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To

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Department of General Pediatrics
(General Pediatrics, Neurology, Metabolism, Gastroenterology, Nephrology)
Prof. Dr. med. G.F. Hoffmann
Chairman
Center for Metabolic Diseases Heidelberg

Metabolic Laboratory

Heidelberg, 27 February 2017

ERNDIM QA Scheme for qualitative urinary organic acid analysis

Annual Report 2016

Participation

The geographical distribution of the active participants of the quality assurance scheme organized and distributed through the centre of Heidelberg in 2016 is shown in Table 1. Sheffield and Heidelberg participate in each other's scheme and the two centers work closely together under the auspices of the ERNDIM Scientific Advisory Committee.

Table 1: Geographical distribution of participants

Country	Number of laboratories		Country	Number of laboratories
Austria	3		Latvia	1
Belgium	1		Lithuania	1
Bulgaria	1		Luxembourg	1
Canada	9		New Zealand	1
Croatia	1		Norway	1
Cyprus	1		Philippines	1
Czech Republic	2		Poland	2
Denmark	1		Serbia	1
Estonia	2		Slovakia	2
France	5		Slovenia	1
Germany	18		Spain	2
Greece	1		Sweden	2
Hong Kong	1		Switzerland	3
Hungary	1		The Netherlands	8
India	4		Ukraine	1
Italy	12		United Kingdom	1
Kingdom of Saudi Arabia	1		USA	12

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Samples and results

Three sets of three samples (total 9; sample numbers H232 - H240) were distributed to **105 laboratories**.

Table 2 shows the number of returned results for each circulation and the number of late returns.

Table 2: Receipt of results

Circulation	In time returns	Late returns	Total
1. circulation	99	4	103
2. circulation	100	1	102
3. circulation	94	7	101

Ninety-four percent of the participants returned results for all three circulations. One laboratory (1%) did not respond to any of the circulations (see also table 3)

Table 3: returned results

Circulations	Number of laboratories	%
3	99	94
2	4	4
1	1	1
0	1	1

Shipment of the samples

Date of sample dispatch: **09 May 2016**

The samples were sent out by the **Quality Control Center Switzerland (CSCQ)**.

As the years, before the samples for all three circulations were shipped together. This is only for organizational reasons, especially to keep the costs for participating in this scheme as low as possible.
Please remember, the idea of the scheme is to measure the samples evenly spread over the year and to report the results near to the closing date!

Table 4: Distribution of scores for individual samples (number of laboratories making returns)

		4	3	2	1	0
Sample H232	Propionic aciduria	101	2			
Sample H233	Normal pattern	103				
Sample H234	Ornithine transcarbamylase (OTC) deficiency	83	14	3	1	2
Sample H235	Short-chain enoyl-CoA hydratase (SCEH/ECHS 1) deficiency	Excluded from scoring				
Sample H236	Normal pattern	101		1		
Sample H237	HMG-CoA lyase deficiency	93	3	6		
Sample H238	Normal pattern	97	4			
Sample H239	Mitochondrial acetoacetyl-CoA thiolase (MAT) deficiency (synonym: beta-ketothiolase deficiency)	21	68	11		1
Sample H240	Aminoacylase I deficiency	66	1	3	8	23

Scoring scheme

In 2013 we changed the scoring system from the former scale (-2, -1, 0, +1, +2) to the four-point system (+1, +2, + 3, +4) which is used also in the DPT schemes. In this system a maximum of two points is given each for analytical results and interpretation, with the latter including suggestions for further testing/actions.

The total score achievable for a single circulation of three samples is twelve and thirty-six for the whole sample set of nine samples per year.

To obtain satisfactory performance a score of 22 or more should be achieved on three returns and 15 or more when two returns have been submitted.

Another criteria for satisfactory performance will be the absence of any “critical error” which is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient.

The final scoring and all proposed critical errors will need to be ratified by the Scientific Advisory Board (SAB).

Further information on the concept of ‘critical error’ can be found in the ERNDIM Newsletters at www.erndim.org.

Comments on performance

Sample H232:

Patient details:

4-month-old boy with metabolic acidosis, sample taken after re-compensation

Known diagnosis:

Propionic aciduria

Analytical details

Marked elevation of 3-hydroxypropionic acid, propionylglycine and methylcitric acid.

Analytical Performance:

96% reported 3-hydroxypropionic acid and/or propionylglycine.

89% reported all three metabolites.

Diagnostic Performance:

Propionic aciduria was diagnosed by 98% of the participants.

For the diagnoses holocarboxylase deficiency and biotinidase deficiency one point was substracted.

