disease
178	*N*-acetylcarnitine	Isovaleric acidemia
179	*N*-acetylglutamic acid	Aminoacylase I deficiency
180	*N*-acetylglutamine	Aminoacylase I deficiency
181	*N*-acetylglycine	Aminoacylase I deficiency
182	*N*-acetylhistidine	Histidinemia
183	*N*-acetylisoleucine	Aminoacylase I deficiency
184	*N*-acetylmethionine	Aminoacylase I deficiency
185	*N*-acetylneuraminic acid	Salla disease
186	*N*-acetylphenylalanine	Phenylketonuria
187	*N*-acetylthreonine	Aminoacylase I deficiency
188	*N*-acetyltryptophane	Isovaleric acidemia

Nr.	compound	main function/disease, further diseases in most cases
189	*N*-acetyltyrosine	Tyrosinemia I+II
190	*N*-acetylvaline	Aminoacylase I deficiency
191	neopterin	Phenylketonuria III
192	*N*-isovaleroylglycine	Glutaric aciduria type II
193	*N*-methylhistamine	Histidinemia
194	noradrenaline	Dopamine beta-hydroxylase deficiency (DßH)
195	*N*-trimethyllysine	TMLHE deficiency
196	octanoic acid	Medium chain acyl-CoA dehydrogenase deficiency
197	octenedioic acid	Medium chain acyl-CoA dehydrogenase deficiency
198	octenylsuccinic acid	Feeding: amino acid formula
199	ornithine	Cystinuria
200	orotic acid	Orotic aciduria
201	orotidine	Citrullinemia
202	oxalic acid	Primary hyperoxaluria I, PH1
203	phenylacetic acid	Phenylketonuria
204	phenylalanine	Phenylketonuria
205	phenyllactic acid	Phenylketonuria
206	phenylpropionylglycine	Medium chain acyl-CoA dehydrogenase deficiency
207	phenylpyruvic acid	Phenylketonuria
208	phosphoethanolamine	Hypophosphatasia
209	pimelic acid	Adrenoleukodystrophy, neonatal
210	pipecolic acid	Hyperprolinemia
211	proline	Iminoglycinuria
212	propionylglycine	Propionic aciduria
213	propionylcarnitine	Propionic aciduria
214	pyruvic acid	Lactic acidosis
215	quinolinic acid	Malabsorption syndromes
216	ribitol	Ribose 5-phosphate isomerase deficiency
217	saccharopine	Saccharopinuria
218	*S*-adenosylhomocysteine	*S*-Adenosylhomocysteine (SAH) hydrolase deficiency
219	salicylic acid	Adenylosuccinate lyase deficiency
220	sarcosine	Glutaric aciduria type II
221	sebacic acid	Glutaric aciduria type II

Nr.	compound	main function/disease, further diseases in most cases
222	serine	Renal dysfunction
223	serotonine	Monoamine oxidase-A deficiency (MAO-A)
224	sialic acid	Sialic acid storage disease
225	*S*-sulfocysteine	Molybdenum cofactor deficiency
226	suberic acid	Glutaric aciduria type II
227	suberylglycine	Medium chain acyl-CoA dehydrogenase deficiency
228	succinic acid	Fumaric aciduria
229	succinylacetone	Tyrosinemia I hepatorenal form
230	sucrose	Malabsorption syndromes
231	taurine	Hyper-ß-alaninemia
232	threonine	Hartnup disorder
233	thymidine	Mitochondrial NeuroGastroIntestinal Encephalopa-thy
234	thymine	Dihydropyrimidinase deficiency
235	thyroxine	Hypothyroidism
236	tiglylgylcine	ß-Ketothiolase deficiency
237	trimethylamine	Trimethylaminuria / fish odor syndrome
238	trimethylamine-N-oxide	Trimethylaminuria / fish odor syndrome
239	tryptophane	Hepatic failure
240	tyramine	Artefacts-bacterial contamination
241	tyrosine	Asphyxia
242	uracil	Tyrosinemia type I
243	uric acid	Citrullinemia
244	uridine	Ornithine carbamoyltransferase deficiency
245	urocanic acid	Urocanic aciduria
246	valine	Hartnup disorder
247	valine-proline	Prolidase deficiency
248	vanilmandelic acid	Neuroblastoma
249	xanthine	Molybdenum cofactor deficiency
250	xanthurenic acid	Hydroxykynureninuria
	currently 250 metabolites	

