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ERNDIM
Diagnostic Proficiency Testing
France 2014

ANNUAL REPORT 2014

In 2014, 23 labs participated to the DPT France scheme.
Scheme Advisor: Dr Christine Vianey-Saban,
Deputy Scheme Advisor : Dr Cécile Acquaviva-Bourdain, Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal, Centre de Biologie et de Pathologie Est, Lyon,
Scheme Organizer: Dr Xavier Albe, CSCQ (Centre Suisse de Contrôle de Qualité), Chemin du Petit-Bel-Air 2, 1225 Chêne-Bourg, Switzerland.

Geographical distribution of participants

Country	Number of labs
France	10
Italy	5
Spain	4
Portugal	2
Switzerland	1
UK	1
Total	23

Logistic of the scheme

- **2 surveys** 2014-1: patient A, B and C
  2014-2: patient D, E and F

Origin of patients:

- Patient A: **Iminodipeptiduria** (prolidase deficiency) – Centre de Biologie Est, Lyon
- Patient B: **Hyperammonaemia Hyperornithinaemia Homocitrullinuria** (HHH syndrome) – Brian Fowler, Switzerland. This sample has been sent to all labs participating to the DPT scheme in Europe
- Patient C: **4-hydroxybutyric aciduria** (succinic semialdehyde dehydrogenase deficiency) – Centre de Biologie Est, Lyon
- Patient D: **Fucosidosis** (alpha-L-fucosidase deficiency) – Céline Caruba, Nice
- Patient E: **L-2-hydroxyglutaric aciduria** (L-2-hydroxyglutarate dehydrogenase deficiency) – Centre de Biologie Est, Lyon
- Patient F: **SCHAD** (short-chain 3-hydroxyacyl-CoA dehydrogenase) **deficiency** – Chris Ottolenghi, Hôpital Necker, Paris

- Mailing: samples were prepared and sent by CSCQ (Centre Suisse de Contrôle de Qualité) at room temperature. One mailing for the 2 surveys.

Timetable of the schemes

• 10 March 2014	Shipment of samples of Survey 1 and Survey 2 by CSCQ
• 17 March 2014	Clinical data available on CSCQ website and start analysis of samples (Survey 1)
• 7 April 2014	Deadline for result submission (Survey 1)
• 14 May 2014	Interim report of Survey 1 by e-mail
• 2 June 2014	Clinical data available on CSCQ website and start analysis of samples (Survey 2)
• 23 June 2014	Deadline for result submission (Survey 2)
• 14 August 2014	Interim report of Survey 2 by e-mail
• 2 September 2014	Meeting in Innsbruck
• 19 March 2015	Scientific Advisory Board meeting: final scoring
• 21 April 2015	Annual report with definitive scoring sent by e-mail

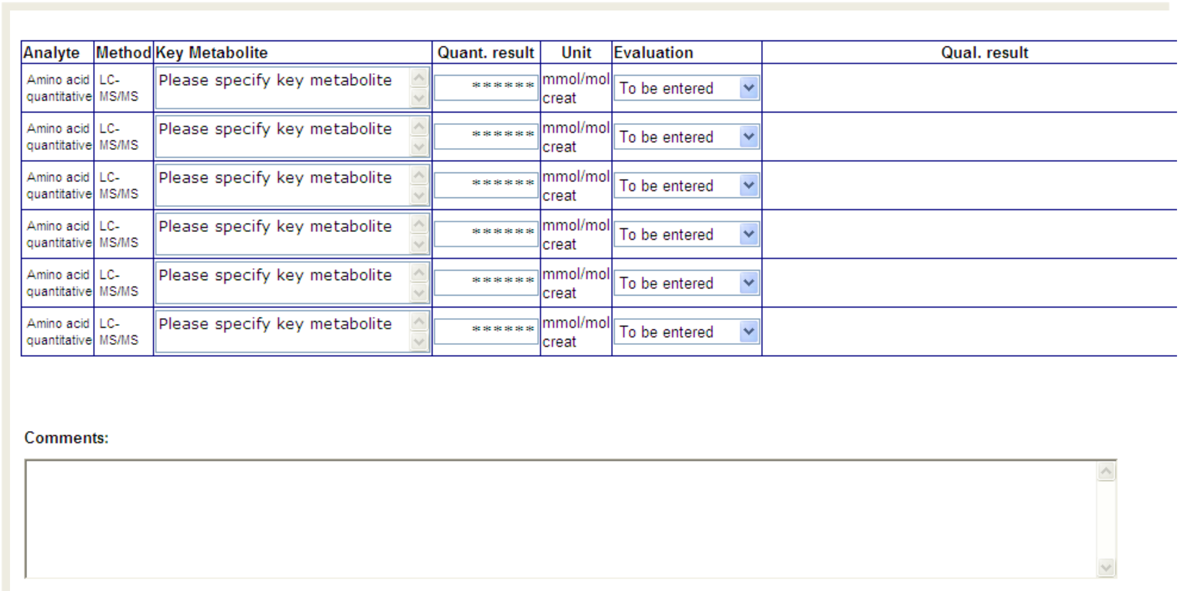
Date of receipt of samples

	Survey 1 + 2
+ 24 hours	23

CSCQ Website reporting

Since 2011, the website reporting system is compulsory for all centres. Please read carefully the following advices:

- Selection of tests: **don't select a test if you will not perform it**, otherwise the evaluation program includes it in the report.
- Results
  - Give quantitative data as much as possible.
  - Enter the key metabolites with the evaluation **in the tables** even if you don't give quantitative data.
  - If the profile is normal: enter "Normal profile" in "Key metabolites".
  - **Don't enter results in the "comments" window**, **otherwise your results will not be included in the evaluation program.**



Analyte	Method	Key Metabolite	Quant. result	Unit	Evaluation	Qual. result
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	

Comments:

- Recommendations = **advice for further investigation**.
  - Scored together with the interpretative score.
  - Advices for treatment are not scored.
  - **Don't give advice for further investigation in "Comments on diagnosis"**: it will not be included in the evaluation program.

	Survey 1	Survey 2
	(3 weeks)	(3 weeks)
Receipt of results	21 labs	23 labs
No answer	**2 labs**	**0**

Scoring of results

The scoring system established by the Scientific Advisory Board (SAB) of ERNDIM has changed in 2013. Two criteria are evaluated:

A	Analytical performance	Correct results of the appropriate tests	2
		Partially correct or non-standard methods	1
		Unsatisfactory of misleading	0
I	Interpretation of results and recommendations	Good, diagnosis is established	2
		Helpful but incomplete	1
		Misleading / wrong diagnosis	0

The **total score** is calculated as the sum of these 2 criteria without weighting. The maximum that can be achieved is 4 for one sample.

Meeting of participants

It took place in Innsbruck on Tuesday 2 September 2014 from 9.00 to 10.30, before the SSIEM Symposium.

❖ Participants

Representatives from 13 labs were present: S Pajares, A Ribes (Hospital Clinic, Barcelona), I Redonnet-Vernhet (Bordeaux), F Sabourdy (Toulouse, cluster lab Bordeaux), E Pasquini (Florence), O Boulat, C Roux (Lausanne), B Merinero (Madrid), M Gastaldi (Marseille), G Pelo (Padova), JF Benoist, O Rigal (Hôpital Robert Debré, Paris) F Habarou, C Ottolenghi (Hôpital Necker, Paris), S Boenzi, C Rizzo (Rome), A Tebani (Rouen), JA Cocho (Santiago de Compostella), J Dalley (Sheffield).

❖ Information from the Executive Board and the Scientific Advisory Board

- **Scoring and certificate of participation**: scoring is done by 2 scheme organizers, who change every year. The results of DPT France 2014 have been also scored by Pr Brian Fowler, from DPT Switzerland. At the SAB meeting in March, the definitive scores have been finalized. The concept of critical error has been introduced in 2014. A critical error is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient. For 2014, the SAB decided that sample B has to be considered as a critical error for the labs who failed to identify an increase of orotic acid. Non-identification of 4-hydroxybutyric acid in sample C has also been advised by the SAB as a critical error.
- Certificate of participation for 2014 will be issued for participation and it will be additionally notified whether the participant has received a performance support letter. This performance support letter is sent out if the performance is less than 62% (score < 15 / 24). Four performance support letters will be sent by the Scheme Advisor for 2014. The partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.
- **Urine samples**: we remind you that every year, each participant must provide to the scheme organizer at least 300 ml of urine from a patient affected with an established inborn error of metabolism or “normal” urine, together with a short clinical report. If possible, please collect 1500 ml of urine: this sample can be sent to all labs participating to one of the DPT schemes. Each urine sample must be collected from a single patient (don’t send urine spiked with pathological compounds). Please don’t send a pool of urines, except if urine has been collected on a short period of time from the same patient. For “normal” urine, the sample must be collected from a symptomatic patient (don’t send urine from your kids!). Annex 1 gives the list of the urine samples we already sent.

