**ERNDIM**
**Diagnostic Proficiency Testing**
**DPT Centre: Netherlands**

# ANNUAL REPORT 2015


| Scheme Organiser | Scientific Advisor | Deputy Scientific Advisor |
|---|---|---|
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## 1. Introduction

As of 2014 DPT schemes are organised by CSCQ, the Swiss EQA organisation. The minimal required test panel for participation in any DPT scheme includes dip sticks, amino acids, organic acids and quantitative GAG. DPT-NL additionally requires the analysis of oligosaccharides and purines-pyrimidines. It is strongly recommended to have the following tests available for DPT-NL: qualitative GAG analysis (electrophoresis/TLC), sialic acid, creatine-guanidinoacetate and polyols-sugars. Please note that in DPT schemes it is allowed to obtain results from neighbouring laboratories if one does not offer a certain test, while such test is deemed necessary for a sample. It is required to indicate in the report that results were obtained from a cluster lab.

## 2. Participants

The 2015 scheme had 20 participating laboratories with the following allocations: Table 1. For both surveys all 20 participants submitted results.

Table 1. Participants in DPT-NL 2015

| Country | Number of participants |
|---|---|
| Australia | 1 |
| Belgium | 5 |
| Germany | 3 |
| The Netherlands | 8 |
| South-Africa | 1 |
| Switzerland | 1 |
| UK | 1 |

## 3. Logistics of the scheme

The samples used in the DPT scheme are authentic human urine samples and were selected by the Scientific Advisors of the scheme. Table 2 provides the sources of the samples. Sample pre-treatment (heat-treatment) was performed in the Scientific Advisor's laboratory, while aliquoting and dispatch of

the samples was done by the Scheme organiser. Two surveys were performed; 2015-1 (samples A, B, C) starting April 7, and 2015-2 (samples D, E, F) starting June 1. Before dispatch to participants one set of samples was sent to the Scientific Advisor and checked. In all six samples the typical metabolic profiles were preserved. Sample dispatch was done March 31, 2015 by DHL. All participants had received the samples by April 7.
Reports of the samples were submitted electronically on the website of the Swiss organisation for quality control (CSCQ) (https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php). The time allotted for submitting reports was 3 weeks after opening of the website. Clinical information on the samples was provided through the website.
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Table 2. Source of the 2015 samples

| Sample | Diagnosis | Provider |
|---|---|---|
| **A** | MPS II | Erasmus Medical Centre, Rotterdam, NL |
| **B** | 2-methylbutyryl-CoA DH deficiency (MBD or SBCAD deficiency) | Erasmus Medical Centre, Rotterdam, NL |
| **C** | Hyperoxaluria type I | SKML DPT sample collection |
| **D** | Homocystinuria due to CBS deficiency | Dr Vianey-Saban, Lyon, France (this was the common sample used in all DPT schemes) |
| **E** | Argininosuccinic aciduria/ASL deficiency | Dutch patient organisation, VKS |
| **F** | Glutaric aciduria type I | Dutch patient organisation, VKS |

## 4. Scoring of results

General scoring criteria are depicted in Table 3. Scoring of the 2015 samples was performed according to the criteria summarised in Table 4. In order to achieve harmonised scoring throughout the five European DPT schemes, the ERNDIM Board has instituted a second scoring officer belonging to one of the partner DPT schemes as of 2011. The external scores are discussed with the scheme's own scientific advisor(s). For the DPT-NL scheme, the second evaluation was performed by the scientific advisors of the DPT CZ scheme in 2015.

Table 3. General criteria for scoring results.

| Item | Criterium | Score |
|---|---|---|
| **Analytical performance:** | Correct results of the appropriate tests | 2 |
| | Partially correct or non-standard methods | 1 |
| | Unsatisfactory or misleading | 0 |
| | | |
| **Interpretative performance:** | Good (diagnosis was established) and adequate recommendations were suggested | 2 |
| | Helpful but incomplete | 1 |
| | Misleading / wrong diagnosis | 0 |
| | | |
| | Total maximal score for each sample | 4 |

Table 4. Specific criteria for scoring results of the 2015 samples.