NMR Analysis of Bodyfluids (Flowchart)

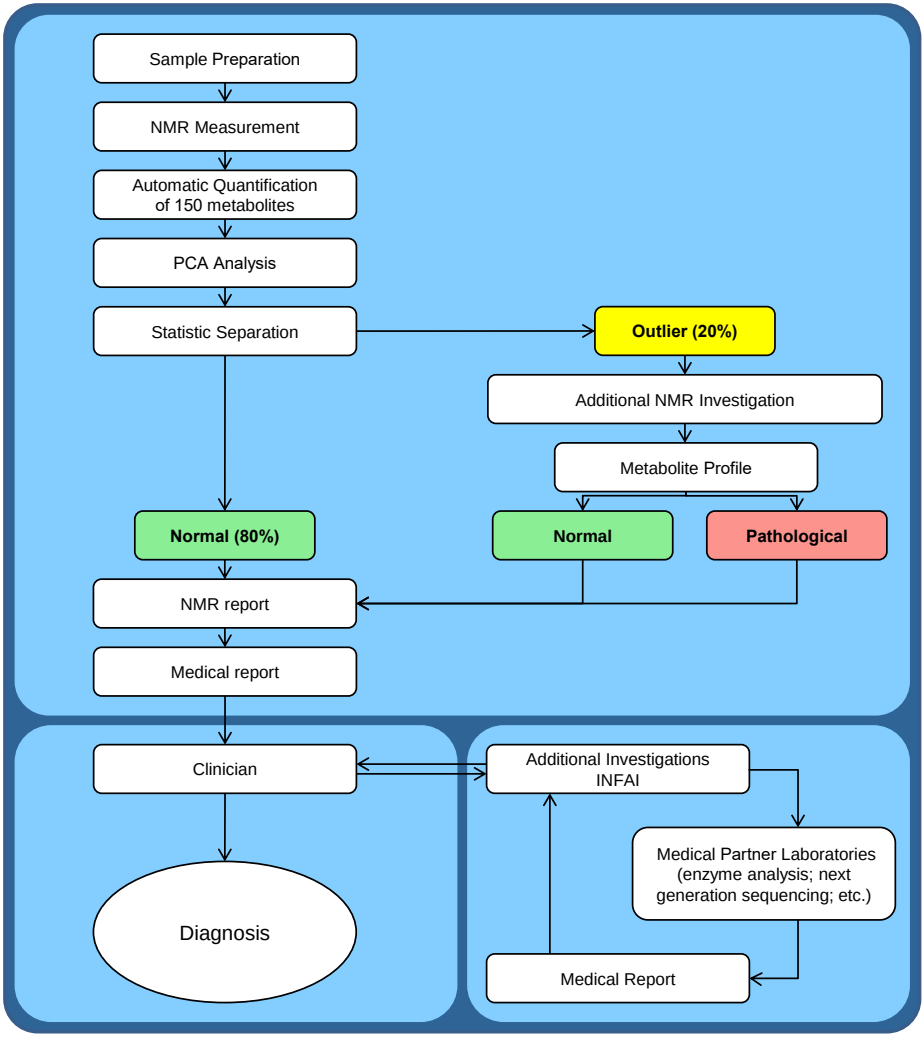


Figure 9: Performance of targeted and untargeted NMR analysis of bodyfluids. Additional investigations (GC-MS, LC-MS, enzyme analysis, gene sequence analysis) can be used.