Sample H233:

Patient details:

2-year-old boy with developmental delay

Known diagnosis:

Normal pattern

Analytical details:

Nothing specifically

Overall Performance: 100%

Sample H234:

Patient details:

8-year-old girl presenting with ataxia and recurring episodes of hyperammonaemia. Currently under medication

Known diagnosis:

Ornithine transcarbamylase (OTC) deficiency

Analytical details:

Elevated amount of orotic acid.

Secondary findings were increased lactic acid and pyruvic acid as well as drug metabolites of phenylbutyric acid and benzoic acid.

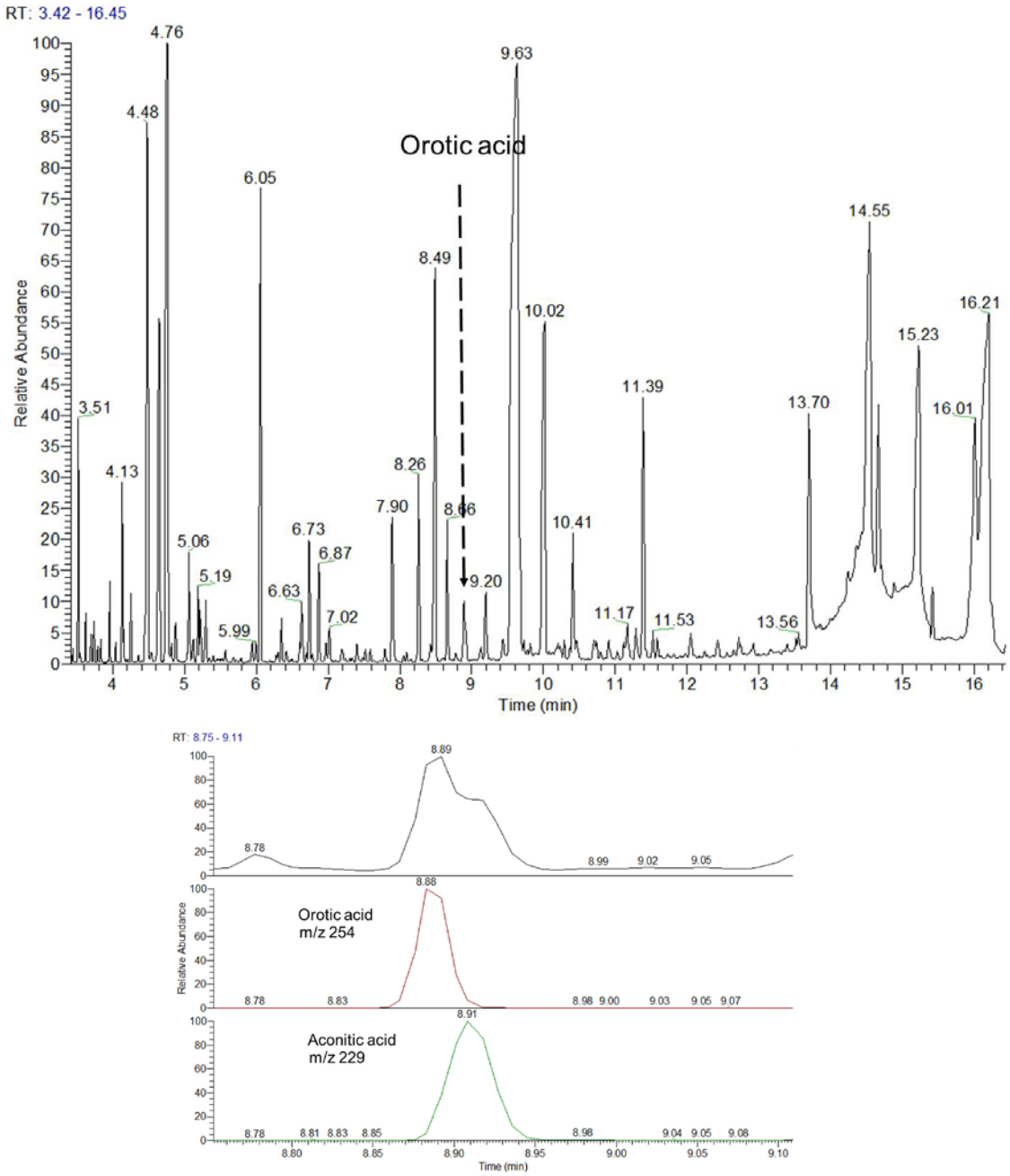


Fig. 1: GCMS chromatogram and mass chromatograms: orotic acid / aconitic acid

Analytical Performance:

Detection of orotic acid is important for the diagnosis and laboratories should make sure that this metabolite could not be overlooked e.g. by carefully checking the mass chromatograms for orotic acid and aconitic acid. Poor extraction efficiency and/or poor performance of the chromatographic system may also be the reason for missing orotic acid.