| Sample | Analytical | points | Interpretation | points |
|---|---|---|---|---|
| **A** | Elevated GAG and/or abnormal electrophoresis/TLC<br>Elevated GAG without electrophoresis/TLC performed | 2<br>1 | MPS II included in DD<br>Other MPS or MPS not defined | 2<br>1 |
| **B** | 2-mebutyrylglycine elevated<br>C5-carnitine elevated (2-methyl-butyrylglycine not reported) | 2<br>1 | MBD/SBCAD deficiency included in DD<br>IVA or other | 2<br>0 |
| **C** | Elevated oxalic and/or glycolic acid | 2 | Hyperoxaluria type I<br>Hyperoxaluria unspecified<br>Advice to measure oxalate/glycolate | 2<br>1<br>1 |
| **D** | Elevated homocystine and/or hcys-cys mixed disulfide | 2 | Homocystinuria due to CBS deficiency<br>Hyperhomocysteinemia | 2<br>1 |
| **E** | Elevated ASA | 2 | Argininosuccinic aciduria/ASL deficiency | 2 |
| **F** | Elevated 3-OH-glutaric acid and/or C5DC | 2 | Glutaric aciduria type I | 2 |

The final decision about scoring of the DPT schemes is made in the Scientific Advisory Board. In accordance with a previous decision by the board, participants who failed to achieve satisfactory performance were those who scored less than 15 points out of the maximum of 24 in this year. Starting with the 2014 schemes the concept of 'critical error' is introduced to the assessment of the DPT schemes. Labs failing to make a correct diagnosis of a sample considered as eligible for this category will be deemed not to have reached a satisfactory performance even if their total points for the year exceed the limit set by the SAB. The classification of samples to be judged for critical error was undertaken at the SAB meeting held on March 17, 2016. The following possible critical errors were identified in the 2015 scheme (Table 5).

Table 5. Critical errors in the 2015 scheme.

| Sample | Critical error | No. of occurences |
|---|---|---|
| **A** | MPS not mentioned | 0 |
| **B** | none | - |
| **C** | none | - |
| **D** | Failure to report elevated homocystine and hcys-cys mixed disulfide and methionine AND no specific recommendations made | 3 |
| **E** | Failure to report elevated ASA and ASL deficiency AND no specific recommendations made | 0 |
| **F** | Failure to report elevated 3-OH-glutaric acid and C5DC-carnitine and glutaric aciduria type I | 1 |

## 5. Communication of results

As previously, we used the CSCQ scheme evaluation programme to generate individual lab reports and these were distributed on May 14th and July 25th. These individual participant reports included the scores obtained.
Discussion of the results took place in Lyon during the ERNDIM workshop held at the SSIEM conference on September 1, 2015. The meeting, as usual open to participants only, was attended by representatives from 9 of the participating institutes. George Ruijter, Erasmus Medical Centre Rotterdam, chaired the meeting and made a presentation of the analytical/diagnostic points of interest. This presentation has been sent to all DPT-NL participants attending the meeting. In addition, analysis of the results submitted and items discussed during the DPT meeting are part of the Annual Report Finally this annual report summarises scheme organisation and results.

ERNDIM provides a single certificate for all its schemes with details of participation and performance.

Five Performance Support letters will be send for the 2015 surveys. One was sent for the 2014 surveys.

## 6. Proficiency of the 2015 surveys

Proficiencies (% of maximal achievable points for all labs) of the 2015 samples are summarized in Table 6. Distribution of scores is given in Table 7.
Maximal scores (24 points) were obtained by 7 out of the 20 participating labs. Samples A, E and F were rather straightforward, while sample B, C and D were more challenging. Overall performance for all six samples was 86%, which is comparable to the performance in 2014 (85%), but considerably better than earlier years.

Table 6. Performance on the DPT 2015 samples.

| Sample | Diagnosis | No. of reports | Proficiency (%) | | |
|---|---|---|---|---|---|
| | | | analytical | interpretation | TOTAL |
| **A** | MPS II | 20 | 93 | 88 | 90 |
| **B** | 2-methylbutyryl-CoA DH deficiency (MBD or SBCAD deficiency) | 20 | 78 | 80 | 79 |
| **C** | Hyperoxaluria type I | 20 | 75 | 70 | 73 |
| **D** | Homocystinuria due to CBS deficiency | 20 | 85 | 83 | 84 |
| **E** | Argininosuccinic aciduria/ASL deficiency | 20 | 95 | 95 | 95 |
| **F** | Glutaric aciduria type I | 20 | 95 | 95 | 95 |