References

1. Aygen S, Dürr U, Hegele P, Kunig J, Spraul M, Schäfer H, Krings D, Cannet C, Fang Fang, Schütz B, Bülbül SFH, Aydin HI, Sarici SÜ, Yalaz M, Örs R, Atalan R, Tuncer O, NMR-Based Screening for Inborn Errors of Metabolism: Initial Results from a Study on Turkish Neonates, JIMD Reports 14 (2014) 326.
2. Clark H, Kelleher AS, Chace DH, Spitzer AR, Gestational Age and Age at Sampling Influence Metabolic Profiles in Premature Infants, Pediatrics 134 (2014) e37.
3. Pyhtila BM, Shaw KA, Neumann SE, Fridovich-Keil JL, Newborn Screening for Galactosemia in the United States: Looking Back, Looking Around, and Looking Ahead, JIMD Reports 14 (2014) 302.
4. Sahai I, Garganta CL, Bailey J, James P, Levy HL, Martin M, Neilan E, Phornphutkul C, Sweetser DA, Zytkovicz TH, Eaton RB, Newborn Screening for Glutaric Aciduria-II: The New England Experience, JIMD Reports 13 (2014) 1.
5. Leal J, Wordsworth S, Oerton J, Khalid JM, Dezateux C, Synthesis framework estimating prevalence of MCADD and sensitivity of newborn screening programme in the absence of direct evidence, J Clin Epidemiol (2014).
6. Aygen S, Dürr U, Coskun T, Dursun A, Different diagnosis: distinction between L-2- and D-2-hydroxyglutaric acid, JIMD 36 (Suppl. 2) (2013) S164.
7. Saudubray JM, van den Berghe G, Walter JH, Inborn Metabolic Diseases - Diagnosis and Treatment, 5th Edition, Springer (2012).
8. Gryff-Keller A, Kraska-Dziadecka A, Kubica D, Detection of acylglycines in urine by 1H and 13C NMR for the diagnosis of inborn metabolic diseases, Acta Biochim. Pol. 59 (2012) 613.
9. Aygen S et al., Urine based newborn screening study applying high-resolution NMR spectroscopy in Turkey, JIMD 34 (Suppl. 3) (2011) S256.
10. Assfalg M, Bertini I, Colangiuli D, Luchinat C, Schäfer H, Schütz B and Spraul M, Evidence of different metabolic phenotypes in humans, PNAS 105 (2008) 1420.
11. Engelke UF, Sass JO, Van Coster RN, Gerlo E, Olbrich H, Krywawych S, Calvin J, Hart C, Omran H, Wevers RA, NMR spectroscopy of amino-acylase 1 deficiency, a novel inborn error of metabolism, NMR Biomed. 21 (2008) 138.
12. Blau N, Duran M, Blaskovics ME, Gibson KM, Laboratory diagnosis of metabolic diseases, Springer (2005) 363.
13. Topcu M, Coskun T et al., L-2-Hydroxyglutaric aciduria: a report of 29 patients, Turkish J. Pediatr. 47 (2005) 1.
14. Blau N, Duran M, Blaskovics ME, Gibson KM, Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases, 2nd Edition, Springer (2003).
15. Moolenaar SH, Engelke UF, Hoenderop SM, Sewell AC, Wagner L, Wevers RA, Handbook of 1H-NMR spectroscopy in inborn errors of metabolism, SPS 2002.
16. Linn T, Santosa B, Grönemeyer D, Aygen S, Scholz N, Busch M, Bretzel RG, Effects of long-term dietary protein intake on glucose metabolism in humans, Diabetologica 43 (2000) 1257.
17. Lindon JC, Nicholson JK, Everett JR, NMR spectroscopy of biofluids, Ann. Rep. NMR Spectr. 38 (1999) 1.
18. Holmes E, Foxall PJD, Spraul M, Farrant RD, Nicholson JK, Lindon JC, 750 MHz 1H NMR spectroscopy characterisation of the complex metabolic pattern of urine..., J. Pharm. Biomed. Anal. 15 (1997) 1647.
19. Aksu F, Aygen S, Vigabatrintherapie bei einem 7jährigen Jungen mit Succinat-Semialdehyd-Dehydrogenase-Mangel, Monatsschrift Kinderheilkunde 144 (1996) 797.
20. Aygen S et al., Hochauflösende 1H NMR Spektroskopie von Körperflüssigkeiten (Serum, Urin, Liquor) zur Diagnose und Therapiekontrolle bestimmter angeborener Stoffwechselerkrankungen im Kindesalter, Aktuelle Neuropädiatrie, Ciba-Geigy Verlag (1994) 273.
21. Yamaguchi S, Koda N, Eto Y, Aoki K, Quick screening and diagnosis of organic acidemia by NMR urinalysis, J. Pediatr. 106 (1985) 620.

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Metabo Test INFAI in Turkey will be performed in cooperation with Bruker Biospin.

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