ERNDIM Administration Office
Manchester Centre for Genomic Medicine
6th floor, St Mary's Hospital
Oxford Road
Manchester
M13 9WL
UK
Email: admin@erndim.org

Scientific Coordination
C. VIANEY-SABAN, C. ACQUAVIVA-BOURDAIN
Service Maladies Héréditaires du Métabolisme
Centre de Biologie et de Pathologie Est
59, Boulevard Pinel
69677 Bron cedex
France
Tel +33 4 72 12 96 94
e-mail
christine.saban@chu-lyon.fr
cecile.acquaviva-bourdain@chu-lyon.fr

Scheme Organisation
CSCQ (Quality Control Centre, Switzerland)
Xavier Albe
2 chemin du Petit-Bel-Air
1225 Chêne-Bourg
Switzerland,
Tel: +41 22 305 52 36
Email: Xavier.Albe@hcuge.ch

Diagnostic Proficiency Testing

Centre: France

Final Report 2018

Prepared by
C. Vianey-Saban and C. Acquaviva-Bourdain

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

The fact that your laboratory participates in ERNDIM schemes is not confidential, however, the raw data and performance scores are confidential and will only be shared within ERNDIM for the purpose of evaluating your laboratories performance, unless ERNDIM is required to disclose performance data by a relevant government agency. For details please see the terms and conditions in the ERNDIM Privacy Policy on www.erndim.org.

1. Geographical distribution of participants

In 2018, 25 labs participated to the Proficiency Testing Scheme DPT France.

Country	Number of participants
France	11
Italia	5
Portugal	2
Spain	5
Switzerland	1
United Kingdom	1

2. Design and logistics of the scheme including sample information

The scheme has been designed and planned by Dr Christine Vianey-Saban as Scientific Advisor and coordinated by Xavier Albe as scheme organizer (sub-contractor on behalf of CSCQ), both appointed by and according to procedures laid down the ERNDIM Board.

CSCQ dispatches DPT EQA samples to the scheme participants and provides a website for on-line submission of results and access to scheme reports. Existing DPT and Urine MPS scheme participants can log on to the CSCQ results submission website at:
https://cscq.hcuge.ch/cscq/ERNDIM/Initial/Initial.php

2 surveys	2018-1: patients A, B and C
	2018-2: patients D, E and F

Samples

- Patient A: DPD def. - This sample has been sent to all labs participating to the DPT scheme in Europe
- Patient B: MPS VII
- Patient C: SCHAD def.
- Patient D: GAI low excr.
- Patient E: OAT def.
- Patient F: DPD def.

Samples have been kindly provided by Petr Chrastina, Prague, Czech Republic, and Fréderique Sabourdy, Toulouse, France. All other urine samples have been provided by the Scientific Advisors.
The samples have been heat-treated. They were pre-analyzed in our institute after 2 weeks at room temperature (to mimic possible changes that might arise during transport). In all six samples the typical metabolic profiles were preserved after this process.
Mailing: samples were sent by DHL, FedEx, or the Swiss Post at room temperature.

3. Tests

Analyses of amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines / pyrimidines are required. If you are not performing one of these assays, you can send the samples to another lab (cluster lab) but you are responsible for the results.

4. Schedule of the scheme

– February 5	Shipment of samples of Survey 1 and Survey 2 by CSCQ
– February 26	Clinical data available on CSCQ website and start analysis of samples (Survey 1)
– March 12	Reminder for website submission
– March19	Deadline for result submission (Survey 1)
– March 26	Interim report of Survey 1 available on CSCQ website
– May 28	Clinical data available on the CSCQ website and start analysis of samples (Survey 2)
– June 11	Reminder for website submission
– June 18	Deadline for result submission (Survey 2)
– June 25	Interim report of Survey 2 available on CSCQ website
– November 29	SAB meeting: definition of critical errors
– January 17	Annual Report with definitive scoring sent by e-mail

5. Results

All labs submitted results for both surveys, but one lab did not enter results for one sample of the 1st survey.

	Survey 1	**Survey 2**
Receipt of results	25	25
No answer	0	0

6. Web site reporting

The website reporting system is compulsory for all centres. Please read carefully the following advice:

- 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.**
- Recommendations = **advice for further investigation**.
  - Scored together with the interpretative score.
  - Advice 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.

7. Scoring and evaluation of results

Information regarding procedures for establishment of assigned values, statistical analysis, interpretation of statistical analysis etc. can be found in generic documents on the ERNDIM website.
The scoring system has been established by the International Scientific Advisory Board of ERNDIM. Two criteria are evaluated: 1) analytical performance, 2) interpretative proficiency also considering recommendations for further investigations.

A	Analytical performance	Correct results of the appropriate tests	2
		Partially correct or non-standard methods	1
		Unsatisfactory or misleading	0
I	Interpretative proficiency & Recommendations	Good (diagnosis was established)	2
		Helpful but incomplete	1
		Misleading or wrong diagnosis	0

The total score is calculated as a sum of these two criteria. The maximum to be achieved is 4 points per sample. The scores were calculated only for laboratories submitting results.

Scoring and certificate of participation: scoring is carried by a second assessor who changes every year as well as by the scientific advisor. The results of DPT France 2018 have been also scored by Dr Brian Fowler, from DPT Switzerland. At the SAB meeting in Leiden on November 29th, the definitive scores have been finalized. The concept of critical error was 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. Thus 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 at the SAB. For 2018, the SAB decided that sample B (MPS VII) has to be considered as a critical error for the labs who did not conclude to a lysosomal storage disorder nor advised to perform mucopolysaccharides analysis since the clinical presentation was highly suggestive. Non identification of an increase of ornithine in sample E (OAT deficiency) has also been considered as a critical error by the SAB.

A certificate of participation 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 evaluated as unsatisfactory. Two performance support letters will be sent by the Scientific Advisor for 2018. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

