

# Selective Screening in urine for inborn errors of metabolism using NMR analysis linked to METAGENE knowledgebase

Fraudienst-Egger G<sup>1</sup>, Goetz H<sup>4</sup>, Cannet C<sup>3</sup>, Beedgen L<sup>2</sup>, Trefz F<sup>5</sup>, Godejohann M<sup>3</sup>, Schäfer H<sup>3</sup>, Spraul M<sup>3</sup>

<sup>1</sup> Kreiskliniken Reutlingen, Reutlingen, Germany, <sup>2</sup> Dietmar-Hopp-Stoffwechszentrum Zentrum für Kinder- und Jugendmedizin, Universitätsklinikum Heidelberg, Germany,

<sup>3</sup> Bruker BioSpin GmbH, Rheinstetten, Germany, <sup>4</sup> TDB Software, Schwabach, <sup>5</sup> Metabolic Consulting, Reutlingen, Germany

**Introduction:** NMR analysis in urine is a new approach for highly quantitative and reproducible measurement of a high number of analytes with different substance classes running on one platform. Because of the huge number of information provided by the NMR report an automatic evaluation providing a suggestion of possible diagnoses is desirable.

**Methods:** Quantitative NMR analysis (1 Bruker Biospin Advance IVDr, B.I.Quant-URTM) was performed automatically for 152 metabolites of 12 substance classes. Metagene, a knowledgebase for diagnostic support of inborn errors of metabolism ([www.metagene.de](http://www.metagene.de)) was adopted for direct interpretation of NMR reports. Ranking of potential diagnoses explainable by the metabolic findings in the report is done by comparison to pathological cut off levels of metabolites in Metagene providing 209 diseases and differential diagnoses potentially detectable by NMR analysis.

**Results:** In 20 out of 27 diagnosed cases data based automatic ranking was correct (74%). Additional adding information as age and clinical symptoms improved rational ranking. Diseases which are well-defined by one characteristic metabolite show the best ranking as: (2) L- Alloisoleucine in MSUD, (3) Galactitol in GALACTOSEMIA, (4) Argininosuccinic acid in ARGININOSUCCINIC ACIDURIA (ASL).

**1 NMR-Analysis and NMR-Report**

**2 MAPLE SIRUP URINE DISEASE**

Metabolite	mmol/mol Crea	mmol/l
Alanine	89,0	0,1
DL-Alloisoleucine / allo-Isoleucine	410,0	0,4
Glycine	520,0	0,5
L-Isoleucine	110,0	0,1
Leucine	290,0	0,3
Valine	120,0	0,1
D-Glucose	400,0	0,4
Creatine	120,0	0,1
Guanidinoacetic acid / Guanidinoacetic	120,0	0,1
Acetoacetic acid	22,0	0,0
Acetone	17,0	0,0
1,3-Dimethyluric acid	79,0	0,1
1-Methylhydantoin	62,0	0,1
2-Hydroxyisovaleric acid	1200,0	1,2
2-Oxoglutaric acid	340,0	0,3
2-Oxoisocaproic acid	150,0	0,1
3-Hydroxyisovaleric acid	150,0	0,1
3-Hydroxypropionic acid	110,0	0,1
3-Methylglutaconic acid	140,0	0,1

**3 GALACTOSEMIA**

metabolite (mmol/mol crea)	(+)	+	++	+++	noticeable	significant
Galactitol					66	10 - 17 >= 18
Galactitol	196				151 - 299	>= 300

**4 ARGININOSUCCINIC ACIDURIA**

metabolite (mmol/mol crea)	(+)	+	++	+++	noticeable	significant
Argininosuccinic acid					780	161 - 319 >= 320
Argininosuccinic acid					2400	100 - 197 >= 198
Cystine					110	39 - 75 >= 76
3-Methylglutaconic acid					120	27 - 51 >= 52
4-Aminobutyric acid	21					21 - 39 >= 40
Guanidinoacetic acid	360					191 - 379 >= 380
Proline betaine	170					121 - 239 >= 240
Benzoic acid	74					72 - 141 >= 142

**Conclusion:** NMR analysis provides an excellent tool for using automatic analysis to further enable high throughput screening of urine samples and to improve yield of genetic metabolic diseases in the metabolic laboratory. However, further work is needed to include additional diagnosed cases and to optimize the rules for developing a comprehensive expert system.