Diagnostic Proficiency Testing
Centre: France
Final Report 2019

prepared by
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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.

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In 2019, 24 labs participated to the Proficiency Testing Scheme France.

1. Geographical distribution of participants

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

2. Design and logistics of the scheme including sample information

The scheme has been designed and planned by Christine Vianey-Saban and Cécile Acquaviva as Scientific Advisors, and coordinated by Xavier Albe as scheme organiser (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	Round 1: patients A, B and C
	Round 2: patients D, E and F

Version Number (& Date)	Amendments
[1] version 2 (04 March 2020)	• Page 20-23 : scores for sample C revised for a number of participants.

Origin of patients: Three urine samples have been provided by the scheme organizers, one by DPT Switzerland, one by DPT Czech Republic, and one by Dr Pedro Ruiz-Sala from Madrid.

Patient A: APRT deficiency
Patient B: Beta-mannosidosis
Patient C: Hyperprolinaemia type II
Patient D: MADD
Patient E: MPSII
Patient F: Argininemia

The samples have been heat-treated. They were pre-analyzed in our institute after 3 days incubation at ambient 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 urines/pyrimidines were required in 2019.

4. Schedule of the scheme

- February 4, 2019: shipment of samples of Survey 1 and Survey
- March 4, 2019: clinical data available on CSCQ website and start analysis of samples (Survey 1)
- March 18, 2019: reminder for website submission
- March 25, 2019: deadline for result submission (Survey 1)
- May 14, 2019: Interim report of Survey 1 available on CSCQ website
- June 3, 2019: clinical data available on CSCQ website and start analysis of samples (Survey 2)
- June 17, 2019: reminder for website submission
- June 24, 2019: deadline for result submission (Survey 2)
- June 23, 2019: Interim report of Survey 2 available on CSCQ website
- September 3, 2019: meeting in Rotterdam
- November 24, 2019: SAB meeting in Manchester; definition of critical errors
- February 17, 2020: Annual Report with definitive scoring available on CSCQ website

5. Results

All participants returned results for both surveys, but one participant returned results for only one sample of the second survey.

	Survey 1	**Survey 2**
Receipt of results	24	24
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 2019 have been also scored by Dr George Ruijter from DPT The Netherlands. At the SAB meeting in Manchester on November 24, 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 2019, the SAB decided that sample C has to be considered as a critical error for the labs who did not perform amino acid analysis and did not identify N-(pyrrole-2-carboxyl) glycine in the organic acid profile. For sample F, non-identification of an increase of orotic acid without recommendation to perform plasma amino acid analysis has also been advised by the SAB as a critical error. The SAB also considered sample B (beta-mannosidosis) as educational and therefore has not been scored.

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. Four performance support letters will be sent by the Scheme Advisor for 2019. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