Table 7. Distribution of final scores in 2015; for each sample the number of participants with score 0/1/2/3/4 points is given.

| Sample | 0 points | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| **A** | 0 | 0 | 3 | 2 | 15 |
| **B** | 4 | 0 | 0 | 1 | 15 |
| **C** | 4 | 1 | 1 | 1 | 13 |
| **D** | 3 | 0 | 0 | 1 | 16 |
| **E** | 1 | 0 | 0 | 0 | 19 |
| **F** | 1 | 0 | 0 | 0 | 19 |

**7. Results of individual samples and evaluation of reporting**

## Sample 2015-A: Mucopolysaccharidosis type II (Hunter syndrome (OMIM 309900).

Clinical description: A male patient diagnosed at age 7 y with joint stiffness and carpal tunnel syndrome. The urine sample was obtained at age 42 y.

Sample A was obtained from an adult MPS II sample with confirmed iduronate sulfatase deficiency. This was a clear MPS sample, which was correctly identified by all 20 participants. The mean GAG level was 26 mg/mmol (range 12-46). Thirteen participants reported elevations of both DS and HS, while 4 reported elevated DS only. Three labs did not perform analysis of the different GAG species. MPS type II was included in the possible diagnoses by 15 participants. Two labs suggested MPS I or VI on the basis of elevated DS.
It has been suggested that the ratio HS/DS is higher in MPS II compared to MPS I. While this has been difficult to establish using qualitative techniques such as electrophoresis or TLC, the development of quantitative tests for the different GAG species shows that indeed MPS II urine samples contain a larger HS fraction than MPS I (Fig. 1, see also Langereis et al 2015 Plos One 10). All participants reccommended to test the relevant MPS enzymes in leukocytes or fibroblasts.

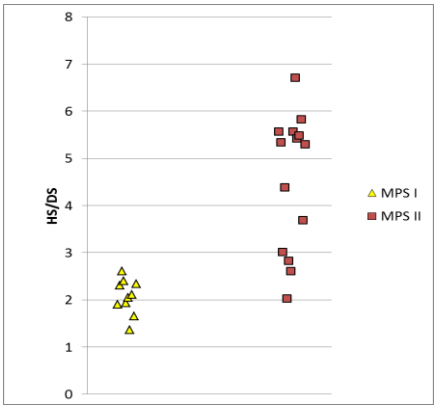


Fig. 1. HS/DS ratio's in a number of MPS I and II urine samples.

## Sample 2015-B: 2-Methylbutyryl-CoA DH (MBD) or Short Branched Chain Acyl-CoA DH (SBCAD) deficiency (OMIM 610006).

Clinical description: This male infant was referred for delayed motor milestones and hypotonia at age 1 y. Currently, at age 6 y, his intellectual development is normal. He is receiving specific treatment.

While presently most MBD deficiencies are identified through newborn screening, the urine sample distributed in this survey was obtained from a patient that presented with clinical symptoms. Enzyme testing showed decreased MBD activity: 0.11 nmol/mg/min (ref 0.50 +/- 0.10).

Results of organic acid analysis (qualitative/interpretation were (number of participants):

| | elevated | normal/trace |
|---|---|---|
| 2-methylbutyrylglycine | 15 | - |
| Isovalerylglycine | 3 | 1 |
| Isobutyrylglycine | 3 | 2 |

Results of quantitative organic acid analysis were (all mmol/mol):

| | n | median | mean | SD | min-max |
|---|---|---|---|---|---|
| 2-methylbutyrylglycine | 6 | 39 | 58 | 41 | 19-130 |
| Isovalerylglycine | 2 | 2.2 | 2.2 | 1.8 | 0.4-3.9 |
| Isobutyrylglycine | 3 | 3.3 | 3.0 | 1.5 | 1.0-4.6 |