81% detected orotic acid (median: 69 mmol/mol creatinine; range 30 – 144; N=10;
Heidelberg: 75 mmol/mol creatinine).

Diagnostic Performance:

93% diagnosed urea cycle defect. Of these 39% specified OTC deficiency.

Critical error:

A critical error was defined by the SAB for this sample if detection of orotic acid was missed and a normal diagnosis or a misleading diagnosis was given with no or unhelpful advice for further investigations.

Sample H235:

Patient details:

2-month-old girl presenting with failure to thrive and lactic acidosis

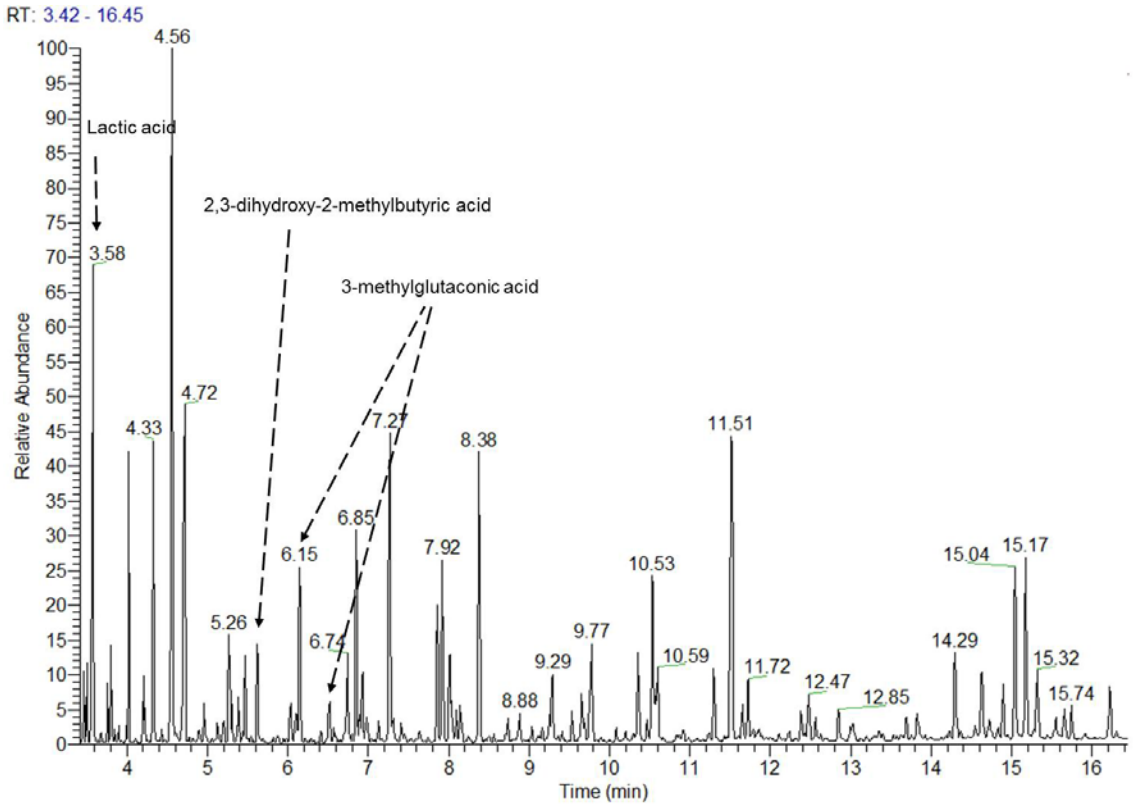
Known diagnosis:

Short-chain enoyl-CoA hydratase (SCEH/ECHS 1) deficiency

Analytical details:

Increased excretion of 3-methylglutaconic acid and lactic acid.

The chromatogram also shows a prominent peak for 2,3-dihydroxy-2-methylbutyric acid, a metabolite which is found in the urine of patients with short-chain enoyl-CoA hydratase (ECHS1) deficiency and 3-hydroxyisobutryl-CoA hydrolase (HIBCH) deficiency (Peters et al; *Molecular Genetics and Metabolism 115 (2015) 168–173)*.