  As soon as possible after collection, the urine sample must be heated at 50 °C for 1 hour. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. Separate 4 aliquots in 10 ml plastic tubes, add stoppers, and freeze these aliquots and the rest of the urine sample in a bulk. Send the bulk and the aliquots on dry ice by rapid mail or express transport to:

  Dr Christine Vianey-Saban, Dr Cécile Acquaviva,
  Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal, 5ième étage,
  Centre de Biologie et de Pathologie Est, Groupement Hospitalier Est,
  59, Boulevard Pinel, 69677 Bron cedex, France.

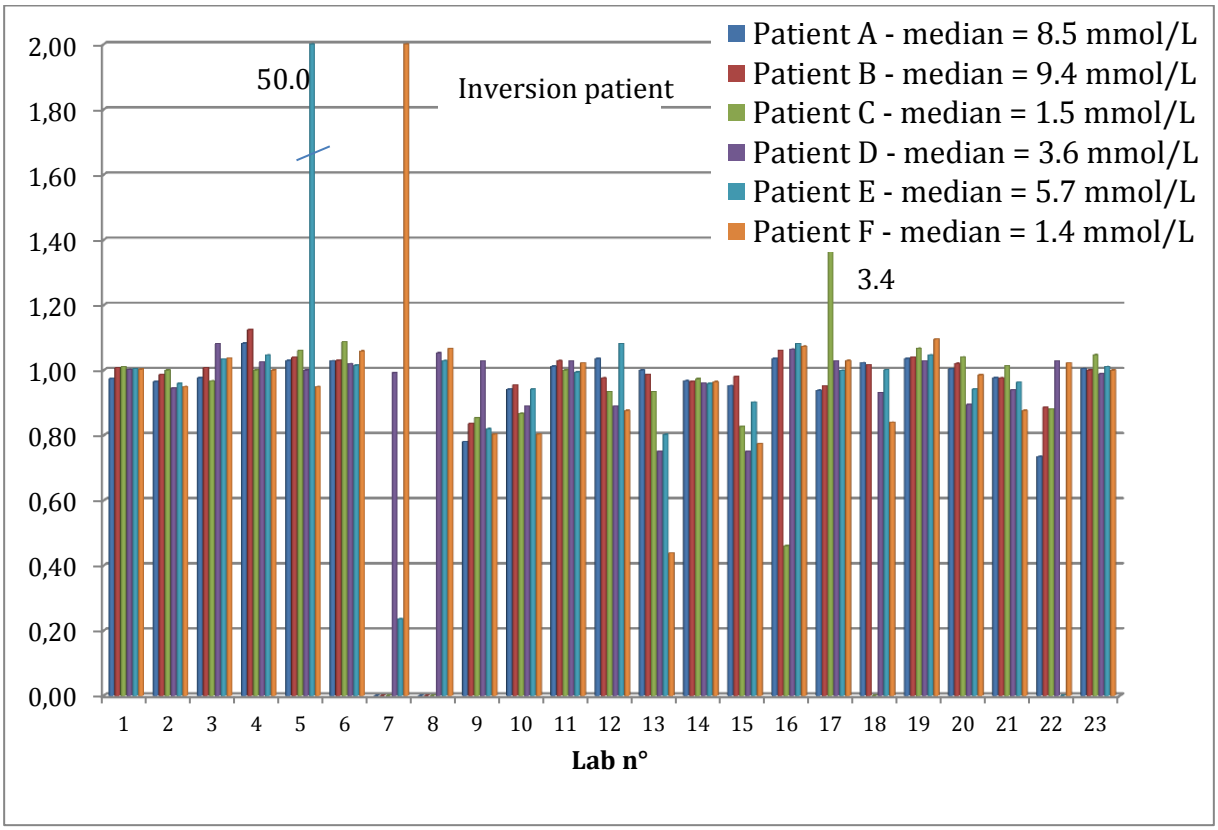
  Please send us an e-mail on the day you send the samples.

❖ Discussion of results

- **Creatinine measurement**

Creatinine determination was satisfying for most labs, except for lab 9, 10, and 15 who have systematically low values (lab 9 and lab 15 had the same problem the last two years). Lab 5, 13, 16 and 17 had one wrong value (excluded for calculation of median and CV). Lab 41 has inverted creatinine of sample E and sample F. Creatinine values are expressed in the figure as the ratio of each measurement over the median for all labs.
CV is < 10 % for all samples (6.0 – 9.7 %), after exclusion of wrong values, and this is rather similar to the interlab CV 2013 for Special Assay in urine (6.1 %, n = 122), and the interlab CV 2013 for Quantitative organic acids (4.9 %, n = 70).

Creatinine: ratio to median



- **Patient A – Iminodipeptiduria (prolidase deficiency – OMIM #170100)**

The patient is a 34 year-old man, born form consanguineous parents. He presented slight psychomotor retardation since infancy. He was investigated at 22 years of age because of the progressive development of diffuse interstitial pneumopathy with clubbing of fingers and several episodes of bacterial infections, chronic obstructive bronchopneumopathy due to tobacco, rheumatoid arthritis with positive antibodies, autoimmune hepatitis, atrophic polychondritis, cryoglobulinemia, and osteoporosis. Diagnosis relied on urinary amino acid analysis by tandem MS. He is now 34 year-old. Interstitial pneumopathy and psychomotor retardation have been ascribed to prolidase deficiency, and possibly the other autoimmune disorders. He has chronic respiratory insufficiency, necessitating artificial ventilation at night. He is receiving treatment with methotrexate for auto-immune disorders. But he never presented skin lesions. He is still a heavy smoker.

Diagnosis

Most likely diagnosis

- Iminodipeptiduria (prolidase deficiency) 16
- No diagnosis 3
- Mucopolysaccharidosis type III 1
- Gaucher disease type 3 1

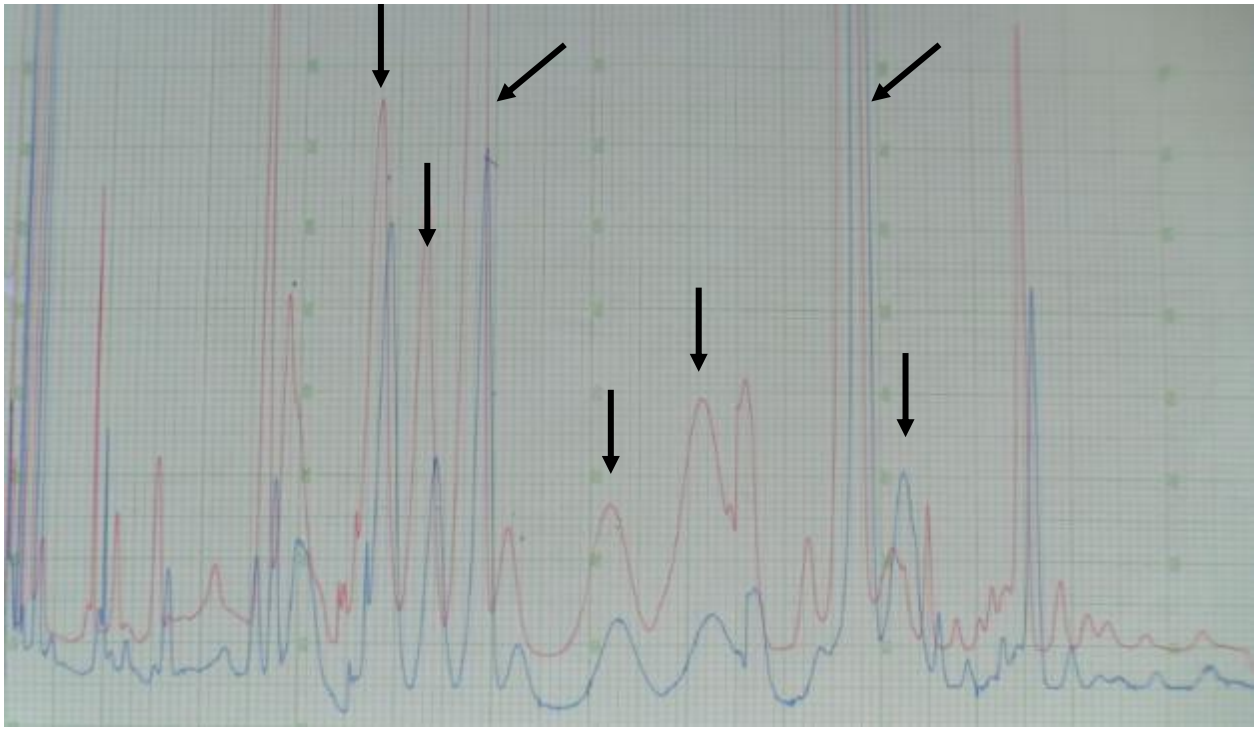
Alternative diagnosis

- Other mucopolysaccharidosis 1
- Biotinidase deficiency 1
- Niemann-Pick type B 1

All 21 participants performed **amino acid** analysis. They reported an increase of:

- Iminodipeptides: 7 labs
- Glycine-proline dipeptide: 4 labs
- Proline after acid hydrolysis: 4 labs
- Glycine after acid hydrolysis: 4 labs

Five participants interpreted the abnormal amino acid profile as interferences, or as an increase of Orn, and Tyr possibly due to artefact, or finally an increase of Cit, Ile, Tyr, and abnormal peaks. Three of them used ion exchange chromatography with ninhydrin detection, the other two used gas chromatography with FID detection (same lab). The figure below gives the urinary amino acid profile of prolidase deficiency using ion exchange chromatography. Arrows indicate dipeptides.