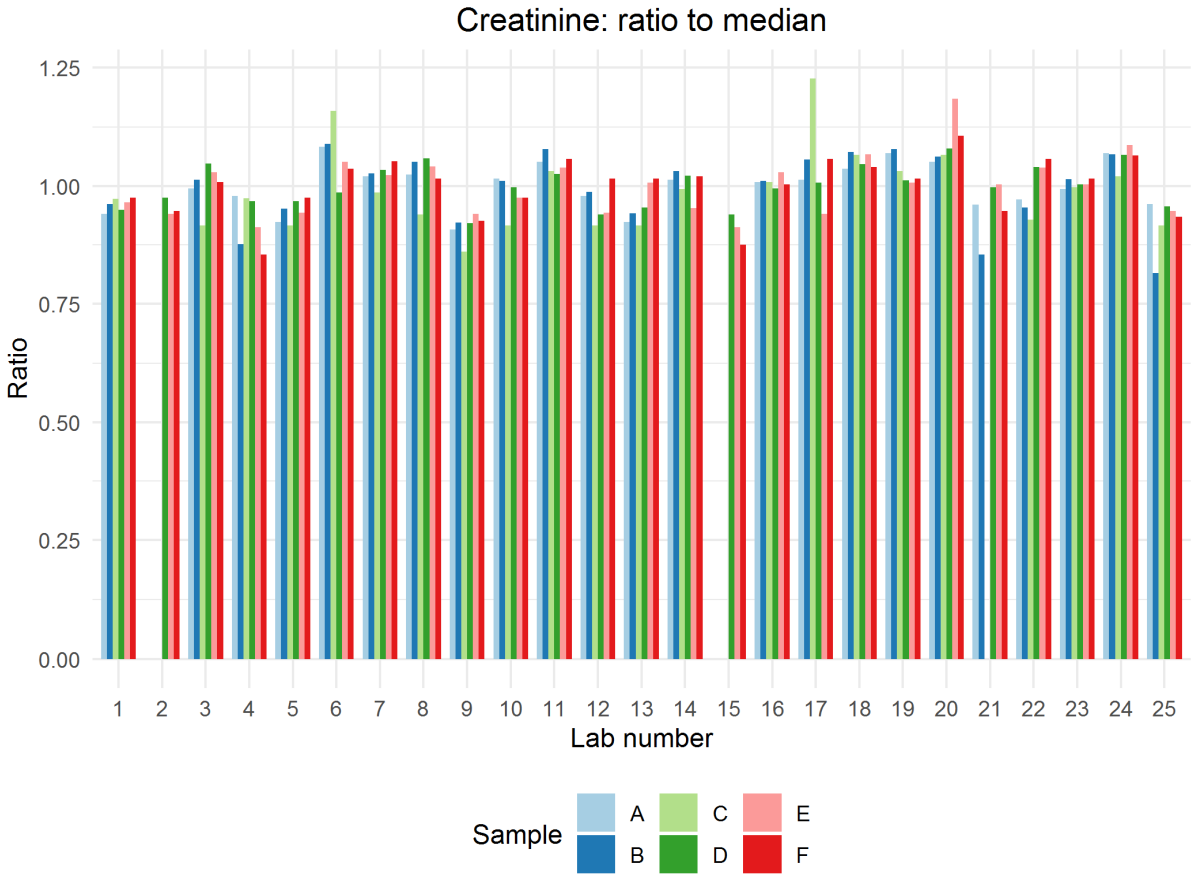
7.1. Score for satisfactory performance

At least 15 points from the maximum of 24 (62%).

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

Creatinine determination was satisfying for all labs in 2018. There were no incorrect values, nor systematic error. Creatinine values are expressed in the figure as the ratio of each measurement over the median for all labs.
CV is <7.4 % for all samples (3.6 % – 7.4 % for low creatinine values), without outliers, and this is similar to the interlab CV 2017 for Special Assay in urine (6.8 %, n = 172).



Sample	A	B	C	D	E	F
Median	5.53	4.51	0.86	7.00	3.19	2.50
SD	0.07	0.11	0.01	0.09	0.04	0.02

Table I

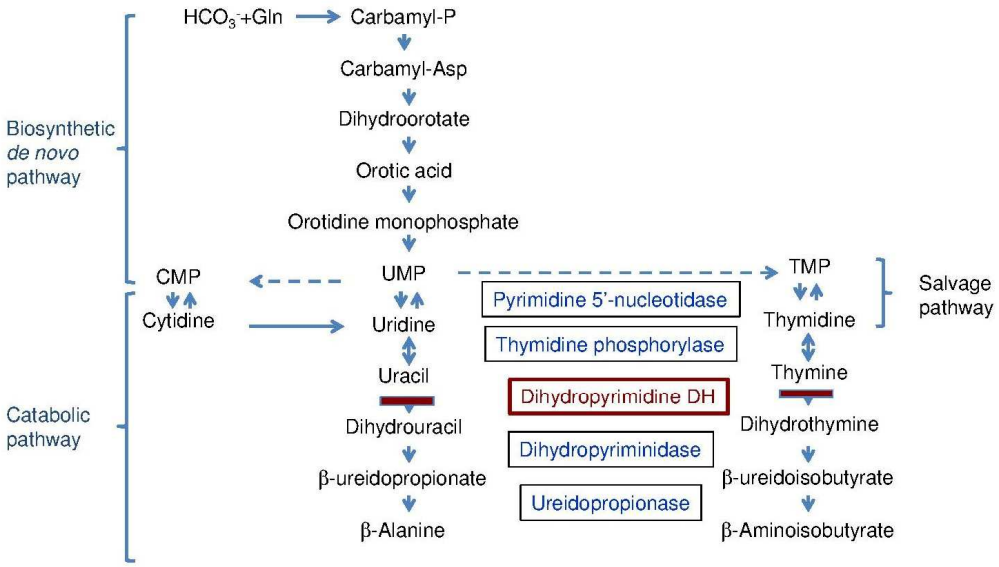
8.2. Patient A – Dihydropyrimidine dehydrogenase (DPD) deficiency (DPYD gene)

Patient details provided to participants

This female patient was referred at the age of 18 years with suspicion for multiple sclerosis based on MRI scan. Since the age of 5 years mental retardation and cognitive impairment was observed. Urine was collected at the age of 20 years.

Patient details

The diagnosis of dihydropyrimidine dehydrogenase (DPD) deficiency was confirmed by measurement of DPD activity and molecular genetic analysis of *DPYD* gene.
This sample has been distributed to all labs participating to DPT schemes. Details have been presented by Petr Chrastina (DPT Czech Republic) during the ERNDIM workshop on September 4th (available on ERNDIM website).



Pyrimidine metabolism

Dihydropyrimidine dehydrogenase deficiency can present with two clinical phenotypes:

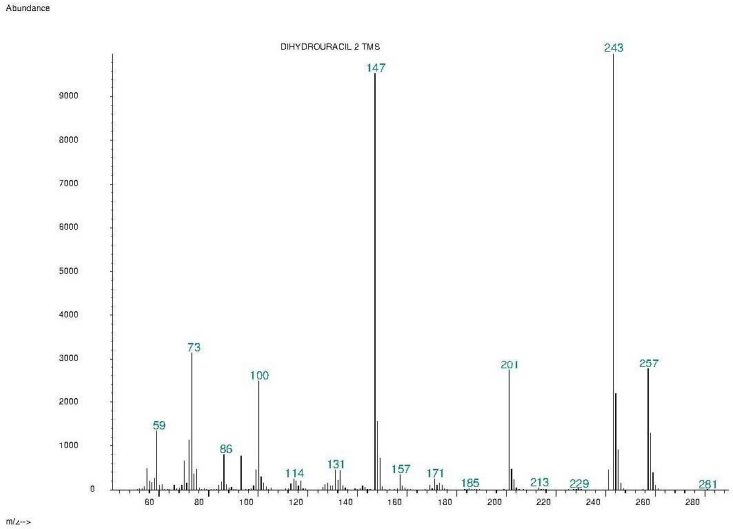
- A paediatric form, with epilepsy, motor and mental retardation, often associated with generalized hypertonia, hyperreflexia, growth delay, dysmorphic features including microcephaly. It is due to a complete DPD enzyme deficiency.
- An adult form: patients are asymptomatic except if they receive 5-flurouracil, a pyrimidine analogue, an antineoplastic drug. The toxicity manifests with profound neutropenia, stomatitis, diarrhoea and neurological symptoms. It is due to partial DPD deficiency

The pathophysiology of the disease is due to accumulation of thymine and uracil, but also to reduction of β-alanine, a neurotransmitter.
There is no treatment for the paediatric form. In adults, discontinuation of 5-flurouracil results in a slow resolution of symptoms

Analytical performance

All labs but one performed **organic acids**, and all of them reported an increase of **thymine** (median 40 mml/mol creat – range: 17.9 – 103; n = 7) and of **uracil** (median 150 mml/mol creat – range: 13.3 – 332; n = 11). Four participants mentioned that there is no increase of dihydrothymine, and three, no increase of dihydrouracil.