The 15 labs that identified the characteristic metabolite, 2-methylbutyrylglycine, included MBD deficiency in the possible diagnoses. A small retention time difference exists between 2-methulbutyrylglycine and isovalerylglycine: ΔRT MBG-IVG = 0.65 min (TMS; DB-1701), ΔRT MBG-IVG = 0.2 min; (methylated, Restek RTX-35). The mass spectra of MBG and IVG are different (Fig. 3), which enables their identification.
Five labs quantified acylcarnitines. The median value of C5-carnitine was 21 mmol/mol. Separation of the different C5-carnitine species clearly showed elevation of (both R- and S-) 2-methylbutyryl-carnitine (Fig. 4).
Two labs mentioned MADD as a possible diagnosis. Since there were no clear elevations of other metabolites that would suggest MADD,such as dicarboxylic acids, 2-OH-glutaric acid and glutaric acid, this is unlikely. A mild MADD presentation, e.g. Brown-Vialetto-Van Laere syndrome, shows clearly elevated levels of these metabolites (Bosch et al 2011, JIMD 34:159-164).
Also Isovaleric academia was suggested by 2 labs. However, isovalerylglycine was not clearly elevated.
The following further investigations were reccommended: acylcarnitine analysis in plasma, repeat OA in urine, enzyme test in WBC or fibroblasts and ACADSB sequencing.

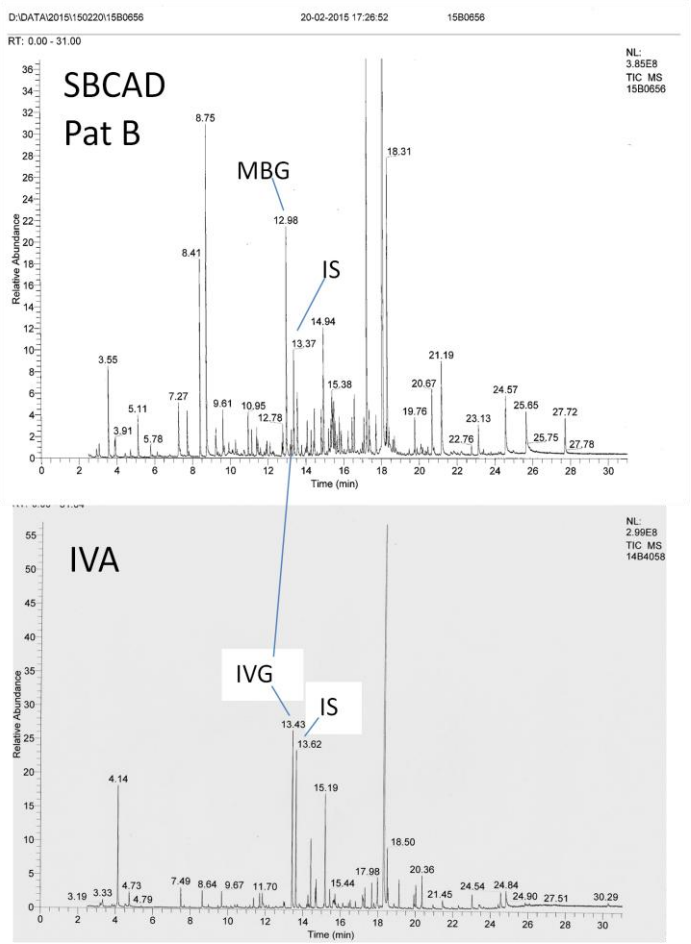

Fig. 2. GC-MS of methylated organic acids: isovaleric acidemia vs MBD/SBCAD. IVG = isovalerylglycine, MBG = 2-methylbutyrylglycine, IS = Internal standard (4-phenylbutyric acid)