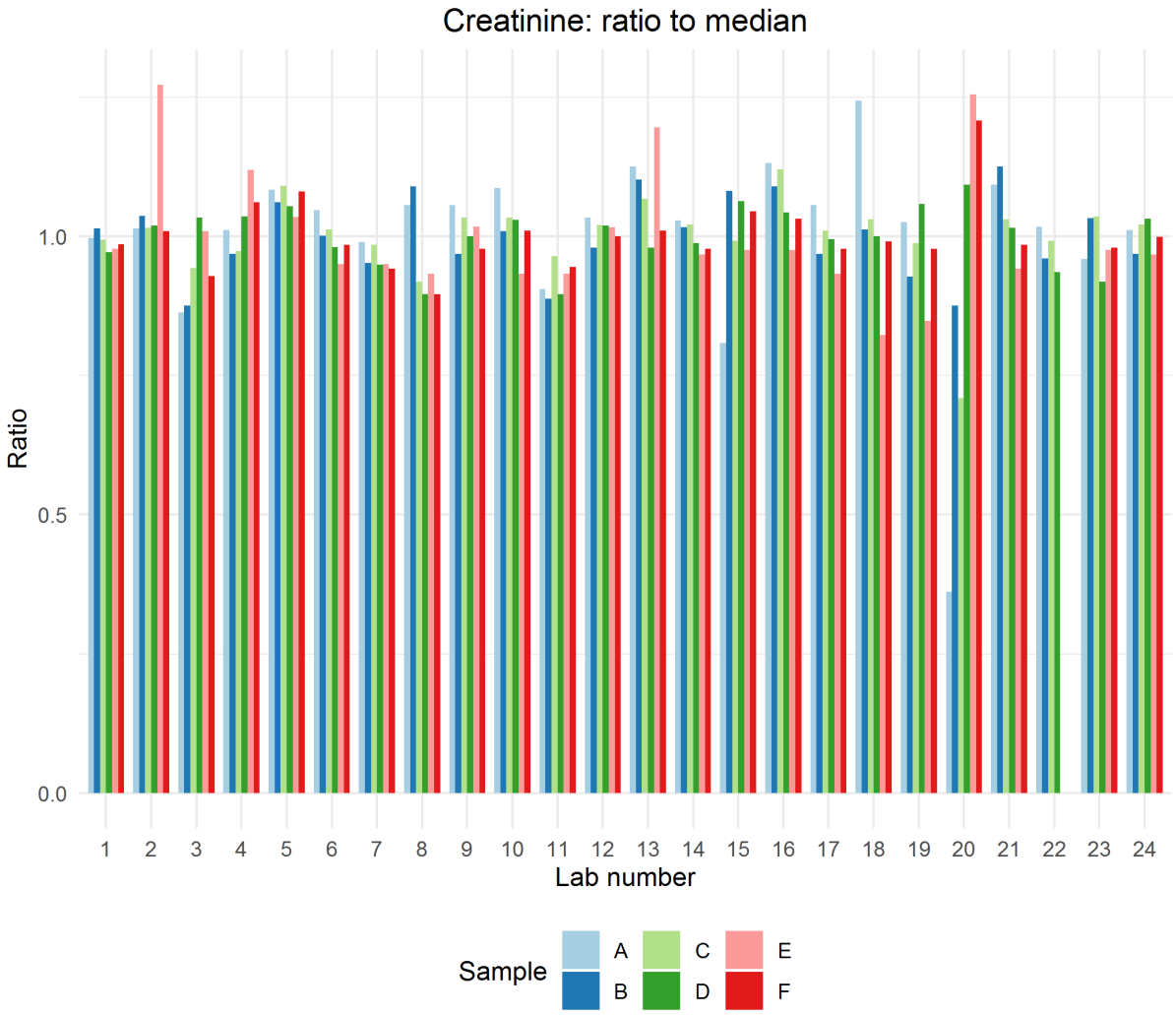
7.1. Score for satisfactory performance

At least 13 points from the maximum of 20 (65%).

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

Creatinine determination was satisfying for all labs this year, except one aberrant value (removed) and lab 20 who has scattered results. Creatinine values are expressed in the figure as the ratio of each measurement over the median for all labs.
CV is < 8.5 % for all samples (5.2 – 8.5 %), except for sample E which had a low creatinine value (CV = 11.3%), and this is rather similar to the interlab CV 2019 for Special Assay in urine (6.1 %, n = 172).



Sample	A	B	C	D	E	F
Median	3.41	2.49	4.42	20.46	1.15	6.05

8.2. Patient A

Adenine phosphoribosyltransferase (APRT) deficiency (APRT gene)

Patient details provided to participants

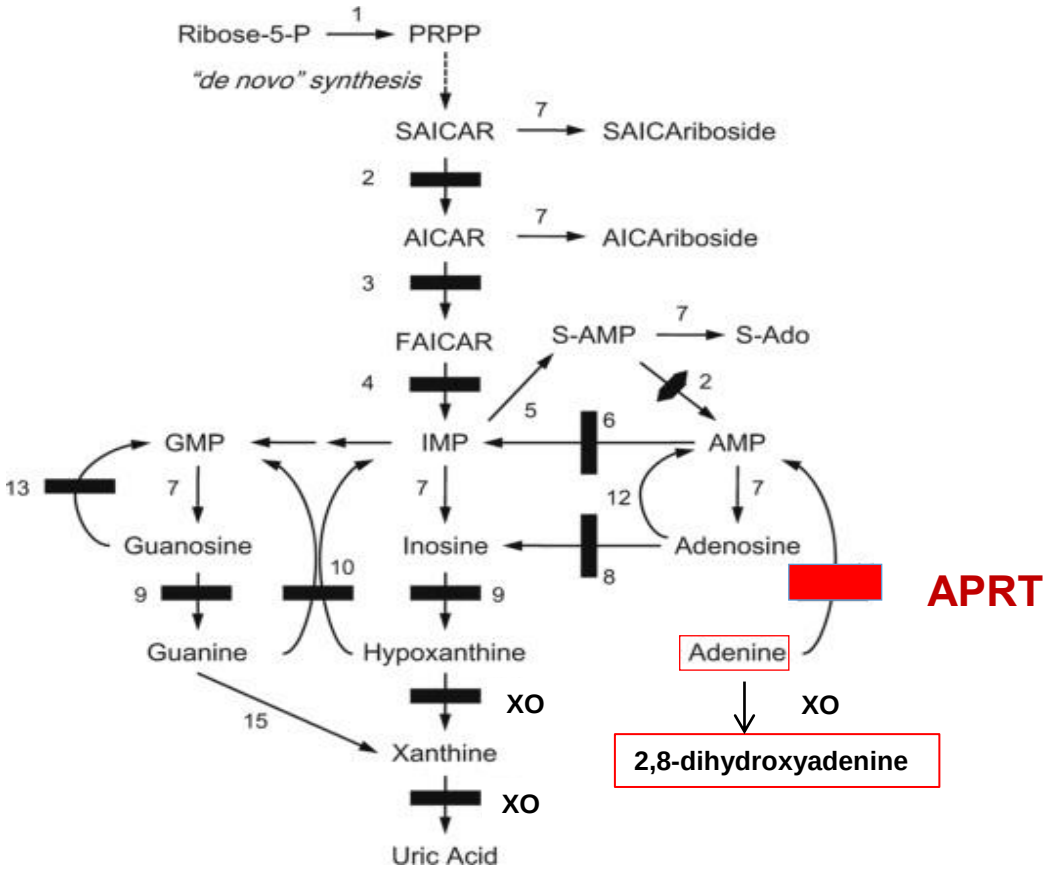
The female was admitted to hospital due to a history of pain on passing urine. Had been treated but urine collected off treatment.