Dihydrouracil and dihydrothymine are increased in dihydropyrimidinase deficiency. Dihydrouracil (spectrum: 147 / 201 / 243 / 257) and dihydrothymine (same spectrum than dihydrouracil +CH2: 147 / 201 / 257 / 271) are both eluted between isovalerylglycine and adipic acid (thanks to Dr Cristiano Rizzo, Dr Odile Rigal, and Dr Pedro Ruiz Sala).



Spectrum of dihydrouracil di TMS

Twelve participants performed purines & pyrimidines analysis. Eleven of them reported an increase of **thymine** (median 71 mml/mol creat – range: 52.9 – 129.3; n = 11) and an increase of **uracil** (median 150 mml/mol creat – range: 42.2 – 178.3; n = 11). Four labs reported an increase of 5-hydroxy-3-methyluracil, while two mentioned that there was no increase of deoxyuridine and thymidine.

Diagnosis / Interpretative proficiency

Most likely diagnosis

- Dihydropyrimidine dehydrogenase def. 23
- MNGIE 1
- Urea cycle disorder 1

Alternative diagnosis

- Dihydropyrimidine dehydrogenase def. 2
- Dihydropyrimidinase deficiency 5
- MNGIE 2
- Ureidopropionase deficiency 1

Recommendations

Only 11 participants advised to avoid 5-fluorouracil treatment.

Scoring

Analytical performance

- Increase of thymine (score 1)
- Increase of uracil (score 1)

Interpretation of results

- Dihydropyrimidine dehydrogenase as first or alternative diagnosis (score 2)

Overall impression

- Analytical performance 100 %
- Interpretative performance 100 %
- Overall performance 100 %

8.3. Patient B – Mucopolysaccharidosis type VII (Sly disease - GUSB gene)

Patient details provided to participants

This boy was referred at the age of 5 months because of hypertelorism, hepatosplenomegaly, inquinal hernias, and vertebrae deformities. The urine sample was collected at the age of 14 years.

Patient details

Diagnosis of mucopolysaccharidosis type VII (Sly disease) was confirmed enzymatically (β-glucuronidase deficiency) and genetically (*GUSB* gene).

Sly syndrome is a very rare and variable disorder. *Hydrops fetalis* is the most common presentation. In our experience from ~1700 amniotic fluids investigated for non-immune *hydrops fetalis,* 107 fetuses were affected with LSDs. Among them, MPS VII was the most frequent etiology: 29 patients - 27% (Vianey-Saban et al, JIMD 2016;39:611). Patients who survive pregnancy have the same clinical presentation than MPS I.
GAG accumulation involves:

- Chondroitine sulphate +++
- Dermatan sulphate +/-
- Heparan sulphate +/-

Analytical performance

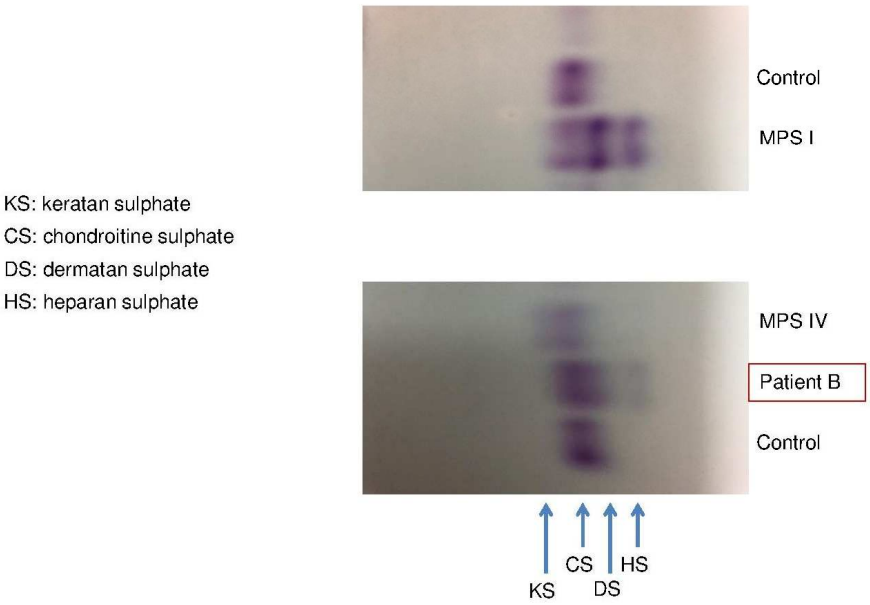
All labs but 2 performed GAG quantification: 17 of them reported elevated GAGs, while 6 reported normal results.

Nineteen participants performed GAG fractionation (19/25). Fifteen reported abnormalities:

- Increase of dermatan sulphate 12
- Increase of heparan sulphate 10
- Increase of chondroitine sulphate 6
- Increase of keratan sulphate 1

while 4 participants reported a normal or borderline profile.

The following figure illustrates the electrophoretic pattern we obtained in urines from patient B.



Electrophoretic pattern MPS patient B

The 12 participants who performed oligosaccharides reported a normal profile.

Diagnosis / Interpretative proficiency

Most likely diagnosis

– MPS VII	13
– MPS I	7
– MPS II	6
– MPS VI	3
– MPS IVA	1
– Mucopolysaccharidosis	1
– No answer	1

Alternative diagnosis

– MPS VII	4
– MPS II	7
– MPS I	5
– MPS VI	2
– MPS IVB	1

Scoring

Analytical performance

– Increase of chondroitine, dermatan and heparan sulphate (score 2)
– Increase of heparan or dermatan or chondroitine sulphate (score 1)
– Increase of GAGs (scored 1)

Interpretation of results

- Mucopolysaccharidosis type VII as first or alternative diagnosis, if GAGs fractionation has been performed (score 2).
- Another mucopolysaccharidosis or diagnosis on clinical signs (score 1)

One particpant did not enter results or recommendation for patient B, whereas they entered results for patient A and patient C. The SAB decided that sample B (MPS VII) has to be considered as a critical error for the participants who did not conclude to a lysosomal storage disorder nor advised to perform mucopolysaccharides analysis since the clinical presentation was highly suggestive.