Mass spectrum of synthetic 2,3-dihydroxy-2-methylbutyric acid

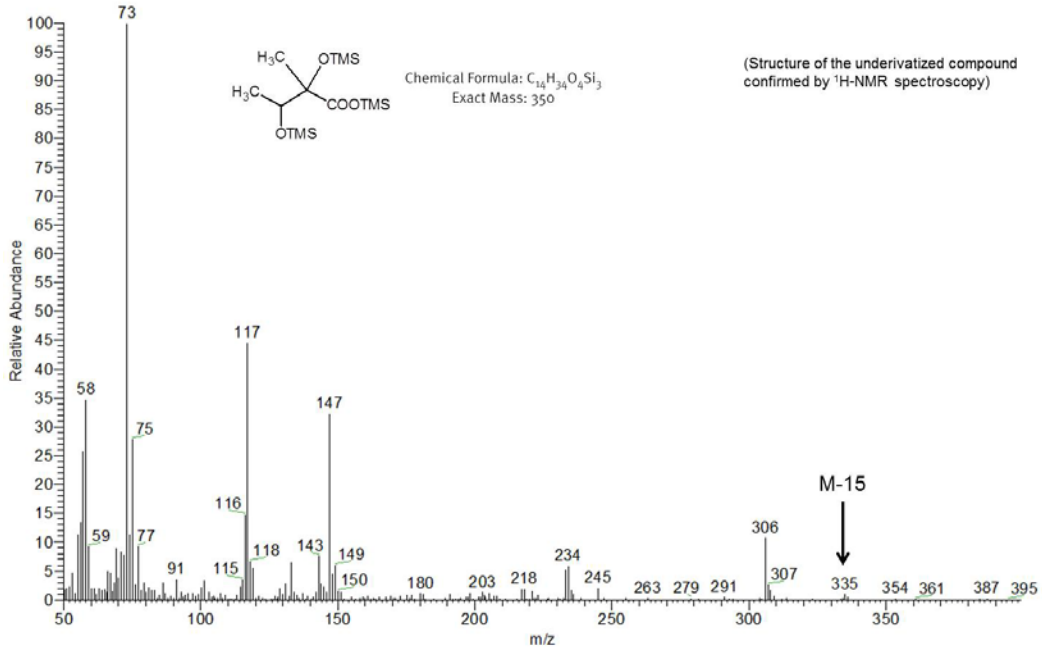


Fig. 2: GCMS chromatogram and mass spectrum of silylated 2,3-dihydroxy-2-methylbutyric acid

2,3-dihydroxy-2-methylbutyric acid was detected by only 6 laboratories. This outcome is possibly related to the mass spectrum of this metabolite which was unknown to most of the labs.

Comment:

This sample was considered by the SAB to be educational and is excluded from the scoring.

Nevertheless in this urine one could find metabolites which, together with the given clinical description could point to mitochondrial disorders. Laboratories should have taken this into account when giving advice on differential diagnosis and further investigations.

All together 76 % of the participants pointed to 3-methylglutaconic acidurias and/or mitochondrial disorders.

Sample H236:

Patient details:

10-year-old boy with autistic behaviour

Known diagnosis:

Normal pattern

Analytical details:

Nothing specifically diagnostic

Overall Performance:

99%. One lab diagnosed neuroblastoma based on the finding of small amounts of homovanillic acid and vanillylmandelic acid

Sample H237:

Patient details:

10-month-old boy with muscular hypotonia and episodes of hypoglycaemia

Known diagnosis:

HMG-CoA lyase deficiency

Analytical details:

Significantly elevated amounts of 3-methylglutaconic acid, 3-hydroxy-3-methylglutaric acid, 3-methylglutaric acid and 3-hydroxyisovaleric acid. 3-methylcrotonylglycine was detectable.