All labs but two also performed **organic acid** analysis, and all of them reported a normal profile. Among the 3 participants who performed mucopolysaccharides analysis, one lab reported an abnormal profile consistent with mucopolysaccharidosis type III.

Scoring

- Analytical performance: increase of glycine-proline and/or iminodipeptides and/or increase of Gly, Pro, Hyp after acid hydrolysis (score 2).
- Interpretation of results and recommendations: prolidase deficiency (score 2).

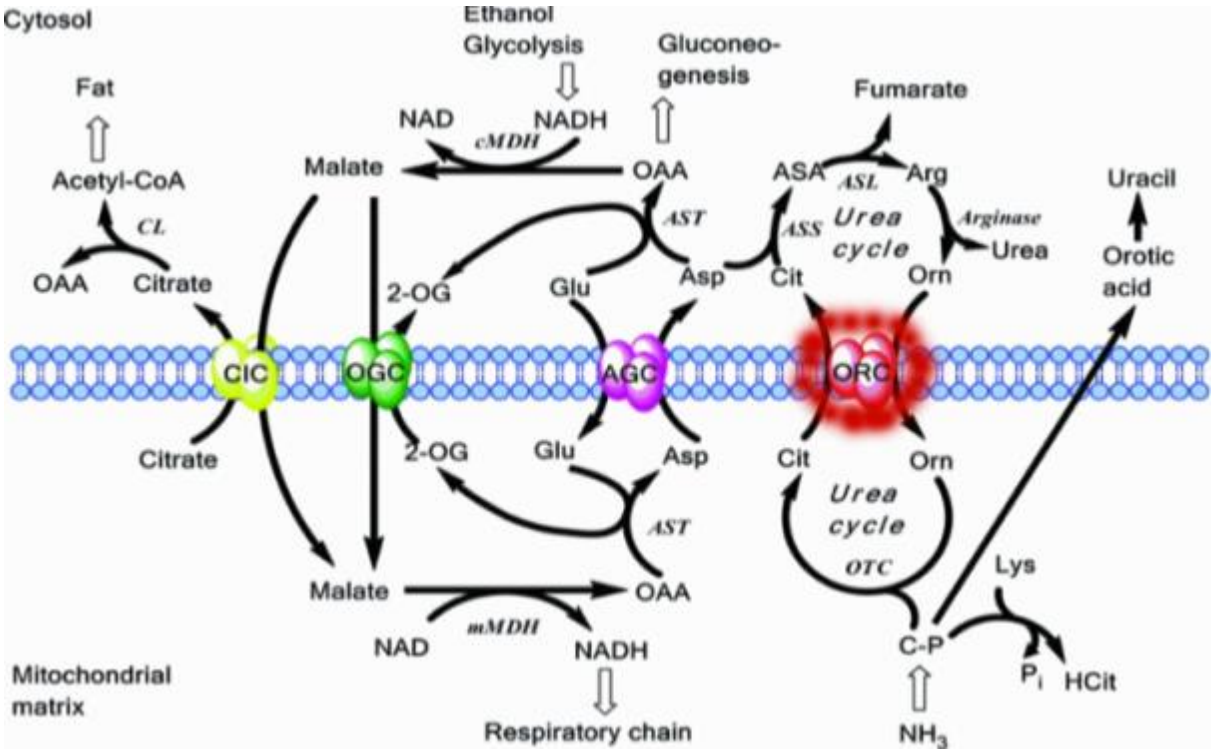
A similar urine sample has been distributed in 2009: the overall performance has increased.

	2009	2014
Analytical performance	70 %	76 %
Interpretative performance	65 %	76 %
Overall performance	67 %	76 %

- **Patient B – HHH (Hyperammonaemia, Hyperornithinaemia, Homocitrullinuria) syndrome (*SLC25A15* gene, OMIM #238970)**

Following uneventful pregnancy and birth, this male child showed mild hypotonia at 6 months of age. A few months later, developmental delay and failure to thrive, with elevated transaminases, was observed. The urine was collected at the age of 8.75 years whilst receiving specific treatment. This sample has been distributed to all participants of the 5 DPT schemes. Details from this patient are available on the ERNDIM website.

Hyperammonaemia, hyperornithinaemia, homocitrullinuria (HHH syndrome) is caused by mutations in *SLC25A15,* the gene that encodes ORNT1 (mitochondrial ornithine transporter 1), which is involved in the transport of ornithine between cytoplasm and mitochondrion.



http://www.treatable-id.org/page23/HHH%20Syndrome.html

The decrease in entry of Orn into mitochondria decreases Cit synthesis and impairs ammonia detoxication. The accumulation of carbamylphosphate due to depleted supply of ornithine for the urea cycle is responsible for the enhanced synthesis of homocitrulline, catalysed by ornithine transcarbamylase (OTC). Another enzyme, lysine transcarbamylase (LTC or carbamoyl-phosphate: L-lysine carbamoyltransferase), is possibly involved, leading to the « lysine urea cycle ».

Diagnosis

Most likely diagnosis

- HHH syndrome 9
- OTC deficiency 8
- Citrullinemia type I 3
- Urea cycle disorder 1

Other possible diagnosis

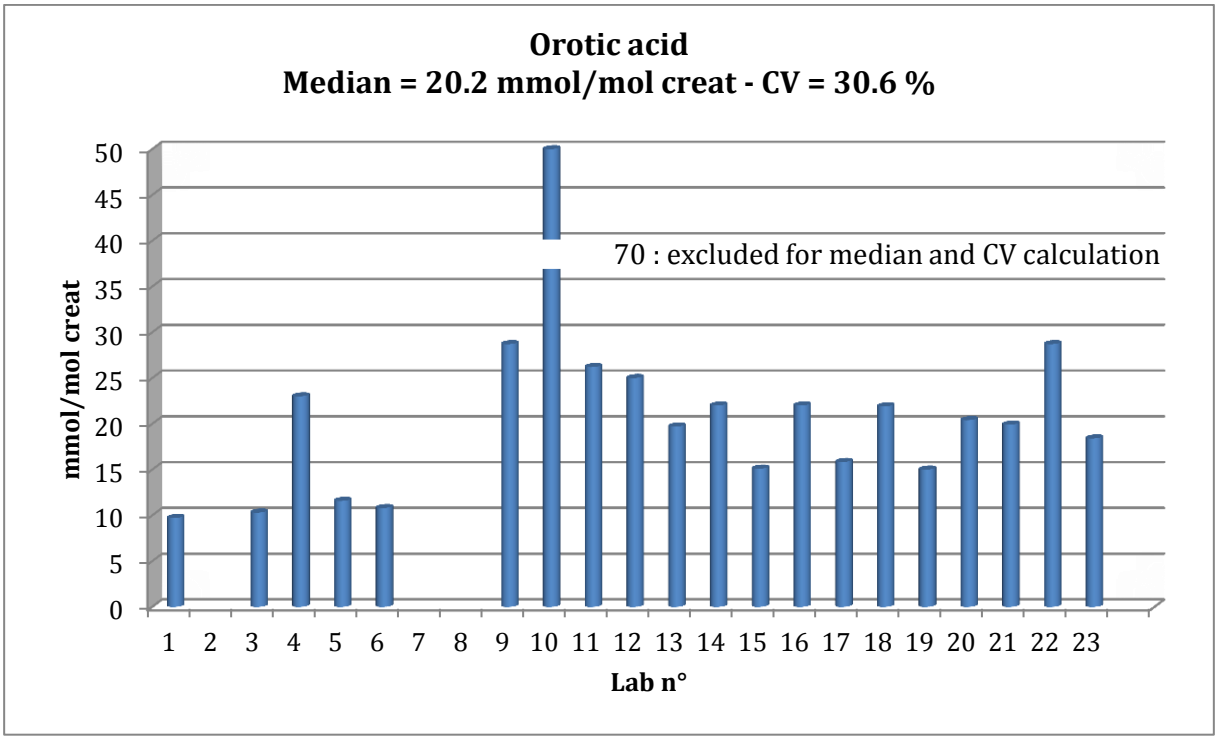
- HHH syndrome 5
- Citrullinemia type II (citrin deficiency) 2
- Citrullinemia type I 1
- OTC deficiency 1
- Hereditary orotic aciduria (UMPS deficiency) 1

All 21 participants performed **amino acid** analysis, and reported an increase of:

- **Homocitrulline** 13
  (median = 25 mmol/mol creat, range : 18 – 30 - CV = 10%)
- Citrulline 21
  (median = 61 mmol/mol creat, range : 36 – 69 CV = 17%)
- Arginine 18
  (median = 37 mmol/mol creat, range : 20 – 44 - CV = 19%)
- Ornithine 15
  (median = 37 mmol/mol creat, range : 31– 48 - CV = 10%)

But 3 of them, using HPLC, MS/MS and IEC/ninhydrin respectively, pointed out that homocitrulline excretion was normal.