Overall impression

- Analytical performance 74 %
- Interpretative performance 80 %
- Overall performance 77 %

8.1. Patient C – Short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) deficiency (HADH gene)

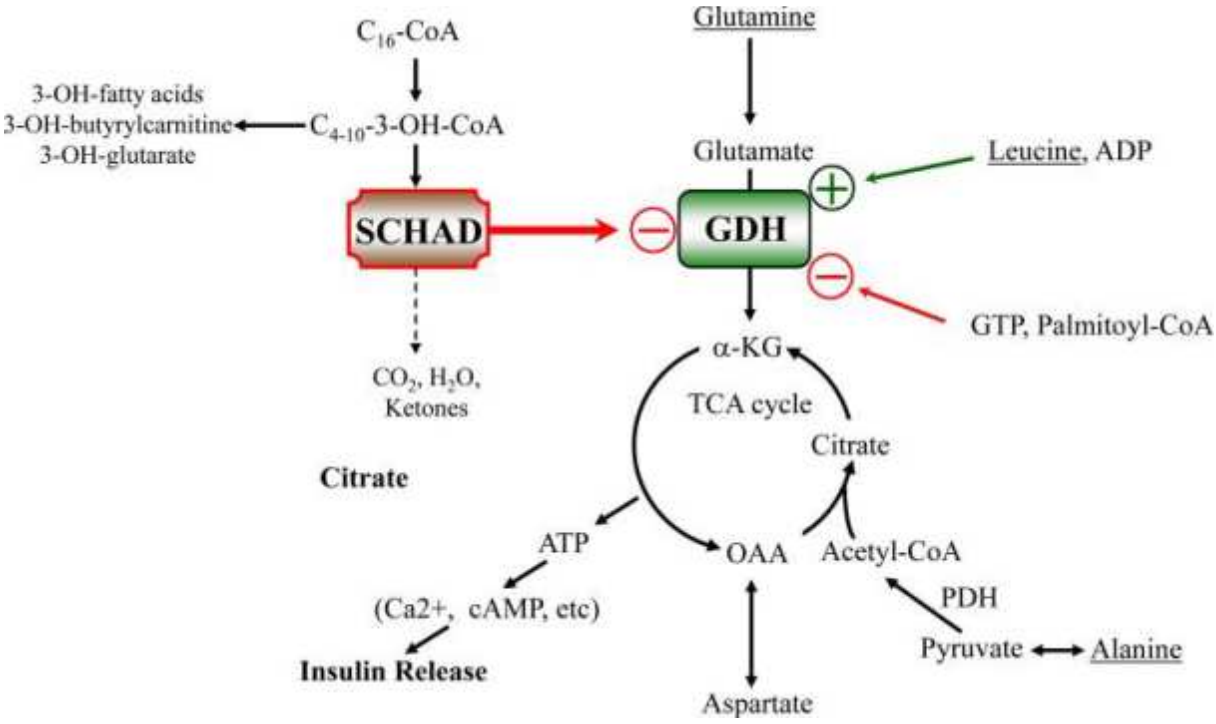
Patient details provided to participants

9-months-old girl. Hypotonia and motor regression from 3 months of age. Seizures at 6 months due to hypoglycemia. Repeated hypoglycemic episodes with increased plasma peptide C and insulin.

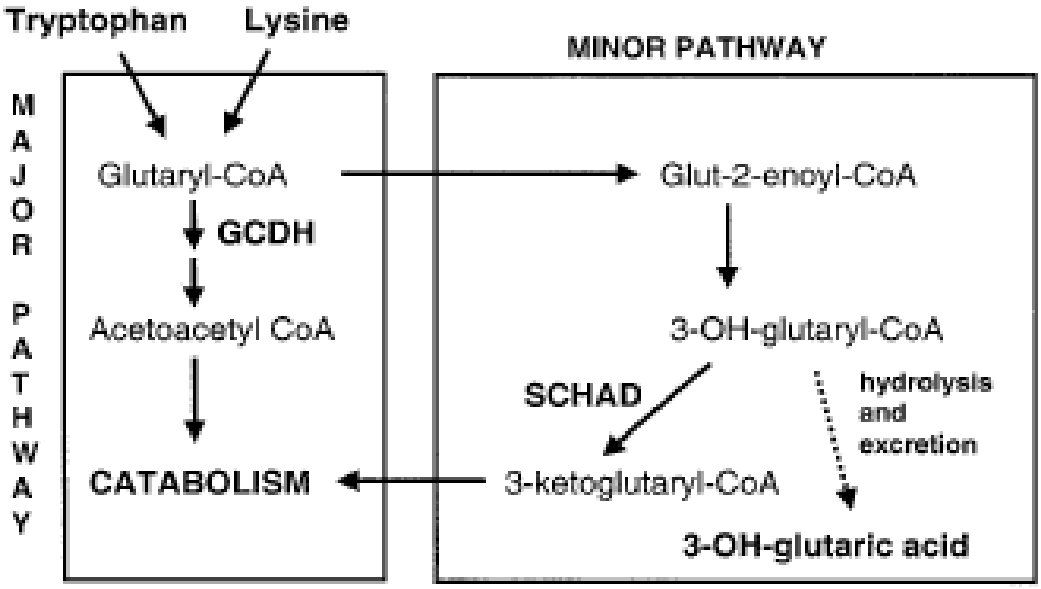
Patient details

This 9-months-old girl presented with hypotonia and motor regression from 3 months of age. She had seizures at 6 months of age due to hypoglycemia. Since then, she presented repeated hypoglycemic episodes with increased plasma peptide C and insulin, suggesting hyperinsulinism. Acylcarnitine profile in plasma showed an increase of 3-hydroxybutyrylcarnitine (C4OH = 0.82 µmol/L - simultaneous control = 0.03 µmol/L). Urinary organic acid profile revealed a slight elevation of ethylmalonic acid (24 mmol/mol creat) and an abnormal peak of 3-hydroxyglutarate (34 mmol/mol creat). No other abnormal dicarboxylic aciduria was noticed. In a second organic acid profile, ethylmalonate (44 mmol/mol creat) and 3-hydroxyglutarate (218 mmol/mol creat) were more significantly increased.
A treatment with diazoxide allowed maintaining a normal glycaemia. The hypotonia slightly improved, and no new seizures were reported.
Diagnosis of short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) deficiency was confirmed by mutation analysis of *HADH* gene.

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



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.