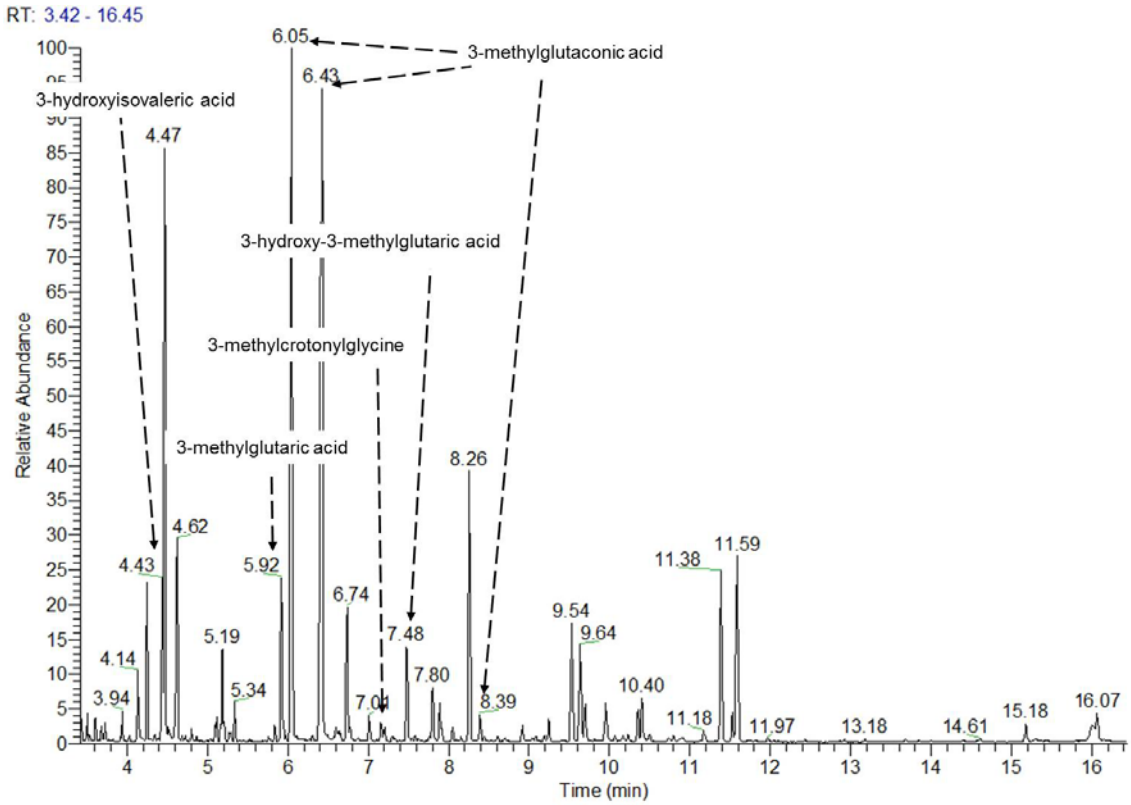


Fig. 3: GCMS chromatogram

Analytical performance:

Two points was given for reporting 3-hydroxy-3-methylglutaric acid, 3-methylglutaconic acid and 3-methylglutaric acid.

99% identified 3-methylglutaconic acid

97% reported 3-hydroxy-3-methylglutaric acid

Diagnostic Performance:

The diagnosis of 3-hydroxy-3-methylglutaric aciduria due to HMG-CoA lyase deficiency resulted in two points for the interpretation. Any type of 3-methylglutaconic aciduria was scored with one point.

93% diagnosed HMG-CoA lyase deficiency

Sample 238:

Patient details:

4-year-old boy with febrile seizure

Known diagnosis:

Normal pattern

Analytical details:

Nothing specifically diagnostic

Overall Performance:

94% suggested a normal profile.

Four participants supposed hyperoxaluria type I based on glycolic acid and/or glycolic acid and oxalic acid

Sample 239:

Patient details:

15-month-old boy admitted due to acute metabolic acidosis after infection

Known diagnosis:

mitochondrial acetoacetyl-CoA thiolase (MAT) deficiency

Analytical details:

high excretion of 2-methyl-3-hydroxybutyric acid, tiglylglycine and 2-methylacetoacetic acid. 2-methyl-3-hydroxybutyric acid appears in the chromatogram as two peaks for the corresponding diastereomeres.