All 21 participants also performed **organic acids**, and 19 of them reported an increase of **orotic acid**, and 15 an increase of uracil. All 19 labs performed quantification of orotic acid.



Scoring

- Analytical performance: increase of homocitrulline (score 1), increase of orotic acid (score 1). Non-identification of an increase of orotic acid in this sample has been advised by the SAB as a critical error.
- Interpretation of results and recommendations: HHH syndrome as first or alternative diagnosis (score 2), other urea cycle (score 1).

A similar urine sample has been distributed in 2012: the overall performance has improved.

	2012	2014
Analytical performance	73 %	81 %
Interpretative performance	73 %	79 %
Overall performance	75 %	80 %

- **Patient C – 4-hydroxybutyric aciduria (succinic semialdehyde dehydrogenase deficiency, OMIM #271980)**

This 2-year-old boy is the first child of consanguineous parents. He was investigated for the first time at 23 months of age because of delayed psychomotor skills: sitting at 11 months, does not walk alone, does not speak. He had a normal neurological examination, and a normal MRI. Biochemical investigation was normal except for organic acids analysis, which revealed a high increased excretion of 4-hydroxybutyric acid (361 mmol/mol de creatinine – controls <5), together with a high increase of erythro and threo-4,5-dihydroxyhexanoic acids, and a moderate increase of glycolic acid, 3-hydroxypropionic, 2,4-dihydroxybutyric and 3,4-dihydroxybutyric acids. His younger sister was 5 month-old when this patient was diagnosed. She also presented with delayed psychomotor skills and 4-hydroxybutyric aciduria. Mutation analysis of *ALDH5A1* gene was performed: both patients are homozygous for a mutation in exon 1, and their parents are heterozygous for this mutation.

Diagnosis

Most likely diagnosis

• 4-hydroxybutyric aciduria	18
• Malonic aciduria	2
• Mucopolysaccharidosis	1

Other possible diagnosis

• GBH poisoning (unlikely)	2

All 21 participants performed **organic acid analysis**, and reported an increase of:

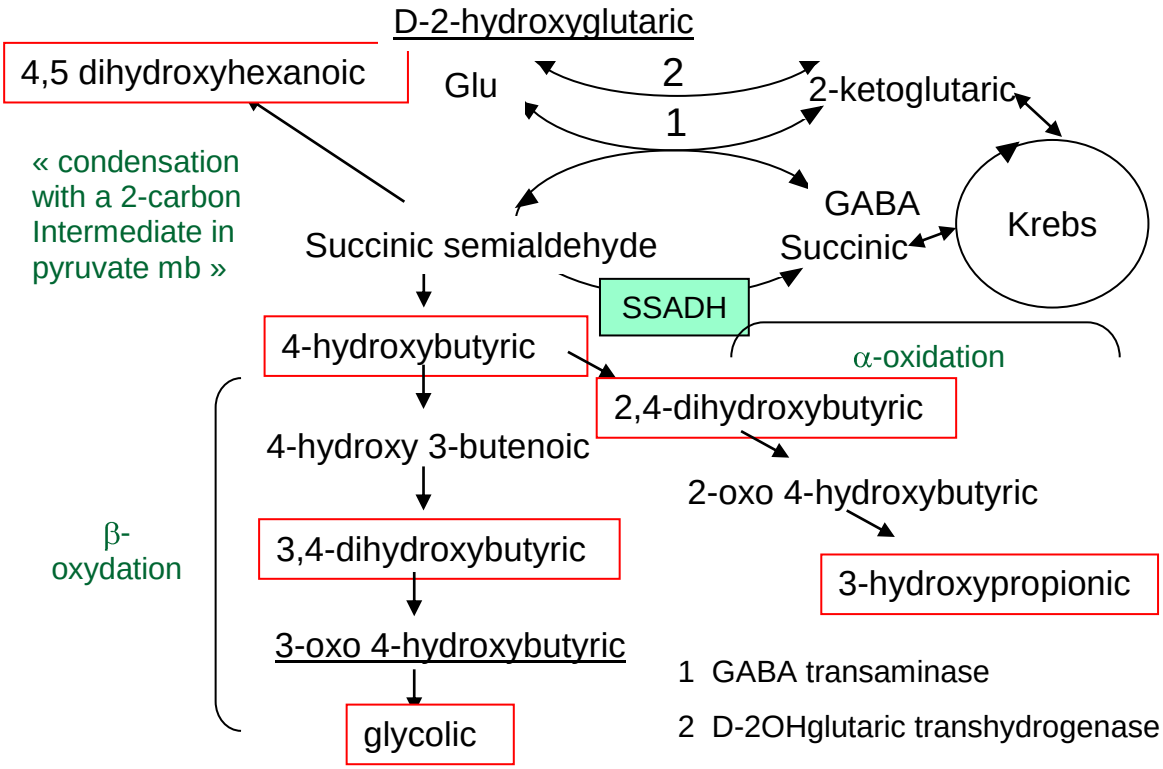
• **4-hydroxybutyric acid** (median = 280 mmol/mol creatinine; range : 106 – 2132)	18
• **Erythro- and/or threo-4,5-dihydroxyhexanoic acids**	14
• 3,4-dihydroxybutyric acid	3
• 2,4-dihydroxybutyric acid	2

Two participants reported an increase of malonic acid (organic acid analysis performed by the same lab), and one reported a normal profile.

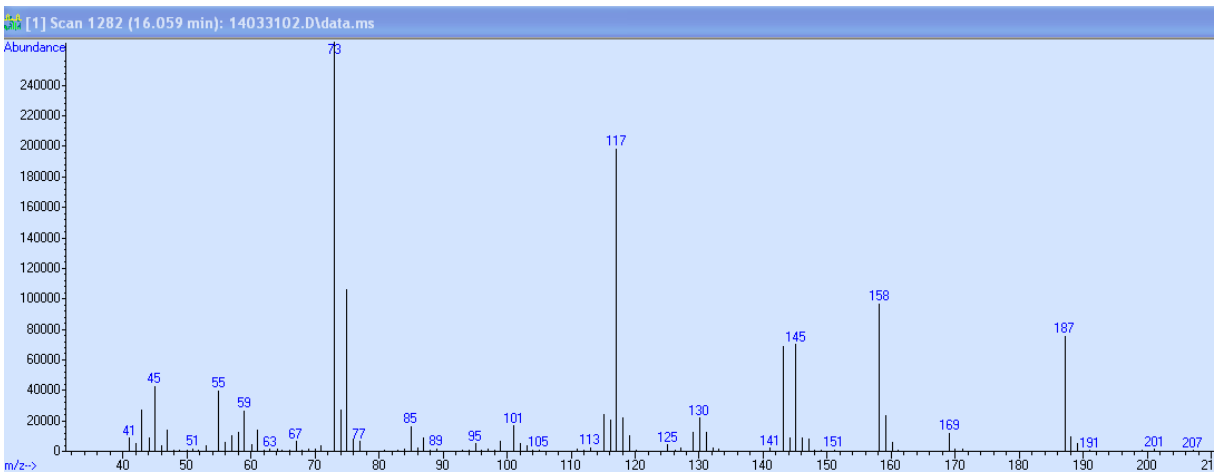
The figure below illustrates the origin of metabolites excreted in SSADH deficiency. Only 4,5-dihydroxyhexanoic acids (erythro, threo and the lactones, which are dependent upon the pH of extraction) are specific of SSADH deficiency, but other metabolites (2,4-dihydroxybutyric, 3-hydroxypropionic, 3,4-dihydroxybutyric and glycolic acids) can be elevated.

In case of exogenous intake (anesthesia with Gamma-OH®, or treatment with Xyrem®, or GBH poisoning), only 4-hydroxybutyric is elevated.

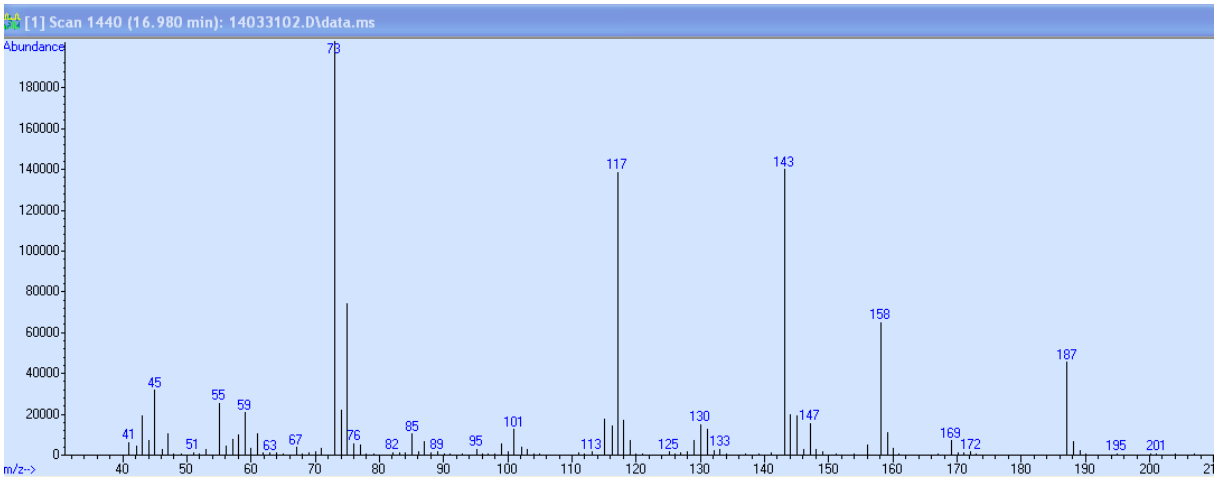
Therefore identification of 4,5-dihydroxyhexanoic acids is important.