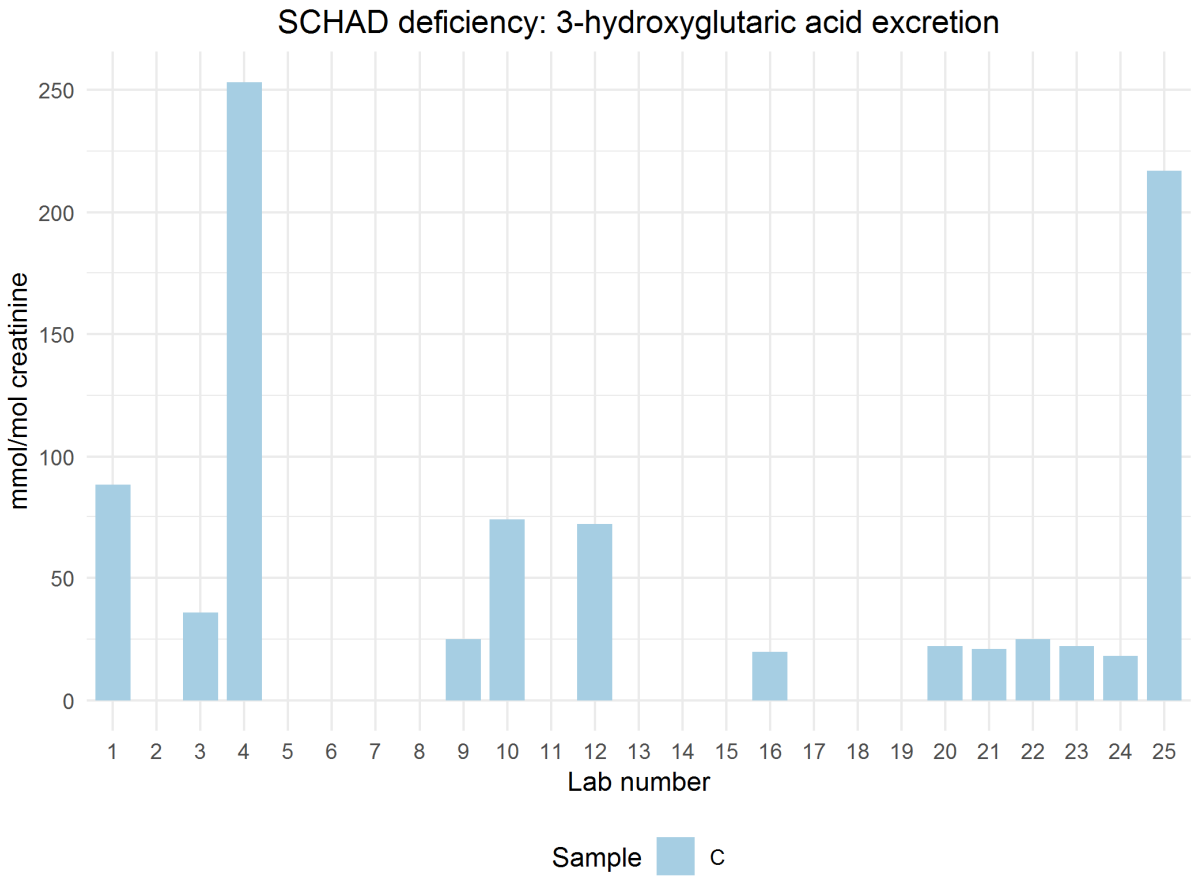


From Molven et al. Diabetes.2004:53:221.

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.

Analytical performance

All labs but one performed **organic acids** analysis, and 23 of them reported an increase of **3-hydroxyglutaric acid** (median = 25 mmol/mol creat ; range: 18.2 – 253 ; n=13), and an increase of ethylmalonic acid (median = 51 mmol/mol creat ; range: 22.6 – 94 ; n=21). Increase of adipic acid was mentioned by 15 labs (median = 48 mmol/mol creat ; range: 20.2 – 99 ; n=15), and an increase of suberic acid by 7 (median = 15.1 mmol/mol creat ; range: 7.5 – 23 ; n=7).
The participant who performed organic acids screening reported an increase of 3-hydroxyglutaric acid (= 124 mmol/mol creat).



Sample	C
Median	25
SD	74.82

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, JIMD 2011;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 glutarate (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	

- Ketotic states : <10 mmol/mol creat (in our experience)

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 ketotic states.

Acylcarnitines was performed by 13 participants and all of them reported an increase of **C4OH** (median = 3.57 mmol/mol creat ; range: 0.99 – 6.08 ; n=7). Three participants mentioned that there was no increase of glutarylcarnitine (C5DC).

The only abnormalities reported by those who performed aminoacids analysis were an increase of glycine (median = 1429 mmol/mol creat ; range: 1013 – 1940 ; n=20), and glutamine (median = 303 mmol/mol creat ; range: 255 – 423 ; n=9)

Diagnosis / Interpretative proficiency

Most likely diagnosis

- SCHAD deficiency 25

(3-hydroxyacyl-CoA dehydrogenase deficiency, hyperinsulinemic hypoglycemia familial 4: HHF4)

Alternative diagnosis

- SCAD deficiency 3
- Multiple acyl-CoA dehydrogenase deficiency 2
- Glutaric aciduria type I, low excretor 2

Recommendations

For those who did not perform urinary acylcarnitines, it is essential to recommend performing plasma / DBS acylcarnitine analysis.

Scoring

Analytical performance

- Increase of 3-hydroxyglutaric acid (score 1)
- Increase of urinary 3-hydroxybutyrylcarnitine or recommendation to perform plasma acylcarnitines (score 1)

Interpretation of results

- SCHAD deficiency (score 2)

Overall impression

- Analytical performance 92 %
- Interpretative performance 100 %
- Overall performance 96 %

Multiple distributions of similar samples

A similar urine sample has been distributed in 2014: the overall performance has significantly increased.

	2014	2018
Analytical performance	91 %	92 %
Interpretative performance	78 %	100 %
Overall performance	85 %	96 %

8.1. Patient D – Glutaric aciduria type I (low excretor)

Patient details provided to participants

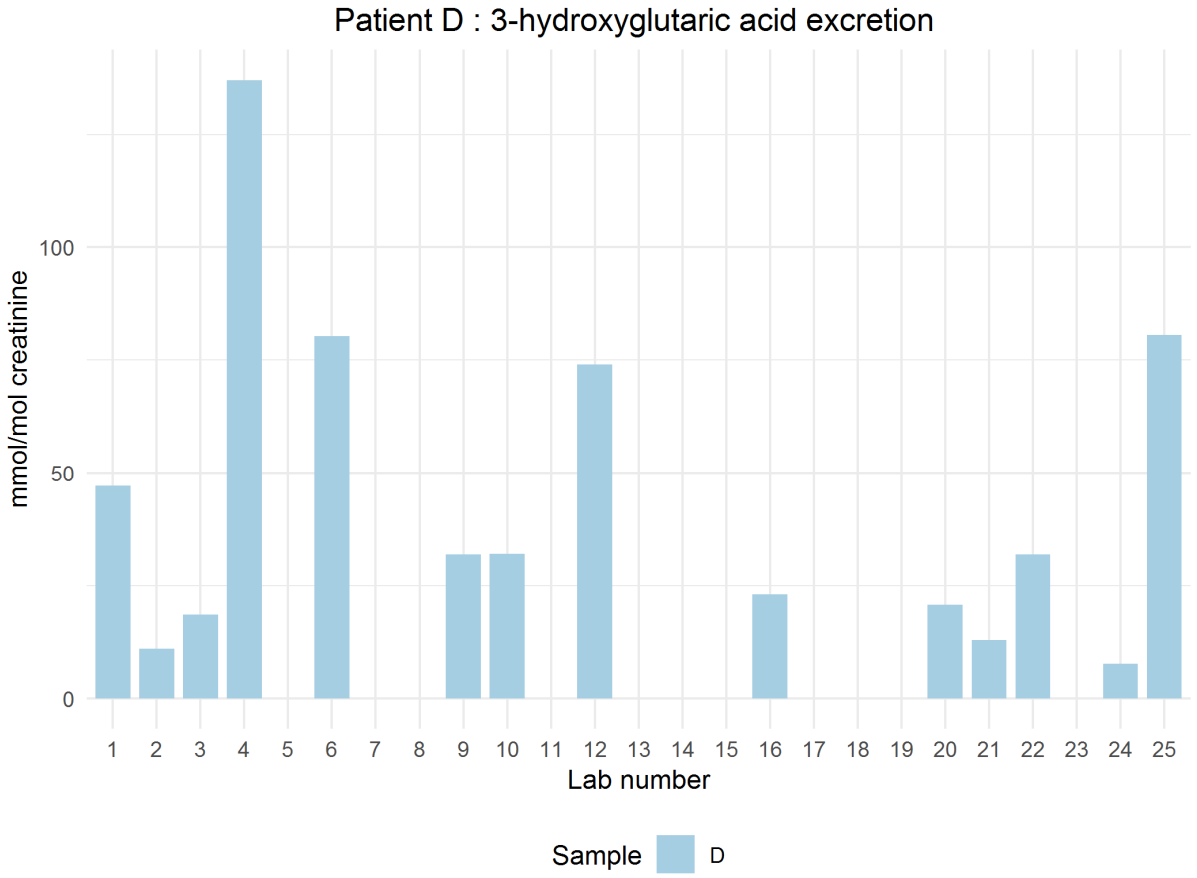
12-year-old boy. Investigated because of hemi-dystonia and hemiparesis, with acute onset of symptoms at 11 months.