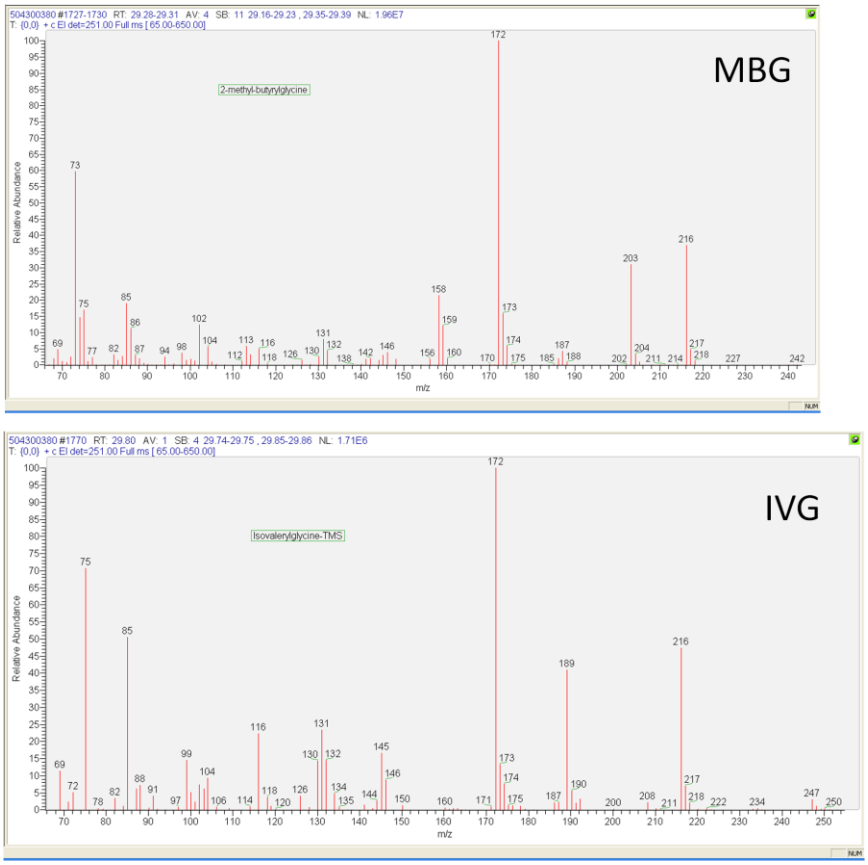


Fig. 3. Mass spectra of MBG and IVG (TMS)

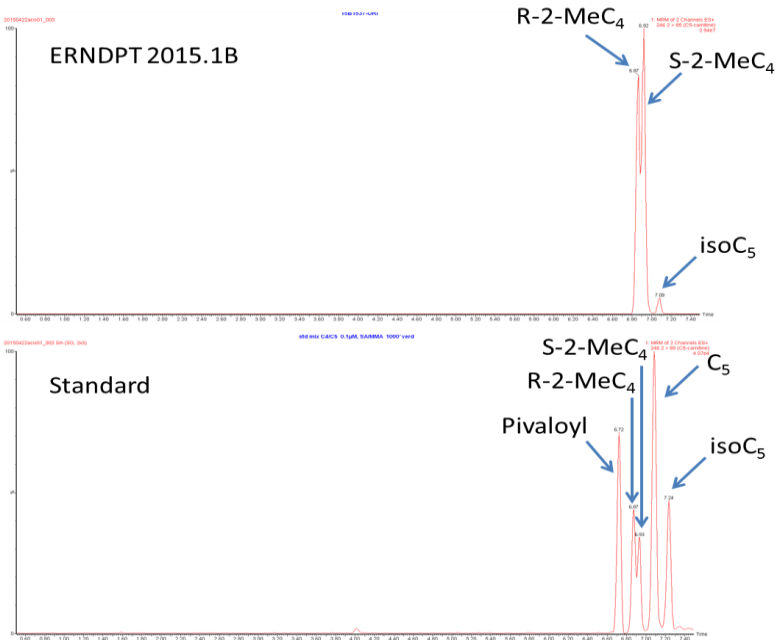


Fig. 4. Separation of different C5-carnitine species by UPLC followed by MS/MS detection. Figure kindly provided by F. Vaz, AMC, Amsterdam.

## Sample 2015-C: Hyperoxaluria type 1 (OMIM 259900).

Clinical description: A 26-y old male suffering from urolithiasis.

Sample obtained from a mild hyperoxaluria type I patient confirmed by decreased activity of alanine:glyoxylate aminotransferase (AGT) in liver: 7.2 nmol/mg/min; control 93.
This was a challenging sample, since oxalate was borderline, regardless of the method used for oxalate quantification (organic acid screening, isotope dilution method or ion chromatography). Glycolic acid was clearly elevated, however.