Patient details

The diagnosis of adenine phosphoribosyltransferase (APRT) deficiency had been suspected at 28 years of age by purines analysis and confirmed by mutation analysis of *APRT* gene. She had been treated but the urine was collected at 35 years of age off treatment.

This is the common sample distributed to all participants to the 5 DPT schemes. Details have been presented by Alessio Cremonesi (DPT Switzerland) at the DPT common meeting in Rotterdam on September 3, 2019. The presentation is available on www.erndim.org "Meetings and Reports".

Purine metabolism



The deficiency of adenine phosphoribosyl transferase (APRT) results in suppression of the salvage of adenine, provided by food and the polyamine pathway. Consequently, adenine is oxidized by xanthine oxidase (XO) into 2,8-dihydroxyadenine, a very poorly soluble compound.

APRT deficiency is clinically characterized by the urinary passage of gravels, small stones and crystals, accompanied by abdominal pain, haematuria and urinary tract infection. It can manifest in childhood, even from birth, or can remain silent for several decades. The urinary precipitates are composed of 2,8-dihydroxyadenine. Allopurinol should be given to inhibit the formation of 2,8-dihydroxyadenine. Dietary purine restriction and high fluid intake are recommended.

Analytical performance

Only 13 labs over 24 performed purine and pyrimidine analysis: only 7 of them reported an increase of 2,8-dihydroxyadenine and 9 an increase of adenine (median 9.0 mml/mol creat – range: 8.5 – 11.0 ; n = 6). Three participants reported a normal profile.

Diagnosis / Interpretative proficiency

Most likely diagnosis

APRT deficiency	10
No diagnosis, no IEM	13
Xanthine oxidase deficiency	1

Alternative diagnosis

APRT or xanthine oxidase deficiency	1

Scoring

- Analytical performance: increase of 2,8-dihydroxyadenine (score 2), increase of adenine without identification of 2,8-dihydroxyadenine (score 1)

- Interpretation of results: APRT deficiency as first or alternative diagnosis (score 2), advise to perform purine & pyrimidine analysis, or crystal and stone analysis (score 1)

Overall impression

The overall proficiency was disappointing: 48%. Only 11 participants concluded to APRT deficiency as first or alternative diagnosis. Although this score was low, the SAB decided to score this common sample.

8.3. Patient B

Beta-mannosidosis due to beta-mannosidase deficiency (MANBA gene)

Patient details provided to participants

A 4 years old girl was referred for failure to thrive, short stature, psychomotor retardation, autistic features, speech impairment and hypotonia.

Patient details

Diagnosis of β-mannosidosis was suspected by oligosaccharide analysis and confirmed by mutation analysis of *MANBA* gene.
The urine sample was collected when the patient was 5-year-old.

β-mannosidosis is a very rare and clinically variable lysosomal storage disorder. It usually presents with severe learning difficulties, challenging behaviour, deafness, and frequent infections. It can be biochemically diagnosed by oligosaccharide analysis and measurement of beta-mannosidase enzyme activity in leukocytes or cultured fibroblasts.

Analytical performance

Only 11 participants performed oligosaccharide analysis and only 5 of them reported an abnormal profile.
The misleading analytical result observed in this patient was an increase of creatine (1.78 – 2520 mmol/mol creat – median = 1821 – n=11): secondary to the disease?

Diagnosis / Interpretative proficiency

Most likely diagnosis

Beta-mannosidosis	3
Oligosaccharidosis	2
Creatine transporter deficiency	8
No IEM	4
MPS III	3
MPS or mucolipidosis	1
FIGLU aciduria	1
Beta-ureidopropionase deficiency	1
Carnosinase deficiency	1

Alternative diagnosis

Creatine transporter deficiency	3
GABA transaminase deficiency	4
MPS III	3

Scoring

Due to the poor proficiency, the SAB considered this sample as educational and therefore it has not been scored.

8.1. Patient C

Hyperprolinaemia type II due to delta 1-pyrroline-5-carboxylate (P5C) dehydrogenase deficiency (ALDH4A1 gene).