Patient details

Diagnosis of glutaryl-CoA dehydrogenase deficiency was confirmed by mutation analysis of *GCDH* gene. The case report of this patient has been published in Demailly et al, Movement Disorders Clinical Practice 2018; 5(4): 436–438.

Analytical performance

All labs performed organic acid analysis and all of them reported an increase of **3-hydroxyglutaric acid** (median = 31.8 mmol/mol creat ; range: 7.64 – 137 ; n=14). Glutaric acid excretion was reported as normal by 14 participants and elevated by 4 participants (median = 2.2 mmol/mol creat ; range: 1.0 – 30 ; n=15).



Sample	D
Median	31.8
SD	35.51

The 15 participants who performed urinary acylcarnitine profile reported an increase of

glutarylcarnitine (C5DC: median = 28.9 mmol/mol creat ; range: 4.9 – 61 ; n=9).

Diagnosis / Interpretative proficiency

Most likely diagnosis

- Glutaric aciduria type I (low excretor) 25

Alternative diagnosis

- Multiple acyl-CoA dehydrogenase deficiency 2
- SCHAD deficiency 2
- Ketosis 1

Recommendations

For those who did not perform urinary acylcarnitines, it is essential to recommend performing plasma / DBS acylcarnitine analysis.

Scoring

Analytical performance

- Increase of 3-hydroxyglutaric acid (score 2)

Interpretation of results

- Glutaric aciduria type I (score 2)

Overall impression

- Analytical performance 100 %
- Interpretative performance 100 %
- Overall performance 100 %

8.1. Patient E – Ornithine aminotransferase (OAT) deficiency ; gyrate atrophy of retina and choroidea

Patient details provided to participants

4-year-old boy, born from consanguineous parents. Investigated because of myopia and retinopathy, without neurological signs.

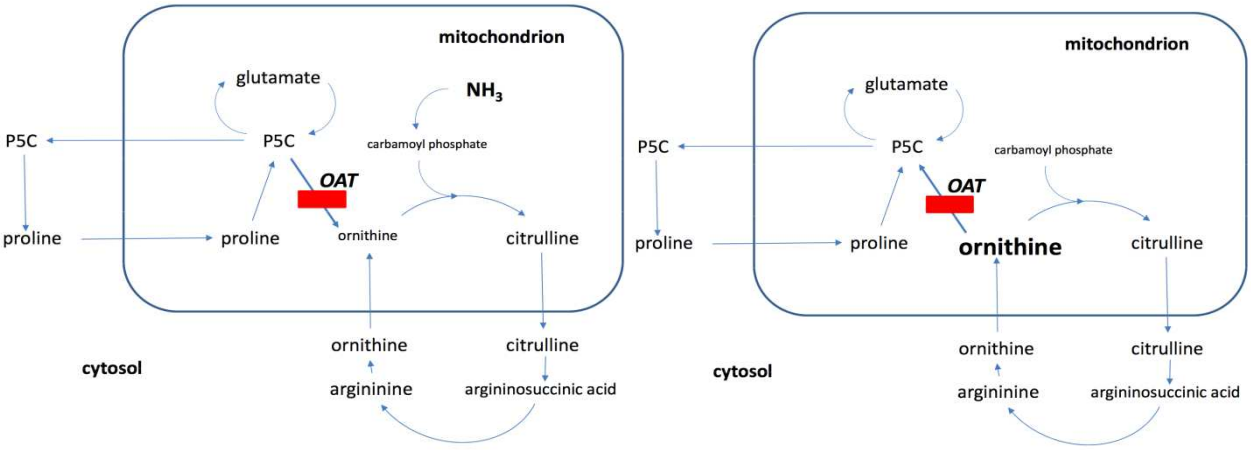
Patient details

Plasma ornithine level at diagnosis was 905 µmol/L. With a restricted protein diet, his plasma Orn levels remain <150 µmol/L.
Diagnosis has been confirmed by mutation analysis of *OAT* gene.

Clinical presentation of OAT deficiency varies with age:

- Neonatal period (few patients): poor feeding, failure to thrive, hyperammonaemia and orotic aciduria, mimicking OCT deficiency. Plasma ornithine levels are usually normal in the neonatal period because OAT is a reversible enzyme: it is active in the direction of Orn synthesis from pyrroline-5-carboxylic acid (P5C) in this period.
- Early to mid-childhood: myopia followed by night blindness, with an increase in Orn levels.
- Progressive chorioretinal degeneration: characteristic fundoscopic appearance of gyrate atrophy of the choroid and retina.
- Blindness occurs between the age of 45 and 65 years

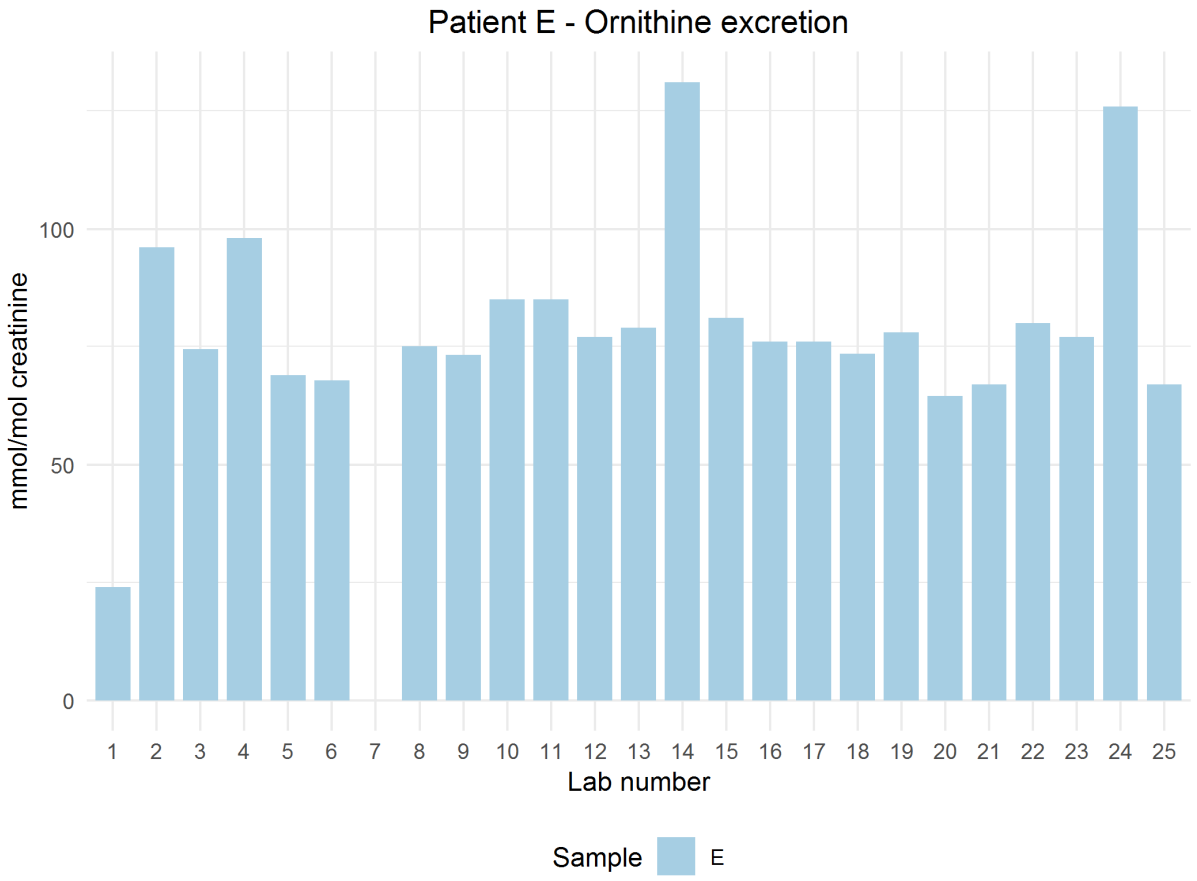
Treatment consists of a dietary restriction of arginine. A few patients demonstrate a significant reduction in plasma Orn level in response to B6 supplementation (300-500 mg/day).