In the scheme advisor system, **erythro-4,5-dihydroxyhexanoic lactone monoTMS** is eluted before succinic acid.



Threo-4,5-dihydroxyhexanoic lactone monoTMS is eluted close to fumaric acid.



Scoring

- Analytical performance: increase of 4-hydroxybutyric (score 1), increase of erythro and/or threo-4,5-dihydroxyhexanoic lactones (score 1). Non-identification of an increase of 4-hydroxybutyric acid in this sample has been advised by the SAB as a critical error.
- Interpretation of results and recommendations: 4-hydroxybutyric aciduria (score 2).

A similar urine sample has been distributed in 2005. The overall performance has decreased since only one lab failed to reach the diagnosis in 2005, whereas 3 labs failed in 2014.

	2005	2014
Analytical performance	91 %	76 %
Interpretative performance	95 %	86 %
Overall performance	93 %	81 %

- **Patient D – Fucosidase deficiency (OMIM #230000)**

The patient is a 7-year-old boy. The only available information is that he presents with psychomotor retardation, ventilation and balance disorders, epilepsy and a non-specific dysmorphia. At MRI, he has abnormalities of the white matter and the basal ganglia. Diagnosis was suspected on urinary oligosaccharide profile and was confirmed by measurement of alpha-L-fucosidase activity in leukocytes:

(µKat/kg protein)	Patient	Control	Reference values
Alpha-L-fucosidase	**0.0**	**21.5**	**11.0 – 26.0**
Total hexosaminidase	**572**	**478**	**240 - 780**

Diagnosis

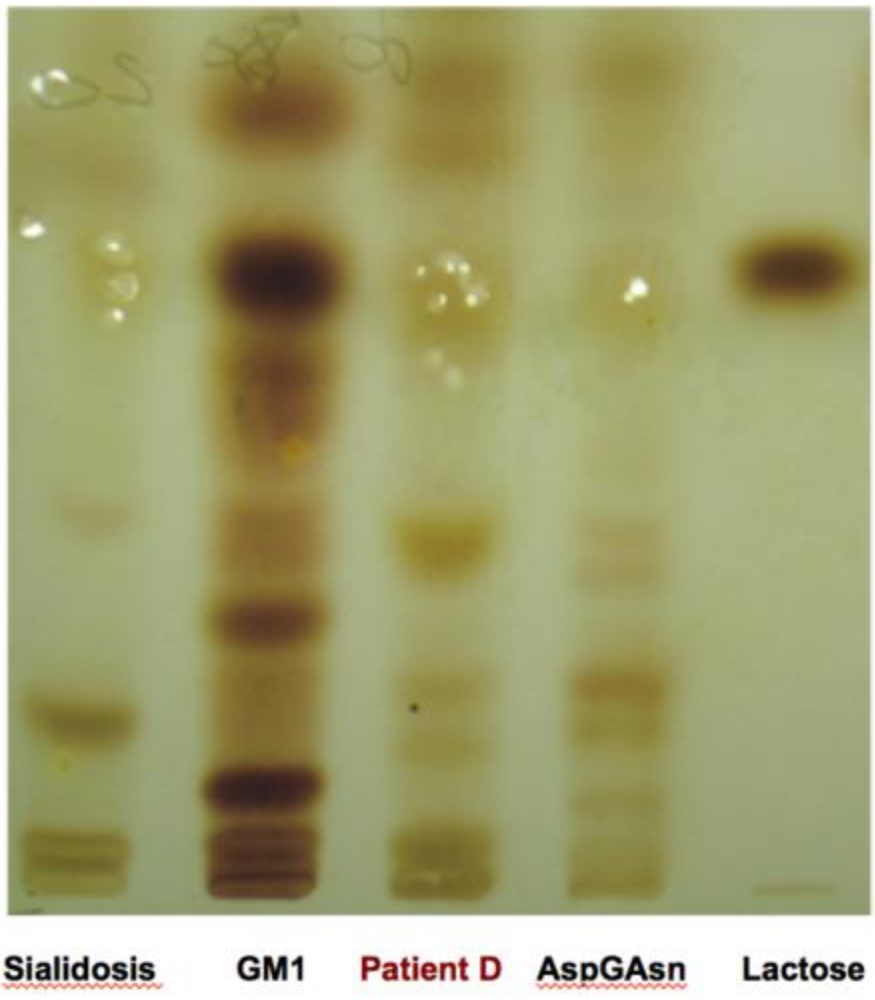
Most likely diagnosis

- Fucosidosis 12
- Oligosaccharidosis 1
- Sialidosis 1
- GM1 gangliosidosis 1
- Aspartylglucosaminuria 1
- No diagnosis / no metabolic disorder 5
- Mitochondrial disorder 1
- Mucopolysaccharidosis type III 1

Alternative diagnosis

- An oligosaccharidosis cannot be excluded 1
- Galactosialidosis 1
- GM1 gangliosidosis 1
- GM2 gangliosidosis 1

Among the 17 participants who performed **oligosaccharides** (over a total of 23), 15 reported an abnormal profile (12 of them in agreement with fucosidosis), whereas 2 pointed out a normal profile. The TLC profile of this urine sample compared to samples from known oligosaccharidoses is given in the figure below:



It is important to note that an educational kit containing 6 common oligosaccharidoses samples is now available to purchase on a not-for-profit basis from MCA Laboratories (a sub-section of SKML) in the Netherlands. Although it does not contain fucosidosis, the samples included in the educational kit are: GM1 gangliosidosis, GM2 gangliosidosis, M. Pompe (infantile), aspartylglucosaminuria, alpha-mannosidosis and Schindler disease. A protocol for qualitative TLC oligosaccharide analysis and also a picture of a TLC separation of the Oligosaccharide kit are both available on the ERNDIM website under Training/ Educational Documents. Further details of the Educational Oligosaccharide kit are available from MCA Laboratories: **http://cms.erndimqa.nl/Educational-Panels.aspx**

Sixteen participants also performed quantification of mucopolysaccharides: 14 reported a normal level and 2 an increased level. A normal mucopolysaccharide profile was reported by the 9 participants who performed fractionation, and a normal screening test for mucopolysaccharides was reported by 2 participants.

Scoring

- Analytical performance: oligosaccharide profile in agreement with fucosidosis (score 2), abnormal oligosaccharide profile (score 1).
- Interpretation of results and recommendations: fucosidosis as first or alternative diagnosis (score 2), unspecified oligosaccharidosis or wrong oligosaccharidosis or perform oligasaccharides (score 1).

A similar urine sample has been distributed in 2004: the overall performance was almost identical.

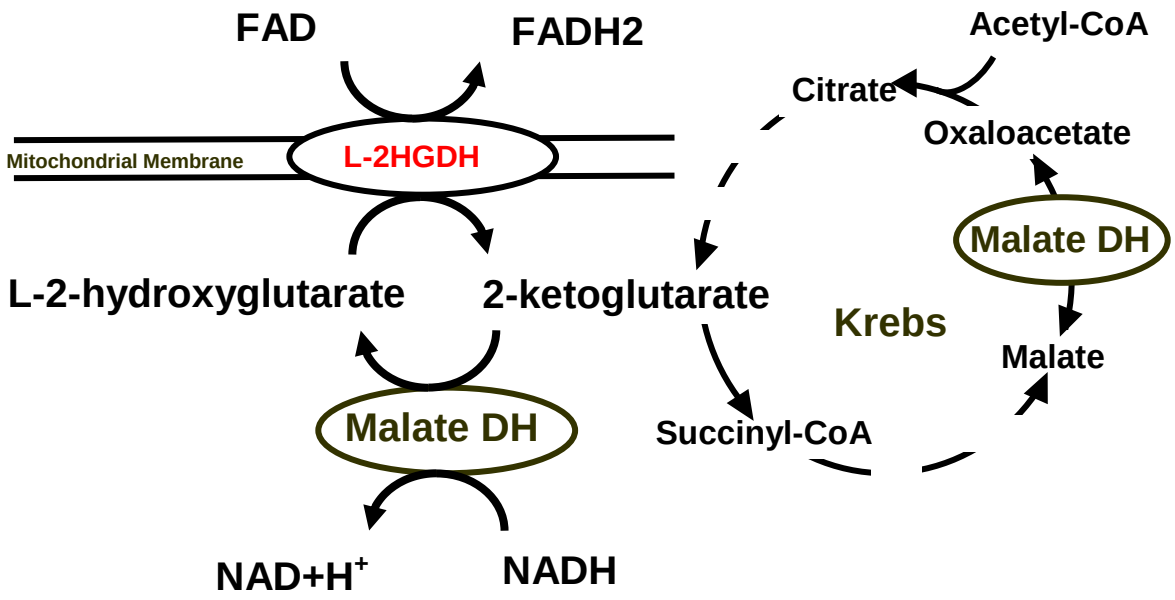
	2004	2014
Analytical performance	66 %	59 %
Interpretative performance	66 %	65 %
Overall performance	69 %	62 %