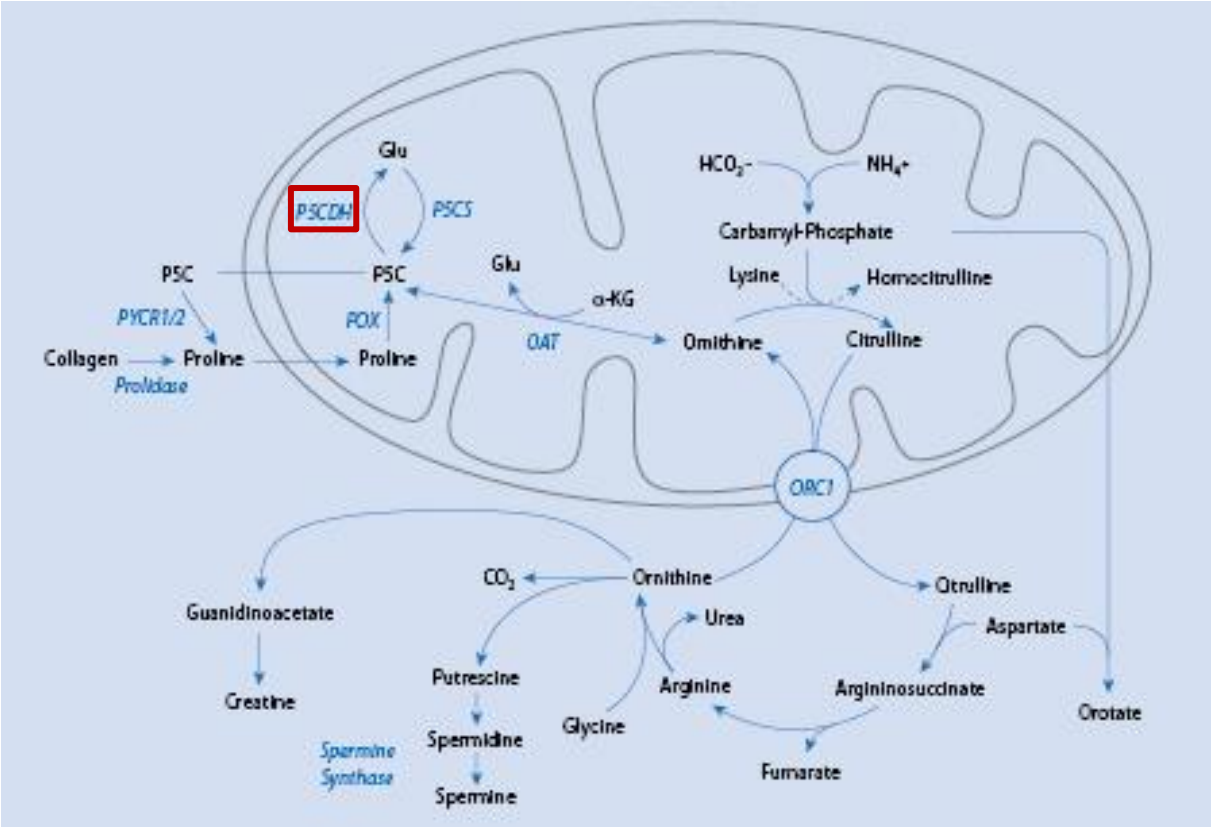
Patient details provided to participants

5-year-old girl. Investigated at 4 years of age because of speech delay, hyperactive behavior and suspicion of autism. EEG was normal.