Results for the relevant organic acids were (qualitative/interpretation):

| | elevated | normal | not reported |
|---|---|---|---|
| Oxalate | 11 | 7 | 2 |
| Glycolate | 13 | 2 | 5 |
| Glycerate | - | 8 | 12 |

Results of quantitative organic acid or special analysis were (all mmol/mol):

| | test | n | median | mean | SD | min-max |
|---|---|---|---|---|---|---|
| Oxalate | OA | 6 | 91 | 94 | ? | 1-231 |
| Oxalate | Special? | 10 | 76 | 87 | 70 | 0.04-261 |
| Glycolate | OA | 13 | 251 | 227 | 71 | 75-323 |

Hyperoxaluria type 1 was reported to be the most likely diagnosis by 12 labs, while 2 labs mentioned this as other possible diagnosis. Hyperoxaluria type 2/3 was mentioned by 2 labs as 'other possible diagnosis'. Hyperoxaluria type 2 is unlikely however in the absence of elevated glycerate. Eight labs reported 'no diagnosis'.
The following further investigations were recommended: genetic analysis of the AGT gene, repeat analysis of oxalate/glycolate in another urine sample with acidification and analysis of oxalate/glycolate in plasma.

This sample was also distributed in 2008 with the following results: oxalate elevated n=10, oxalate normal n=9, glycolic acid elevated n=13, glycolic acid normal n=6. Thus these numbers were similar in 2008 and 2015. Also the concentrations of acids are comparable (in 2008 oxalate median value 100 mmol/mol, glycolate median value 267 mmol/mol).
Proficiency was 71% in 2008 and 73% in 2015, suggesting that detection of mild hyperoxaluria type I patients has not improved since 2008.

The questions remains whether this is a suitable sample for hyperoxaluria screening. Clearly, screening of oxalate in urine samples is not sufficiently sensitive to find all PH1 patients. One suggestion made is to always measure glycolate and glycerate as well, preferably by isotope dilution methodology.

## Sample 2015-D: Homocystinuria due to CBS deficiency(OMIM 236200).

This sample was the common sample circulated to all DPT participants and was discussed during the ERNDIM workshop at the SSIEM symposium in Lyon, September 1, 2015.

## Sample 2015-E: Argininosuccinate lyase deficiency (OMIM 207900)

Clinical description: A female referred at the age of 5 y for ataxia. Upon physical examination, brittle hair was noticed. The urine sample was obtained at the age of 28 years

Sample E was obtained with the help of the Dutch Patient organisation VKS. This sample was also circulated in 2011 (sample ID: 2011-E). Proficiency in 2011 (96%) was similar to 2015 (95%).
This was a straightforward sample, for both analysis and interpretation. The differential diagnosis of brittle hair is rather limited; includes ASL deficiency, biotinidase deficiency, Trichothiodystrophy (TTD), Menkes syndrome.
This sample was slightly nitrite-positive (reported by 13 labs), but this was not considered to be a problem.
Elevated ASA was reported by 19 labs, while 7 also mentioned the presence of ASA anhydrides. Two labs suggested the possible presence of antibiotics peaks. The median value for argininosuccinate values was 1707 mmol/mol (range 171-9299). Presumably the range of reported values is extended due to variable anhydride formation on column.
Only traces of orotic acid were observed and most participants (16/20) reported normal orotic acid (0-2.5 mmol/mol creat). Uracil was reported elevated by 4 labs. The origin of uracil (17-25 mmol/mol) in this sample is most probably bacterial degradation of pseudo-uridine.
Advice for further investigations included: blood ammonia, plasma amino acid analysis, enzyme assay in RBC/fib and mutation analysis.