- **Patient E – L-2-hydroxyglutaric aciduria due to L-2-hydroxyglutarate dehydrogenase deficiency (gene *L2GDH*, OMIM #236792)**

The patient, a boy, is the first child of consanguineous parents, with a normal psychomotor development. He was hospitalized at almost 3 years of age because of a head trauma, followed the day after by high fever (40.6°C) with troubles of consciousness, endobuccal and labial vesicle rush. CT scan revealed abnormal supratentorial white matter, and MRI showed peripheral leucodystrophy with prominent signal abnormalities in the globus pallidum and the dentate nucleus, and to a lesser extent in caudate nucleus and putamen. Infectious investigation demonstrated that labial vesicles were positive for herpes PCR, but viral encephalitis was excluded. Because of MRI abnormalities, biochemical investigation was performed: **2-hydroxyglutaric** acid excretion was 1 734 mmol/mol creat (controls <50), and plasma lysine level was 200 µmol/L (controls: 73 – 144), consistent with L-2-hydroxyglutaric aciduria. This was confirmed by analysis of enantiomers and mutation analysis of *L2GDH* coding L-2-hydroxyglutarate dehydrogenase: the patient is homozygous for the c.241A>G mutation, identified in other patients with L-2-HGA. A treatment with Levocarnil (1g/day) and Riboflavin (100 mg/day) was started but with poor compliance. The patient is now 4 year-old: he walks normally, rides tricycle, speaks Arab, but does not seem to understand French.

L-2-hydroxyglutaric aciduria is due to L-2-hydroglutarate dehydrogenase deficiency, a membrane bound FAD dependent enzyme (inner mitochondrial membrane) which catalyses the dehydrogenation of L-2-hydroxyglutarate into 2-ketoglutarate. It had been demonstrated that urinary excretion of L-2-hydroxyglutaric acid (L2OHGA) was independent of feeding, and came exclusively from endogenous production (muscle: 75%, liver: 25%); but the metabolic function of L2OHGA remained unknown for a long time.
L2HGDH gene has been identified in 2004. In 2007, Rzem et al (J Inher Metab Dis 2007;30:681) demonstrated that the production of L2OHGA has no metabolic function, but is due to the lack of

specificity of malate dehydrogenase. Degradation of L2OHGA by L-2-hydroxyglutarate dehydrogenase is a repair mechanism, and therefore this enzyme belongs to the group of « house-cleaning » enzymes.



(Lactate DH testis)

Diagnosis

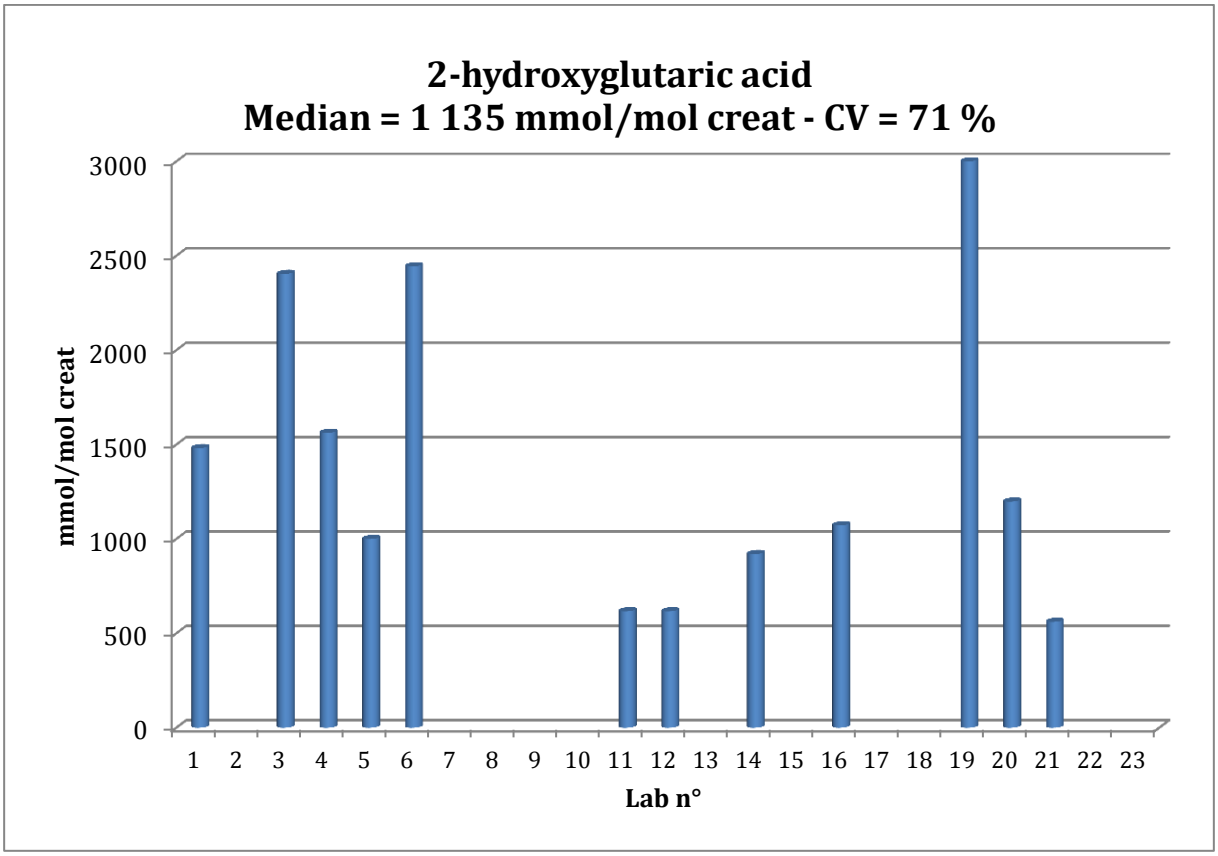
Most likely diagnosis

- L-2-hydroxyglutaric aciduria 13
- D-2-hydroglutaric aciduria 4
- L- or D-2-hydroxyglutaric aciduria 6

Alternative diagnosis

- D-2-hydroglutaric aciduria 4
- L-2-hydroxyglutaric aciduria 3
- Combined D, L-2-hydroxyglutaric aciduria 2
- Neoplasic disorder with isocitrate dehydrogenase deficiency 1

All 23 participants performed **organic acid** analysis: all of them reported an increase of **2-hydroxyglutaric acid** (see figure below), 6 an increase of 2-hydroxyglutaric lactone, and 18 an increase of succinic acid. The 2 labs who performed separation of D- and L- enantiomers, identified an increase of L-2-hydroxyglutaric acid.



No striking abnormality was reported by the 20 participants who performed amino acids analysis.

Scoring

- Analytical performance: increase of 2-hydroxyglutaric acid (score 2).
- Interpretation of results and recommendations: 2-hydroxyglutaric aciduria or L-2-hydroxyglutaric aciduria (score 2).

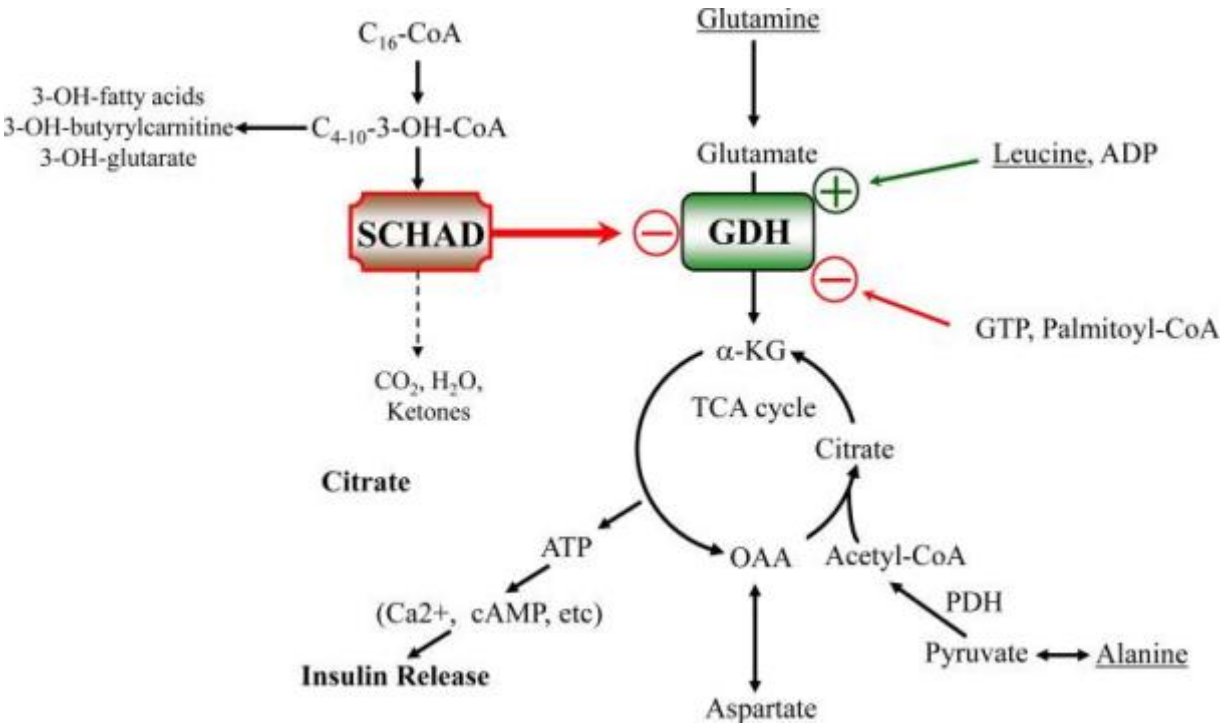
A similar urine sample has been distributed in 2008: the overall performance is the same.