Patient details

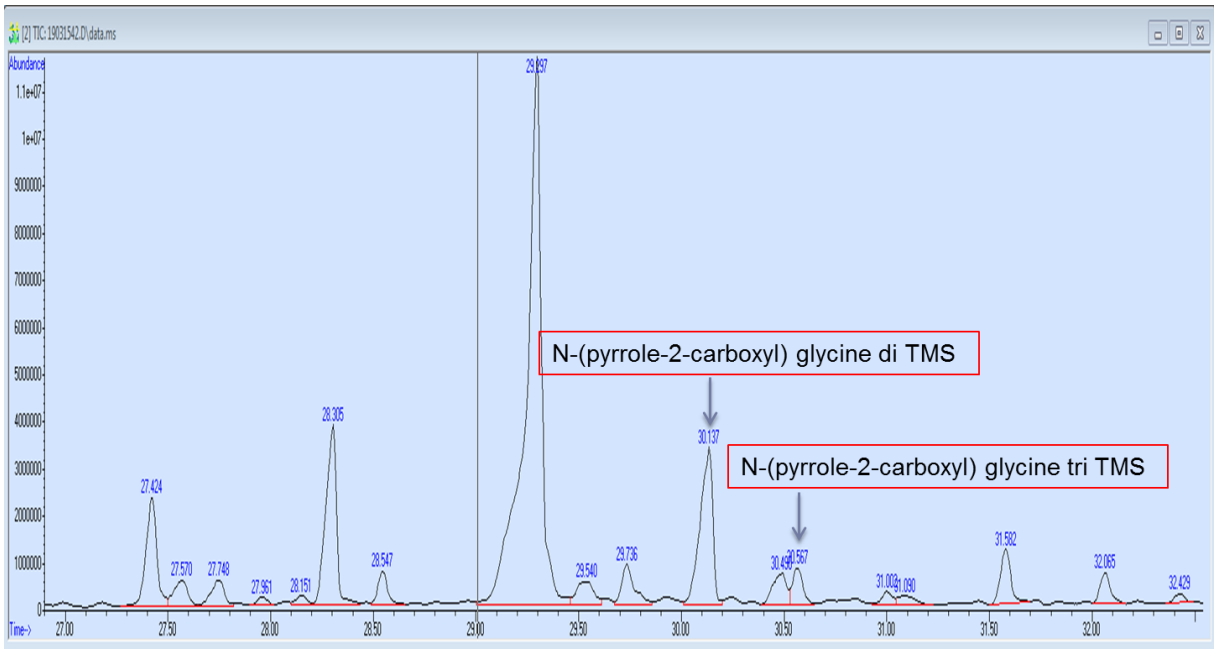
Hyperprolinaemia type II was suspected at 4 years of age because of a high increase of proline in plasma = 2350 µmol/L, with an increase of pyrroline-5-carboxylic acid (evaluated at 6 µmol/L) by LC/MS-MS. Proline was also highly increased in urine = 1059 mmol/mol de creatinine, with an increase of pyrroline-5-carboxylic acid (evaluated at 63 mmol/mol creat by LC/MS-MS). Moreover N-(pyrrole-2-carboxyl) glycine was identified in her urinary organic acid profile.
The diagnosis was confirmed by mutation analysis of *ALDH4A1* gene.

Hyperprolinaemia type II is caused by a deficiency of pyrroline-5-carboxylate dehydrogenase, a mitochondrial inner-membrane enzyme which converts pyrroline-5-carboxylic acid into glutamic acid.

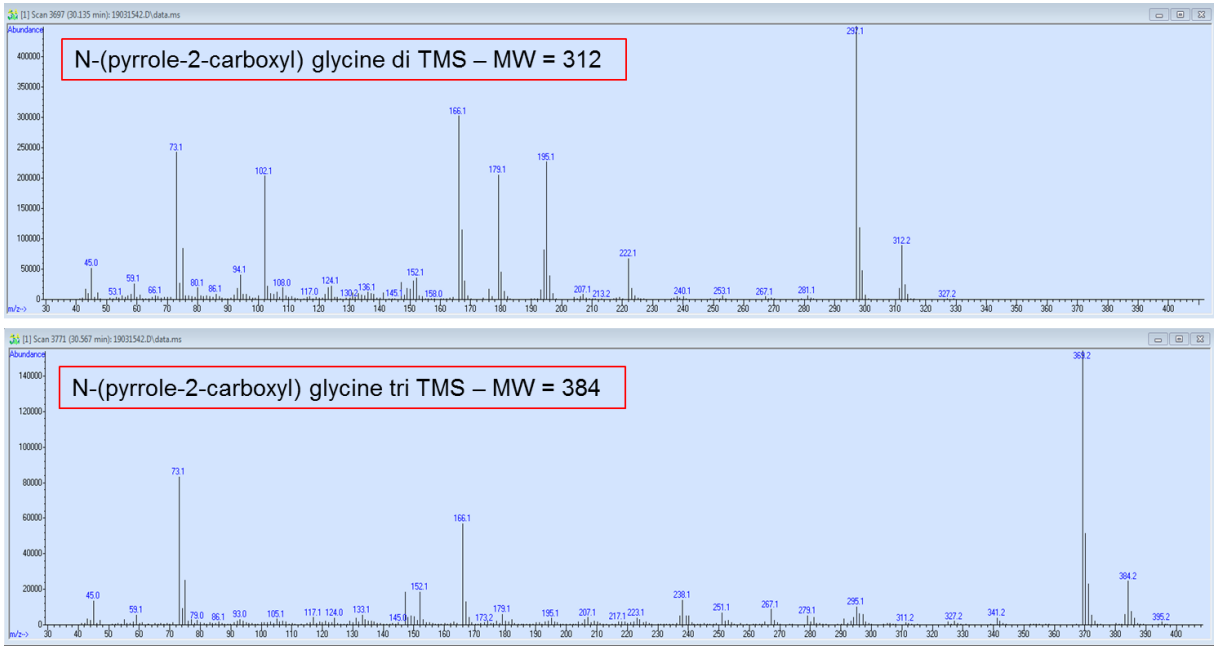


From Inborn Metabolic Diseases, Diagnosis and Treatment, Saudubray, Baumgartner, Walter Eds, Springer

Hyperprolinaemia type II is generally associated with epilepsy and mental retardation, but asymptomatic patients have been described.
Pyrroline-5-carboxylic acid (P5C), which accumulates in this disease, is an antagonist of vitamin B6 (pyridoxine), and seizures can be due in part to B6 inactivation. Seizures are B6 responsive.
Identification of P5C by tandem MS allows differentiating between type II and type I hyperprolinaemia, as well as identification of **N-(pyrrole-2-carboxyl) glycine** in the urinary organic acid profile. Plasma proline levels are higher in hyperprolinaemia type II (usually > 2000 µmol/L) than in type I (usually < 2000 µmol/L).
N-(pyrrole-2-carboxyl) glycine is eluted 1 minute later than hippuric acid.