Newborn | Adult

Analytical performance

All labs but one performed amino acids and reported an increase of **ornithine** (median = 76.5 mmol/mol creatinine ; range: 24 – 131; n = 24). Nine participants specified that there was no increase of arginine and lysine, and 4 no increase of cystine.



Sample	E
Median	76.5
SD	19.9

When performed (6 labs), organic acids were without significant abnormality. Orotic acid excretion was normal (0,10 – 3.0 mmol/mol creatinine; n=7).

Diagnosis / Interpretative proficiency

Most likely diagnosis

- Ornithine amino transferase deficiency (gyrate atrophy of choroid and retina) 24
- No abnormality 1

Alternative diagnosis

- HHH syndrome 4

Scoring

Analytical performance

- Increase of ornithine (score 2)

Interpretation of results

- Ornithine aminotransferase deficiency (score 2)

Non identification of an increase of ornithine in sample E (OAT deficiency) has been considered as a critical error by the SAB.

Overall impression

- Analytical performance 96 %
- Interpretative performance 96 %
- Overall performance 96 %

8.1. Patient F – Dihydropyrimidine dehydrogenase (DPD) deficiency

Patient details provided to participants

6-year-old boy. Psychomotor retardation and behavioural problems.

Patient details

The diagnosis was confirmed by mutation analysis of *DPYD* gene.
By chance, two samples of DPD deficiency have been distributed this year: the DPT scientific advisors are not informed of the diagnosis of the common sample.

Analytical performance

All but one participants performed **organic acid** analysis: all of them reported an increase of **uracil** (median 139 mml/mol creat – range: 0.21 (?) – 397 ; n = 9), and 22 of them an increase of **thymine** (median 59 mml/mol creat – range: 53 – 198 ; n = 5). Three labs mentioned that there was no increase of dihydrouracil and dihydrothymine.

The 14 labs who performed purines & pyrimidines determination, reported an increase of **uracil** (median 291 mml/mol creat – range: 72.7 – 624 ; n = 13) and **thymine** (median 173.5 mml/mol creat – range: 151 – 362 ; n = 13).

Diagnosis / Interpretative proficiency

Most likely diagnosis

– Dihydropyrimidine dehydrogenase def.	24
– Dihydropyrimidinase deficiency	1
– MNGIE	1
– Urea cycle disorder	1

Alternative diagnosis

– Dihydropyrimidine dehydrogenase def.	1
– Dihydropyrimidinase deficiency	7
– MNGIE	1
– Urea cycle disorder	1

Recommendations

Only 12 participants advised to avoid 5-fluorouracil treatment.

Scoring

Analytical performance
- Increase of thymine (score 1)
- Increase of uracil (score 1)

Interpretation of results
- Dihydropyrimidine dehydrogenase as first or alternative diagnosis (score 2)

Overall impression

– Analytical performance	100 %
– Interpretative performance	100 %
– Overall performance	100 %

9. Scores of participants

All data transfer, the submission of data as well as the request and viewing of reports proceed via the DPT-CSCQ results website. The results of your laboratory are confidential and only accessible to you (with your username and password). The anonymous scores of all laboratories are accessible to all participants and only in your version is your laboratory highlighted.

Detailed scores – Round 1

Lab n°	Patient A DPD def.			Patient B MPS VII			Patient C SCHAD def.			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	2	2	4	2	2	4	12
2	2	2	4	--	--	--	2	2	4	8
3	2	2	4	2	2	4	2	2	4	12
4	2	2	4	2	2	4	2	2	4	12
5	2	2	4	1	1	2	2	2	4	10
6	2	2	4	2	2	4	1	2	3	11
7	2	2	4	1	1	2	1	2	3	9
8	2	2	4	1	2	3	1	2	3	10
9	2	2	4	2	2	4	2	2	4	12
10	2	2	4	1	2	3	2	2	4	11
11	2	2	4	1	2	3	2	2	4	11
12	2	2	4	2	1	3	2	2	4	11
13	2	2	4	2	1	3	2	2	4	11
14	2	2	4	2	2	4	2	2	4	12
15	2	2	4	1	1	2	2	2	4	10
16	2	2	4	2	2	4	1	2	3	11
17	2	2	4	1	1	2	2	2	4	10
18	2	2	4	1	1	2	2	2	4	10
19	2	2	4	1	2	3	2	2	4	11
20	2	2	4	2	2	4	2	2	4	12
21	2	2	4	2	2	4	2	2	4	12
22	2	2	4	2	2	4	2	2	4	12
23	2	2	4	1	1	2	2	2	4	10
24	2	2	4	1	2	3	2	2	4	11
25	2	2	4	2	2	4	2	2	4	12

Detailed scores – Round 2

Lab n°	Patient D GAI low excr.			Patient E OAT def.			Patient F DPD def.			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	2	2	4	2	2	4	12
2	2	2	4	2	2	4	2	2	4	12
3	2	2	4	2	2	4	2	2	4	12
4	2	2	4	2	2	4	2	2	4	12
5	2	2	4	2	2	4	2	2	4	12
6	2	2	4	2	2	4	2	2	4	12
7	2	2	4	0	0	0	2	2	4	8
8	2	2	4	2	2	4	2	2	4	12
9	2	2	4	2	2	4	2	2	4	12
10	2	2	4	2	2	4	2	2	4	12
11	2	2	4	2	2	4	2	2	4	12
12	2	2	4	2	2	4	2	2	4	12
13	2	2	4	2	2	4	2	2	4	12
14	2	2	4	2	2	4	2	2	4	12
15	2	2	4	2	2	4	2	2	4	12
16	2	2	4	2	2	4	2	2	4	12
17	2	2	4	2	2	4	2	2	4	12
18	2	2	4	2	2	4	2	2	4	12
19	2	2	4	2	2	4	2	2	4	12
20	2	2	4	2	2	4	2	2	4	12
21	2	2	4	2	2	4	2	2	4	12
22	2	2	4	2	2	4	2	2	4	12
23	2	2	4	2	2	4	2	2	4	12
24	2	2	4	2	2	4	2	2	4	12
25	2	2	4	2	2	4	2	2	4	12