	2008	2014
Analytical performance	100 %	96 %
Interpretative performance	100 %	100 %
Overall performance	100 %	98 %

- **Patient F – SCHAD (short-chain 3-hydroxyacyl-CoA dehydrogenase) deficiency (*HADH* gene, OMIM #231530)**

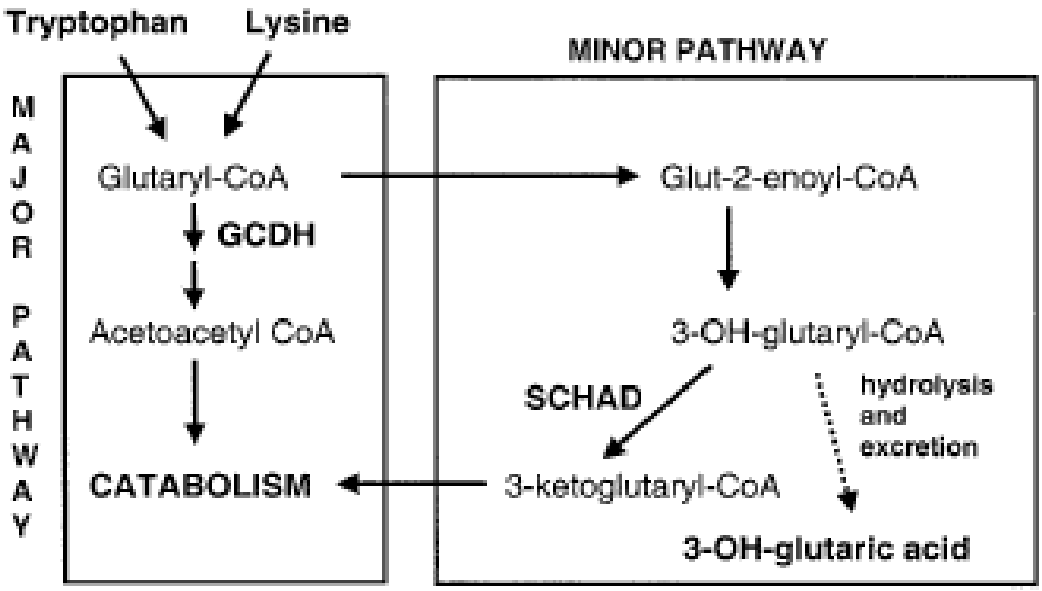
The patient is a girl. She was referred at 6 months of age for suspicion of a fatty acid oxidation defect because of seizures, hepatomegaly, and hypoglycaemia (0.1 g/L). Her plasma acylcarnitine profile performed during hypoglycaemia was consistent with ketosis: low free carnitine, increase of acetylcarnitine (C2), and C4OH (0.7 µmol/L - controls <0.3). She was reinvestigated at 14 months of age, and hyperinsulinism was demonstrated. At that time, her acylcarnitine profile was almost normal: C4OH = 0.3 µmol/L, and she was discharged with diazoxide treatment. At 18 months of age, she presented with a new episode of hypoglycaemia, and urinary organic acids were performed: diagnosis of SCHAD deficiency was evocated because of an isolated increase of urinary 3-hydoxyglutaric acid (= 29 mmol/mol creat – controls <5). Diagnosis was confirmed by mutation analysis of *HADH* gene: the patient is homozygous for a deleterious mutation and her parents are heterozygous for this sequence variation. She has a good clinical outcome under treatment with Diazoxide (30 mg x 3 / day), and Octreotide (70 µg x 3 / day - subcutaneous injections).

SCHAD deficiency causes hyperinsulinism by activation of GDH via loss of inhibitory regulation of GDH by SCHAD in pancreatic islets.



From Li et al. J Biol Chem 2010;285:31806

Molven and coworkers (2004) proposed the following explanation for 3-hydroxyglutarate excretion (see figure below): glutaryl-CoA dehydrogenase is the mitochondrial enzyme required for the catabolic degradation of tryptophan and lysine, amino acids that presumably can be degraded also by a minor pathway that includes conversion of 3-hydroxyglutaryl-CoA to 3-ketoglutaryl-CoA by SCHAD. When this step is blocked, hydrolysis of 3-hydroxyglutaryl-CoA followed by excretion of 3-hydroxyglutaric acid occurs.



From Molven et al. Diabetes.2004:53:221.

Diagnosis

Most likely diagnosis

• SCHAD deficiency	15
• Glutaric aciduria type I (low excretor)	3
• CPT1 deficiency	1
• No diagnosis, no IEM	3
• Glycogenosis type IA	1

Alternative diagnosis

• Glutaric aciduria type I (low excretor)	2
• SCHAD deficiency	1
• Other fatty acid oxidation disorder	1

All 23 participants performed **organic acid** analysis, and 21 of them reported an increase of **3-hydroxyglutaric acid** (median = 50 mmol/mol creatinine; range: 20 – 145; n = 6).

All participants also performed **amino acids** analysis: 17 reported an increase of glycine (median = 1272 mmol/mol creatinine; range: 334 – 1890; n = 6), possibly due to a poor storage of the urine sample (median pH = 7.0).

Increased excretion of 3-hydroxyglutaric acid has been reported in SCHAD deficiency but also in other conditions such as: glutaric aciduria type I (problem of the differential diagnosis of low excretors of glutaric acid), carnitine palmitoyl transferase 1 (CPT1) deficiency, and severe ketotic episodes.

We tried to compare excretion of 3-hydroxyglutaric acid in the different conditions:

- **Glutaric aciduria type I, low excretors of glutaric acid** (Busquets, Merinero et al, Pediatric Research 2000;48: 315–322): results from 17 patients

mmol/mol creat	Range	Median	Mean	Controls
3-hydroxyglutarate	**18 - 571**	**101**	**160**	2 – 14
Glutarate	2 - 84	10	21	2 – 10

- SCHAD deficiency (*Vilarinho et al Mol Genet Metab 2012;106:277, **Martins et al, JIMD2011;34:835, ***Patient from Centre de Biologie Est)

	1*	2**	3**	4**	5**	8***
Plasma C4OH acylcarnitine (µmol/L)	1.22			0.5 - 0.6	0.7	0.7 - 1.6
Urinary 3-hydroxy glutate (mmol/mol creat)	**113**	**12 - 45**	**22 - 45**	**33 - 114**	**55**	**13 - 31**

- CPT 1 deficiency (Korman et al, Mol Genet Metab 2005;86:337)

mmol/mol creat	Patient 1	Patient 2	Patient 3	Controls
Age	14 months	3 years	23 months	
3-hydroxyglutarate	**9.8**	**24.7**	**14.7**	0.88 – 4.5
Glutarate	3.4	1.2	2.3	0.5 – 10.8
Dicarboxylic acids	C6 – C12	C6 – C12	C6 – C12	

Therefore, it seems that 3-hydroxyglutarate excretion is higher in glutaric aciduria type I than in SCHAD deficiency, with even lower excretion in CPT1 deficiency and also probably in ketotic states.

Scoring

- Analytical performance: increase of 3-hydroxyglutaric (score 2).
- Interpretation of results and recommendations: SCHAD deficiency as first or alternative diagnosis (score 2), perform plasma/DBS acylcarnitines (score 1).