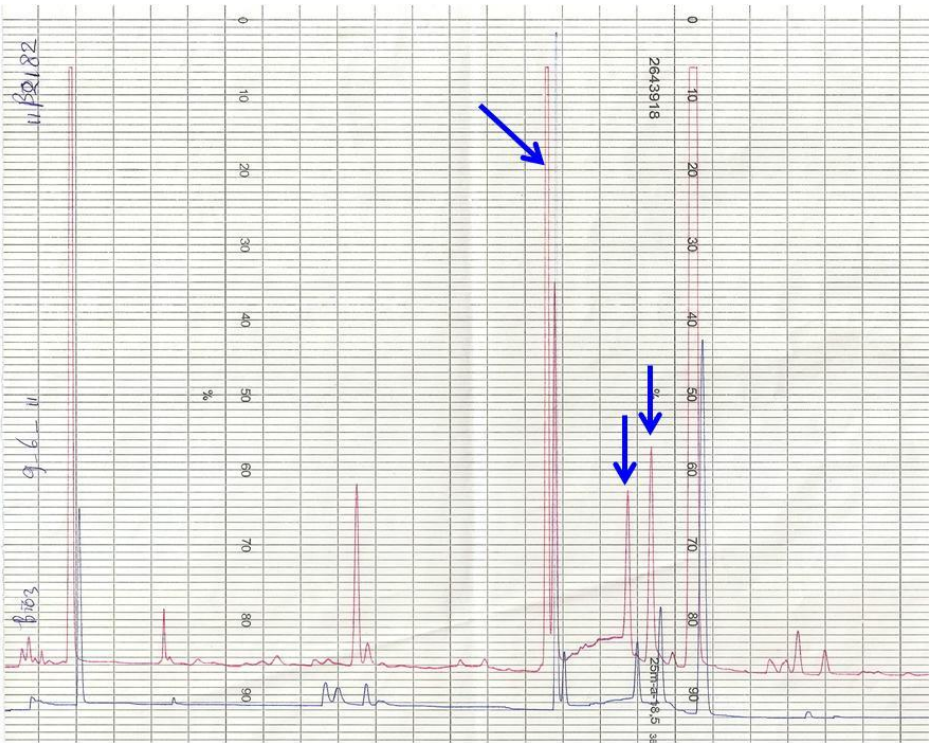

Fig. 5. Amino acid analysis of sample E. Arrows indicate ASA (left) and ASA andrides (2; right).

## Sample 2015-F: (OMIM 238970)

Clinical description: This boy was investigated for progressive macrocephaly at age 6 months. The urine sample was collected at age 3 y, while receiving specific therapy.

Sample F was also obtained with the help of the Dutch Patient organisation VKS.

The differential diagnosis of macrocephaly includes: Alexander diease, GM2 gangliosidosis, α-mannosidosis, Krabbe disease, MPS, Canavan disease, L-2-OH-glutaric aciduria, Glutaric acidemia type I and mitochondrial disorders.

Results for the relevant metabolites (qualitative/interpretation):

| | elevated | normal | not reported |
|---|---|---|---|
| 3-OH-glutarate | 18 | - | 2 |
| Glutaric acid | 8 | 8 | 4 |
| C5DC | 12 | - | 8 |

Quantitative results of organic acid or special analysis (all mmol/mol):

| Quantitative | n | median | mean | SD | min-max |
|---|---|---|---|---|---|
| 3-OH-glutarate | 9 | 32 | 32 | 22 | 6-75 |
| Glutaric acid | 12 | 8 | 11 | 7 | 4-26 |
| C5DC | 7 | 16 | 14 | 6 | 2-23 |

Proficiency was high with 95%. This patient clearly is a 'low-excretor' type GA I.
The differential diagnosis of elevated 3-OH-glutarate includes GA-I, GA-II (MADD) and SCHADD. MADD is unlikely with no other abnormalities in tthe organic acids (such as EMA and 2-OH-glutarate). SCHADD is less likely in view of the clinical symptoms (hyperinsulinism not mentioned). Glutarylcarnitine (C5DC) has been reported to be the best marker for diagnosis of GA I. It is elevated in urine of all GA I patients, classic and low excretor types (Tortorelli et al, Mol Gen Metab (2005) 84, 137-143).

**8. Preview of the 2016 scheme**

The format and logistics of the DPT-NL scheme in 2016 will be identical to 2015.

Tentative planning:

| | |
|---|---|
| Shipment of samples by CSCQ (all six samples will be dispatched in one box): | February 1, 2016 |
| Analysis start survey 1: | February 22, 2016 |
| Deadline for reporting results of survey 1: | March 14, 2016 |
| Interim report survey 1 available: | April 2016 |
| Analysis start survey 2: | May 23, 2016 |
| Deadline for reporting results of survey 2: | June 13, 2016 |
| Interim report survey 2 available: | July 2016 |
| Discussion of results (ERNDIM workshop at SSIEM symposium, Rome): | September 6, 2016 |
| Annual report 2016 | March 2017 |

**9. Minutes of the ERNDIM DPT NL 2015 discussion**

The minutes of the annual DPT meeting have been circulated September 21, 2015 to all DPT Netherlands participants.

Rotterdam, April 3, 2016

Dr George Ruijter
Scientific Advisor

Note: This annual report is intended for participants of the ERNDIM DPT-NL scheme. The contents should not be used for any publication without permission of the scheme advisor