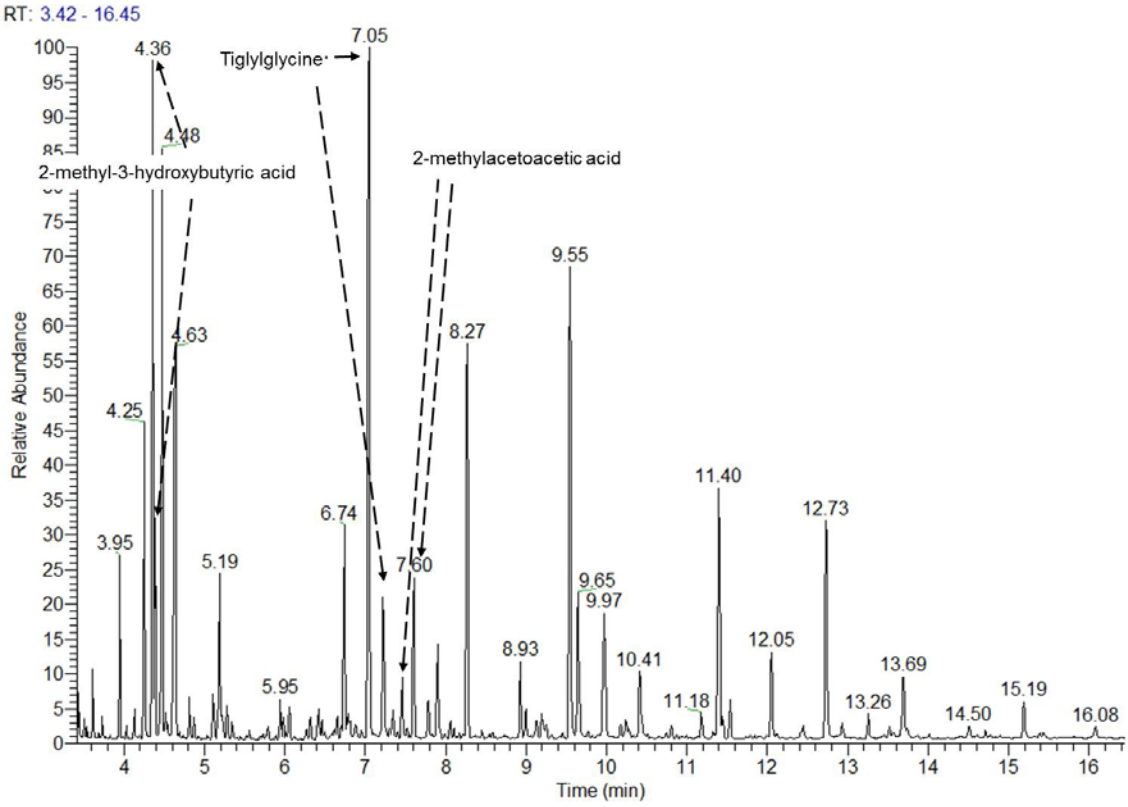


Fig. 4: GCMS chromatogram

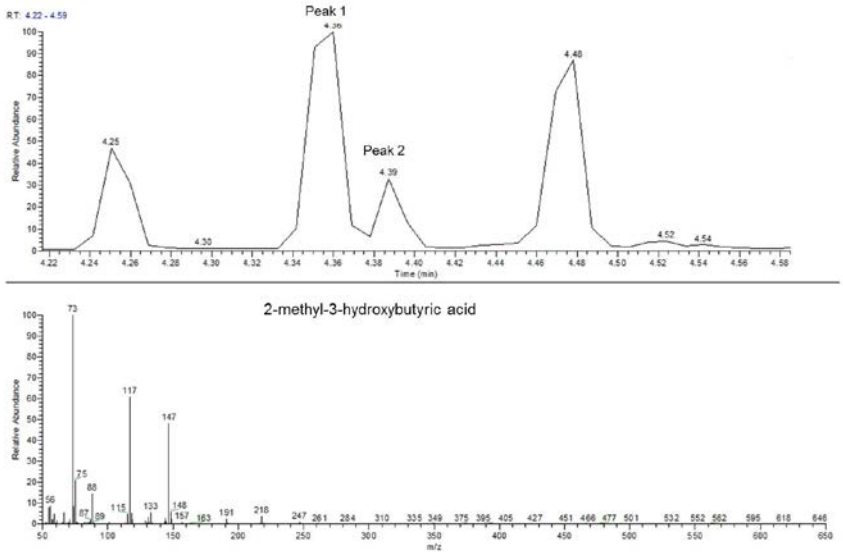


Fig. 5: detail of GCMS chromatogram and mass spectrum of 2-methyl-3-hydroxybutyric acid

Analytical Performance:

2-methyl-3-hydroxybutyric acid was reported by 98% and tiglylglycine by 97% laboratories.

Only 24% could identify 2-methylacetoacetic acid.

Diagnostic Performance:

66% diagnosed MAT deficiency and scored two points.

Mentioning only MHBD deficiency resulted in one point.