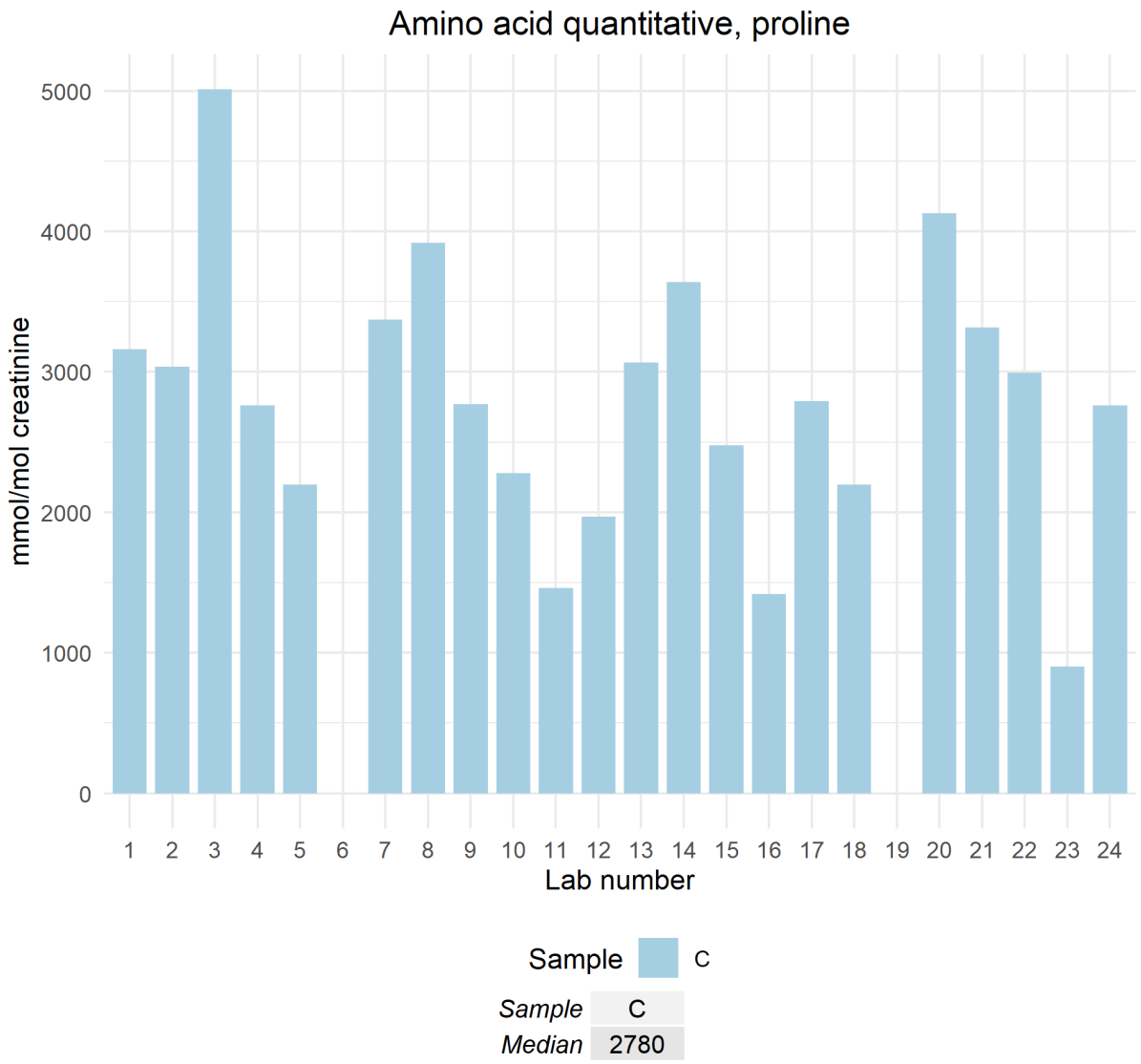
Organic acid profile hyperprolinaemia type II