Scores of participants

- **Survey 2014-1**

Lab n°	Patient A Iminodipetiduria			Patient B HHH syndrome			Patient C 4-hydroxybutyric aciduria		
	A	I	Total	A	I	Total	A	I	Total
1	0	0	0	2	2	4	1	2	3
2	2	2	4	2	1	3	2	2	4
3	2	2	4	1	1	2	2	2	4
4	2	2	4	1	2	3	2	2	4
5	0	0	0	2	2	4	1	2	3
6	2	2	4	2	0	2	0	0	0
7	No answer								
8	No answer								
9	0	0	0	1	1	2	0	0	0
10	2	2	4	2	2	4	2	2	4
11	2	2	4	2	2	4	1	2	3
12	2	2	4	2	2	4	2	2	4
13	2	2	4	2	2	4	2	2	4
14	2	2	4	2	2	4	2	2	4
15	0	0	0	2	2	4	2	2	4
16	2	2	4	2	2	4	2	2	4
17	2	2	4	1	1	2	2	2	4
18	2	2	4	1	0	1	2	2	4
19	2	2	4	2	2	4	2	2	4
20	2	2	4	2	2	4	2	2	4
21	2	2	4	1	2	3	1	2	3
22	0	0	0	1	1	2	0	0	0
23	2	2	4	1	2	3	2	2	4

❖ **Survey 2014-2**

Lab n°	Patient D Fucosidosis			Patient E L-2-hydroxyglutaric aciduria			Patient F SCHAD deficiency		
	A	I	Total	A	I	Total	A	I	Total
1	2	2	4	2	2	4	2	0	2
2	0	1	1	2	2	4	2	2	4
3	2	2	4	2	2	4	2	1	3
4	2	2	4	2	2	4	2	2	4
5	0	0	0	2	2	4	0	0	0
6	0	0	0	2	2	4	2	0	2
7	0	1	1	2	2	4	2	2	4
8	1	1	2	2	2	4	2	2	4
9	2	2	4	2	2	4	2	2	4
10	2	2	4	2	2	4	2	2	4
11	2	2	4	2	2	4	2	2	4
12	2	2	4	2	2	4	2	2	4
13	0	1	1	2	2	4	2	1	3
14	1	1	2	2	2	4	2	2	4
15	0	0	0	2	2	4	2	2	4
16	0	0	0	2	2	4	2	1	3
17	0	0	0	2	2	4	0	1	1
18	2	2	4	2	2	4	2	2	4
19	2	2	4	2	2	4	2	2	4
20	2	2	4	2	2	4	2	2	4
21	1	1	2	2	2	4	2	2	4
22	2	2	4	2	2	4	2	2	4
23	2	2	4	0	2	2	2	2	4

❖ **Total scores**

Lab n°	Survey 2014-1	Survey 2014-2	Cumulative score (max = 24)	Cumulative score (%)
1	7	10	17	71%
2	11	9	20	83%
3	10	11	21	88%
4	11	12	23	96%
5	7	4	11	46%
6	6	6	12	50%
7	0 (no answer)	9	9	38% (75%)
8	0 (no answer)	10	10	42% (83 %)
9	2	12	14	58%
10	12	12	24	100%
11	11	12	23	96%
12	12	12	24	100%
13	12	8	20	83%
14	12	10	22	92%
15	8	8	16	67%
16	12	7	19	79%
17	10	5	15	63%
18	9	12	21	88%
19	12	12	24	100%
20	12	12	24	100%
21	10	10	20	83%
22	2	12	14	58%
23	11	10	21	88%

Performance

	Number of labs	% total labs
Excellent performers (100 % of good responses)	4	17 %
Poor performers (< 60 % good responses)	4 / 21	19 %
Partial non submitters	2	9 %

❖ **Summary of scores**

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
Patient A	Iminodipetiduria	**76 %**	**76 %**	**76 %**
Patient B	HHH syndrome	**81 %**	**79 %**	**80 %**
Patient C	4-hydroxybutyric ac.	**76 %**	**86 %**	**81 %**
Patient D	Fucosidosis	**59 %**	**65 %**	**62 %**
Patient E	L-2-hydroxyglutaric ac.	**96 %**	**100 %**	**98 %**
Patient F	SCHAD deficiency	**91 %**	**78 %**	**85 %**

DPT-scheme in 2015

- Two surveys of 3 urines, including “normal” patients, sent by CSCQ
- Results have to be sent within 3 weeks
- **Reporting** on CSCQ (Centre Suisse de Contrôle de Qualité) website, before the deadline. Read carefully the advices on page 2.
- **Scoring**: performed by two different scheme organizers. The concept of critical error will be maintained.

We remind you that to participate to the DPT-scheme, you must perform at least:

- Amino acids
- Organic acids
- Oligosaccharides
- Mucopolysaccharides

If you are not performing one of these assays, or if purine and pyrimidine analysis is required, you can send the samples to another lab (cluster lab) but you are responsible for the results.

Please send quantitative data for amino acids and, as much as possible, for organic acids.

Meeting in 2015

It will take place during the SSIEM meeting in Lyon **Tuesday 1 September 2015** from 9.00 to 10.30 am.

We remind you that attending this meeting is an important part of the proficiency testing. The goal of the program is to **improve** the competence of the participating laboratories, which includes the critical review of all results with a discussion about improvements.

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ANNEX 1

PROFICIENCY TESTING – SOUTHERN EUROPE, LYON CENTER

URINE SAMPLES ALREADY SENT

• **1998 : 1**	**A**	**OCT**	
	B	**Propionic**	
• **1999 : 1**	**C**	**MPS I or II**	
	E	**Cystinuria**	SKZL
• **1999 : 2**	**D**	**CblC**	
	F	**HMG-CoA lyase**	
• **2000 : 1**	**G**	**Iminodipeptiduria**	SKZL
	H	**Glutathion synthetase**	
• **2001 : 1**	**P1**	**Mevalonate kinase**	
	P2	**L-2-OH glutaric**	
• **2001 : 2**	**P3**	**Methylmalonic**	SKZL
	P4	**MPS IIIA San Fillippo**	
• **2002 : 1**	**P1**	**LCHAD**	
	P2	**Sulphite oxidase**	
• **2002 : 2**	**P3**	**Biotinidase**	SKZL
	P4	**MPS I**	
• **2003:1**	**P1**	**Tyrosinemia type I**	
	P2	**SC-BCAD deficiency**	
	P3	**Argininosuccinic aciduria**	
• **2003:2**	**P4**	**MCC deficiency**	
	P5	**Sialidosis**	SKZL
	P6	**MSUD**	

• **2004:1**	**P1**	**Tyrosinemia type I, treated patient**
	P2	**Propionic acidemia**
	P3	**Non metabolic disease, septic shock**
• **2004:2**	**P4**	**Mevalonic aciduria (common sample)**
	P5	**Fucosidosis**
	P6	**Alkaptonuria**
• **2005:1**	**P1**	**Isovaleric acidemia**
	P2	**Tyrosinemia type II (common sample)**
	P3	**Disorder of peroxysome biogenesis**
• **2005:2**	**P4**	**Multiple acyl-CoA dehydrogenase deficiency**
	P5	**Alpha-mannosidosis**
	P6	**4-hydroxybutyric aciduria**
• **2006:1**	**P1**	**Aromatic amino acid decarboxylase deficiency**
	P2	**Hyperoxaluria type I**
	P3	**Mucopolysaccharidosis type VI**
• **2006:2**	**P4**	**Hypophosphatasia (common sample)**
	P5	**Lysinuric protein intolerance**
	P6	**MCAD deficiency**
• **2007:1**	**P1**	**Mitochondrial acetoacetyl-CoA thiolase (MAT)**
	P2	**Homocystinuria due to CBS deficiency**
	P3	**Hyperlysinemia (common sample)**
• **2007:2**	**P4**	**Aspartylglucosaminuria**
	P5	**Phenylketonuria**
	P6	**SCAD deficiency**
• **2008:1**	**P1**	**Cbl C/D**
	P2	**Mucopolysaccharidosis type III (common sample)**
	P3	**2-hydroxyglutaric aciduria**
• **2008:2**	**P4**	**Glycerol kinase deficiency**
	P5	**α-mannosidosis**
	P6	**3-methylcrotonyglycinuria**
• **2009:1**	**P1**	**Mucopolysaccharidosis type III**
	P2	**Salla disease (common sample)**
	P3	**No metabolic disorder**
• **2009:2**	**P4**	**Glutaric aciduria type I**
	P5	**Iminodipetiduria**
	P6	**Multiple acyl-CoA dehydrogenase deficiency**
• **2010:1**	**P1**	**Mevalonic aciduria**
	P2	**Aminoacylase I deficiency**
	P3	**No metabolic disorder**

• **2010:2**	**P4**	**Sialidosis type I (common sample)**
	P5	**Glutaric aciduria type I**
	P6	**Aspartylglucosaminuria**
• **2011:1**	**A**	**Molybdenum cofactor deficiency**
	B	**GAMT deficiency (common sample)**
	C	**Methylmalonic semialdehyde dehydrogenase def.**
• **2011:2**	**D**	**Mucopolysaccharidosis type IVA (Morquio)**
	E	**Phenylketonuria**
	F	**Citrullinemia type I**
• **2012:1**	**A**	**Intermittent MSUD (common sample)**
	B	**HHH syndrome**
	C	**Mucopolysaccharidosis type I**
• **2012:2**	**D**	**“RedBulluria”**
	E	**CblC**
	F	**SCAD deficiency**
• **2013:1**	**A**	**NFU1 deficiency**
	B	**MNGIE syndrome (educational)**
	C	**Lysinuric protein intolerance**
• **2013:2**	**D**	**Mitochondrial acetoacetyl-CoA thiolase deficiency**
	E	**Morquio disease (MPS IV)**
	F	**Glycerol kinase deficiency**