Overall Performance:

89% for the differential diagnosis of MAT deficiency and MHBD deficiency

Sample 240:

Patient details:

12-year-old girl, severe intellectual disability and autism

Known diagnosis:

Aminoacylase I deficiency

Analytical details:

Increased excretion of N-acetylated amino acids

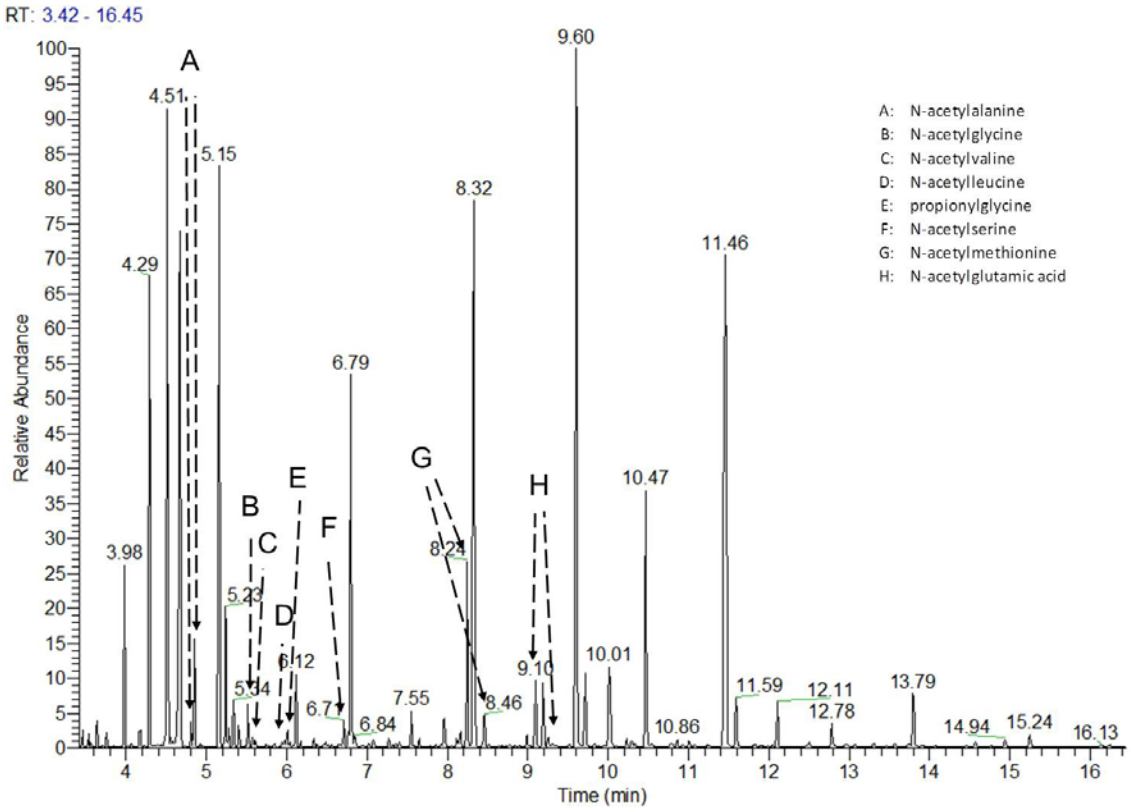


Fig. 6: GCMS chromatogram

Analytical Performance:

67% of the labs reported N-acetylated amino acids which was awarded with two points.

Mentioning at least propionylglycine gave one point.

Diagnostic Performance:

65% of the participants gave the correct diagnosis.

Three labs diagnosed propionic aciduria. Two others suggested SSADH deficiency or N-acetyl glutamate synthase or carbamoyl phosphate synthase deficiency.

The participants´ cumulative scores are shown in table 5 and in figure 7.
Cumulative scores are the scores for the whole year.

Table 5: Cumulative total scores 2013 - 2016
Number of all participants: all registered laboratories
Number of nonresponders: no results returned for any of the three circulations

	Percent of all participants			
Cumulative scores	**2016**	**2015**	**2014**	**2013**
36	*Sample H235 not scored*	81	54	82
35		3	13	-
34		8	12	7
33		-	1	-
32	10	5	6	1
31	34	-	3	-
30	13	-	2	-
29	5	-	1	-
28	9	1	1	-
27	10	-	-	-
26	6	-	-	-
25	4	-	-	-
24	3	2	4	5
23	1	-	-	-
22	1	-	-	-
21	-	-	1	-
20	3	-	-	-
19	-	-	-	-
18	-	-	-	-
17	-	-	-	-
16	-	-	-	-
15	-	-	-	-
14	-	-	-	-
13	-	-	-	-
12	1	-	-	1
11	-	-	-	-
10	-	-	-	-
9	-	-	-	-
8	-	-	-	-
7	-	-	-	-
6	-	-	-	-
5	-	-	-	-
4	-	-	-	-
3	-	-	-	-
2	-	-	-	-
1	-	-	-	-
0	-	3	2	3
Number of all participants	105	103	101	94
Number of Nonresponders	1	4	2	3