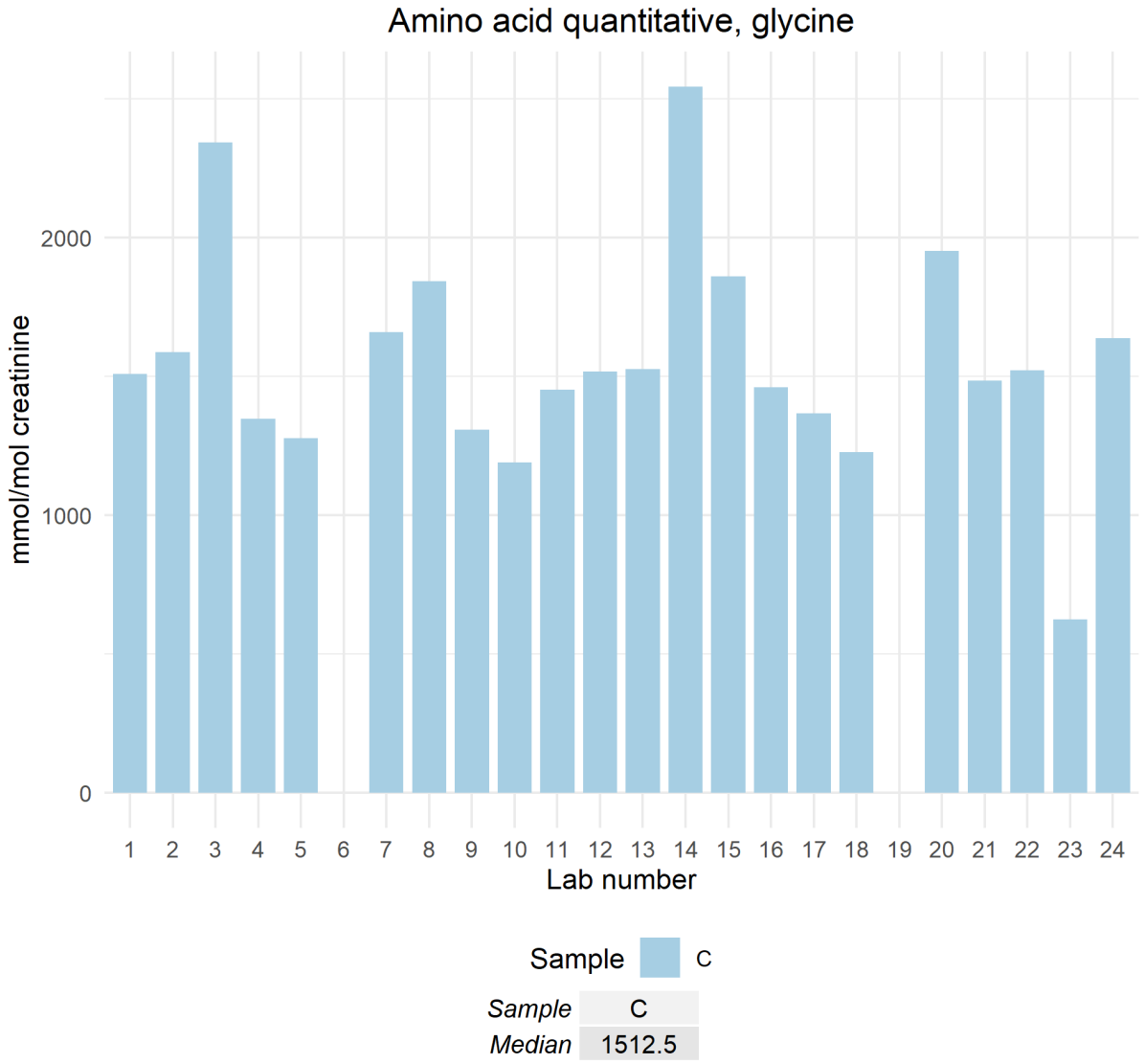
Spectrum of N-(pyrrole-2-carboxyl) glycine di and tri TMS

Analytical performance

Twenty two out of the 24 participants performed amino acid analysis and all of them reported a high increase of proline (median = 2780 mmol/mol creat ; range: 903 – 5012 ; n=22), as well as an increase of glycine (median = 1512 mmol/mol creat ; range: 623 – 2545 ; n=22). Only 12 of the 19 participants who performed organic acids identified N-(pyrrole-2-carboxyl) glycine.



Sample	C
Median	2780



Sample	C
Median	1512.5

Diagnosis / Interpretative proficiency

Most likely diagnosis

Hyperprolinaemia type II	16
Hyperprolinaemia type I	4
Hyperprolinaemia	1
Iminoglycinuria	1
No diagnosis	1
Peroxisomal disorder	1

Alternative diagnosis

Hyperprolinaemia type II	2
Hyperprolinaemia type I	2
Iminoglycinuria	4

Scoring

- Analytical performance: Increase of proline (score 1), increase of N-(pyrrole-2-carboxyl) glycine or of pyrroline-5-carboxylic acid (score 1).

- Interpretation of results: Hyperprolinaemia type II (score 2), recommendation to perform measurement of pyrroline-5-carboxylic acid in plasma or urine (score 1) .

The SAB decided that sample C has to be considered as a critical error for the participants who did not perform amino acid analysis and did not identify N-(pyrrole-2-carboxyl) glycine in the organic acid profile.