Total scores

Lab n°	A	B	C	D	E	F	Cumulative score	Cumulative score (%)	Critical error
1	4	4	4	4	4	4	24	100	
2	4	--	4	4	4	4	20	83	CE
3	4	4	4	4	4	4	24	100	
4	4	4	4	4	4	4	24	100	
5	4	2	4	4	4	4	22	92	
6	4	4	3	4	4	4	23	96	
7	4	2	3	4	0	4	17	71	CE
8	4	3	3	4	4	4	22	92	
9	4	4	4	4	4	4	24	100	
10	4	3	4	4	4	4	23	96	
11	4	3	4	4	4	4	23	96	
12	4	3	4	4	4	4	23	96	
13	4	3	4	4	4	4	23	96	
14	4	4	4	4	4	4	24	100	
15	4	2	4	4	4	4	22	92	
16	4	4	3	4	4	4	23	96	
17	4	2	4	4	4	4	22	92	
18	4	2	4	4	4	4	22	92	
19	4	3	4	4	4	4	23	96	
20	4	4	4	4	4	4	24	100	
21	4	4	4	4	4	4	24	100	
22	4	4	4	4	4	4	24	100	
23	4	2	4	4	4	4	22	92	
24	4	3	4	4	4	4	23	96	
25	4	4	4	4	4	4	24	100	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 60 % of adequate responses)	23	92
Unsatisfactory performers (< 60 % adequate responses and/or critical error)	2	8
Partial and non-submitters	0	0

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
Sample 2018-A	DPD def.	100	100	100
Sample 2018-B	MPS VII	77	83	80
Sample 2018-C	SCHAD def.	92	100	96
Sample 2018-D	GAI low excr.	100	100	100
Sample 2018-E	OAT def.	96	96	96
Sample 2018-F	DPD def.	100	100	100

10. Annual meeting of participants

It took place in Athens on September 4th 2018 from 9.00 to 10.30, before the SSIEM Meeting.

Participants

Representatives from 15 labs were present: M Unceta (Baracaldo), JA Arranz (Barcelona), I Redonnet-Vernhet (Bordeaux), S Funghini, G La Marca, S Malvagia (Florence), C Corne, S Vergnaud (Grenoble), O Boulat, O Braissant, C Roux (Lausanne), I Rivera (Lisbon), P Ruiz Sala (Madrid), M Gastaldi (Marseille), F Habarou (Necker, Paris), G Polo (Padova), D Quelhas (Porto), S Bekri (Rouen), JA Cocho, C Colon (Santiago de Compostella), C Rizzo (Rome).

We remind you that attending the annual 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.

11. Information from the Executive Board and the Scientific Advisory Board

- New **reference materials** are now provided by SKML: they are not related to EQA samples anymore. There are two concentration levels for each group of analytes. The most suitable low and high concentration levels are defined by the respective scientific advisors. Analytes and their concentrations will be approximately the same in consecutive batches of control materials. These reference materials can be ordered through the ERNDIM website. Participants are encouraged to use them as internal control, but they cannot be used as calibrants. On the website a new section

for data management completes the ERNDIM internal Quality Control System. Laboratories have the option to submit results and request reports showing their result in the last run in comparison to defined acceptance limits, their own historical data and the mean of all laboratories using the same batch control material.

- A set of **organic acid mixtures** has been developed by Dr Herman ten Brink in Amsterdam, following request and advice from ERNDIM. The product is currently available at: HJ.tenBrink@VUmc.nl
- **Training**: SSIEM Academy training courses.
  - A 2 days course will be been organized on Monday and Tuesday April 29th & 30th 2019 near Zurich. The program for biochemists includes:
    - Glycogen storage disorders
    - CDG syndromes
    - Mitochondrial diseases
    - Neurotransmitters disorders
  - The lectures will be available on the SSIEM website
- **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 sent for DPT France.

  As soon as possible after collection, the urine sample must be heated at 56 °C for 20 minutes. 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:

  C. VIANEY-SABAN, C. ACQUAVIVA-BOURDAIN
  Service Maladies Héréditaires du Métabolisme
  Centre de Biologie et de Pathologie Est
  59, Boulevard Pinel
  69677 Bron cedex
  France
  Tel +33 4 72 12 96 94
  e-mail
  christine.saban@chu-lyon.fr
  cecile.acquaviva-bourdain@chu-lyon.fr

  Please send us an e-mail on the day you send the samples. You will get a 20% discount on your DPT registration fee the following year.

12. Reminders

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

- Amino acids
- Organic acids
- Oligosaccharides
- Mucopolysaccharides
- Purines & pyrimidines

If you are not performing one of these assays, 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.

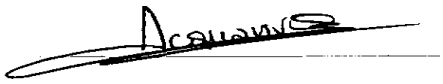
13. Tentative schedule and fee in 2019

Sample distribution	5 February 2019
Start of analysis of Survey 2019/1 Website open	March 4
Survey 2019/1 - Results submission	March 25
Survey 2019/1 - Reports	April
Start of analysis of Survey 2019/2	June 3
Survey 2019/2 – Results submission	June 24
Survey 2019/2 - Reports	July
Annual meeting of participants	Sept 3 Rotterdam SSIEM
Annual Report 2019	December

14. ERNDIM certificate of participation

A combined certificate of participation covering all EQA schemes will be provided to all participants who take part in any ERNDIM scheme. For the DPT scheme this certificate will indicate if results were submitted and whether satisfactory performance was achieved in the scheme.

Date of report, 2019-02-11
Name and signature of Scientific Advisor
C. VIANEY-SABAN, C. ACQUAVIVA-BOURDAIN
Service Maladies Héréditaires du Métabolisme
Centre de Biologie et de Pathologie Est
59, Boulevard Pinel
69677 Bron cedex
France
Tel +33 4 72 12 96 94
e-mail
christine.saban@chu-lyon.fr
cecile.acquaviva-bourdain@chu-lyon.fr



ANNEX 1

DIAGNOSTIC PROFICIENCY TESTING (DPT) FRANCE

URINE SAMPLES ALREADY SENT

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

• 2013:2	D	Mitochondrial acetoacetyl-CoA thiolase deficiency
	E	Morquio disease (MPS IV)
	F	Glycerol kinase deficiency
• 2014:1	A	Iminodipeptiduria
	B	HHH syndrome (common sample)
	C	4-hydroxybutyric aciduria
• 2014:2	D	Fucosidosis
	E	L-2-hydroxyglutaric aciduria
	F	SCHAD deficiency
• 2015:1	A	Combined malonic & methylmalonic aciduria
	B	Homocystinuria-CBS deficiency (common sample)
	C	Mucopolysaccharidosis type VI
• 2015:2	D	N-acetylaspartic aciduria
	E	D-2-hydroxyglutaric aciduria type II
	F	GM1 gangliosidosis
• 2016:1	A	Primary hyperoxaluria type II (common sample)
	B	Methionine S-adenosyltransférase (MAT) def.
	C	Glycerol kinase deficiency
• 2016:2	D	Ethylmalonic encephalopathy (*ETHE1* gene)
	E	Mucopolysaccharidosis type IVA
	F	Argininosuccinic aciduria
• 2017:1	A	Citrullinaemia type I (common sample)
	B	MNGIE
	C	Formiminoglutamic aciduria
• 2017:2	D	GM1 gangliosidosis
	E	No IEM
	F	Imerslund-Gräsbeck