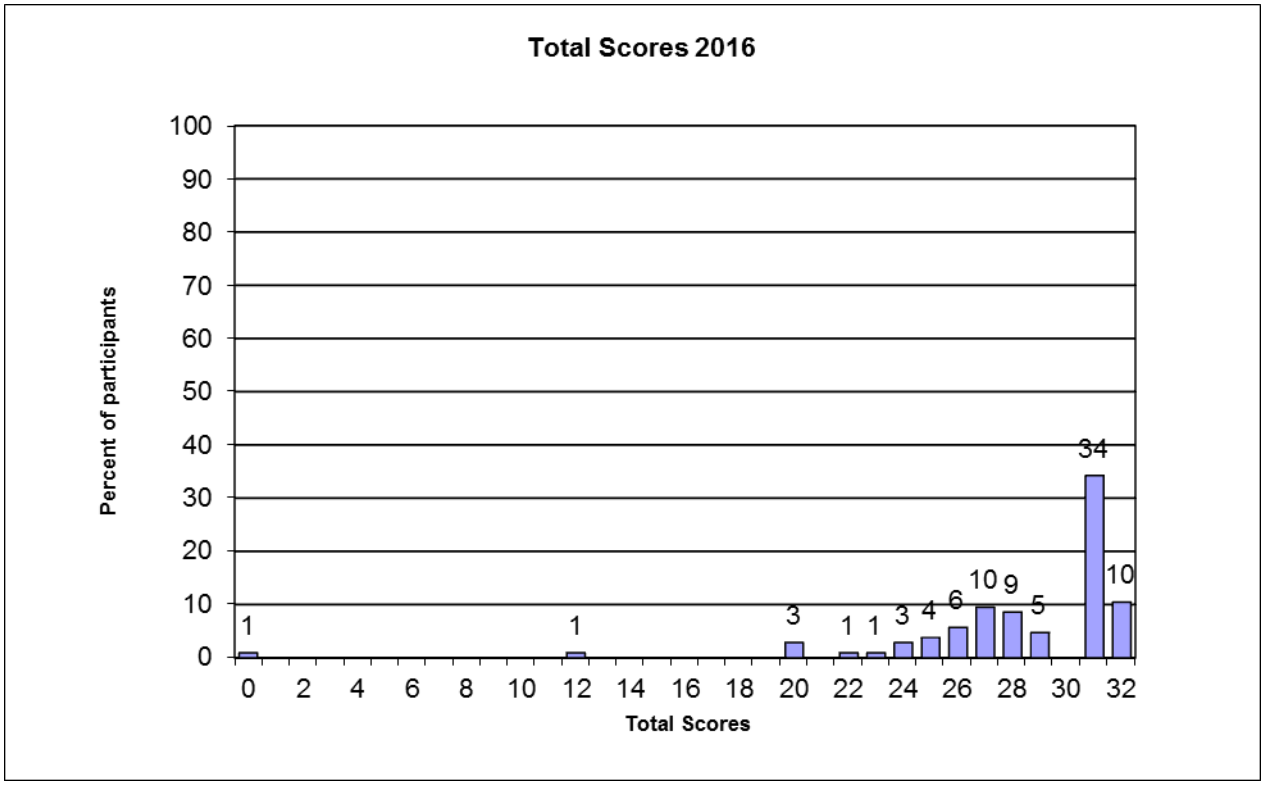


Fig. 7: Cumulative scores 2016 (maximum achievable score: 32)

Your individual scores for Sample H232 – H240:

Sample H232:
Sample H233:
Sample H234:
Sample H235:
Sample H236:
Sample H237:
Sample H238:
Sample H239:
Sample H240:

Your total score 2016

Your total score for 2016 was:

Your number of returns in 2016 was:

General comments

We would just like to point out here that we are not able to accept returns sent in after the report for the corresponding circulation has been mailed because this would not be compatible with the overall intention of the scheme. We are conscious of the fact that posted results could get lost on a variety of ways. Therefore it would be a good advice to send in results by more than one route (e.g. FAX and email, regular mail and FAX or email).

Special thank for the laboratories that supported us last year with samples. This is critical for the success of the program and will keep the scheme interesting. Please **continue to support us with urine from patients. It is most appreciated. Please send us at least 300 ml urine of any interesting patients you may have. We will cover the costs.**

In 2016 samples were contributed by

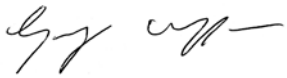
- Dr Maria Grazia Alessandrì (IRCCS Fondazione Stella Maris Calambrone; Italy)

and

- Dr Martin Lindner (University Hospital Frankfurt, Germany)

Yours sincerely,





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