Overall impression
The overall proficiency was 77%.

8.1. Patient D

Multiple acyl-CoA dehydrogenase deficiency (MADD) due to ETF deficiency (ETFA gene).

Patient details provided to participants

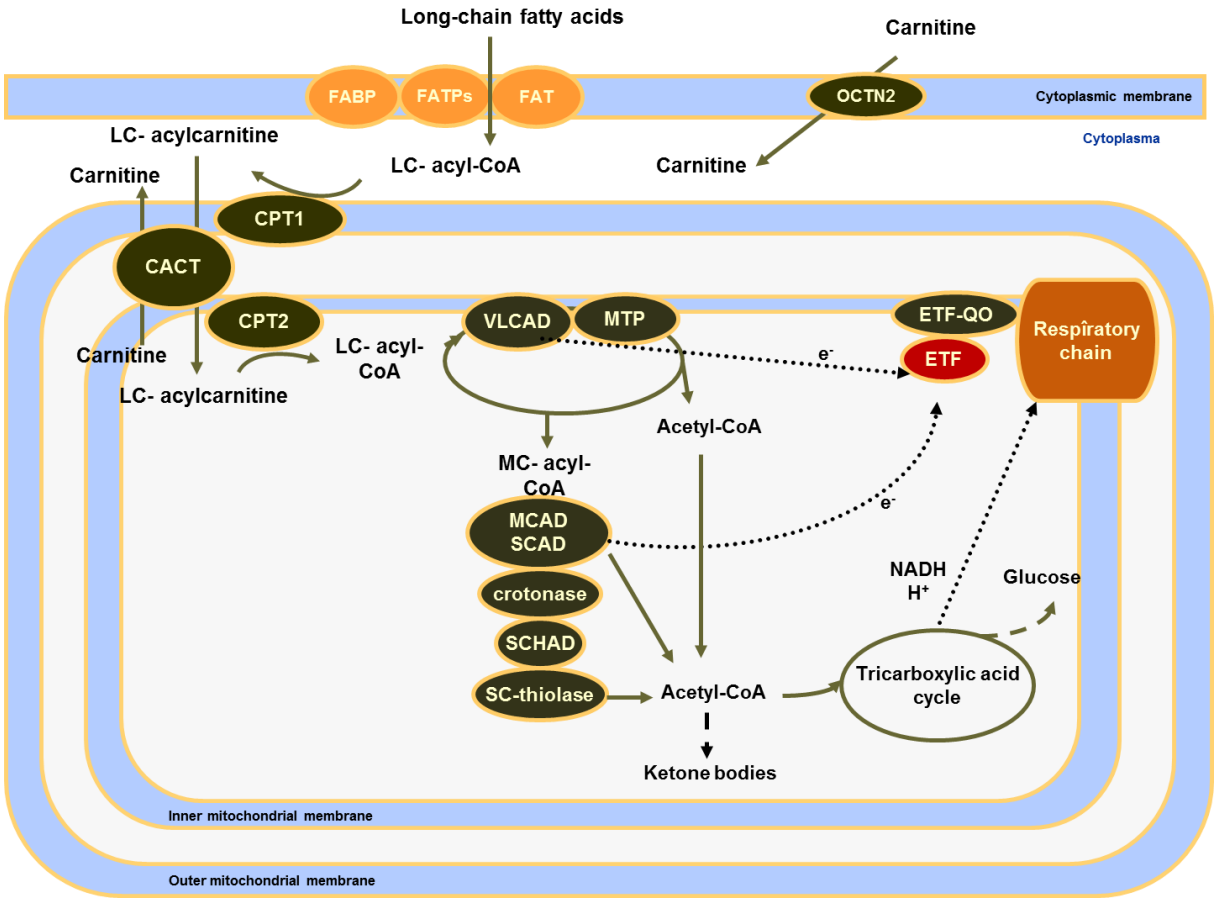
27-year-old boy. His elder brother died in a "Reye-like" syndrome at 9 months of age. He is almost asymptomatic under treatment.

Patient details

The patient's mother was pregnant at the time his brother died. Multiple acyl-CoA dehydrogenase deficiency (MADD) was diagnosed at birth by analysis of plasma acylcarnitines and urinary organic acids. Diagnosis of ETF deficiency was assessed by measurement of ETF activity in fibroblasts and by mutation analysis of *ETFA* gene. He is now 27-year-old and is almost asymptomatic under treatment.

Plasma acylcarnitine profile was strikingly abnormal when the urine sample was collected, with an increase of all chain-length acylcarnitines.

Multiple acyl-CoA dehydrogenase deficiency is due either to a deficiency of electron transfer flavoprotein (ETF – genes *ETFA* and *ETFB*) or to a deficiency of ETF ubiquinone oxidoreductase (EFFQO – gene *ETFDH*), the 2 electron transporters which transfer electrons from ETF dependent dehydrogenases to respiratory chain.



Simplified scheme of mitochondrial fatty acid oxidation

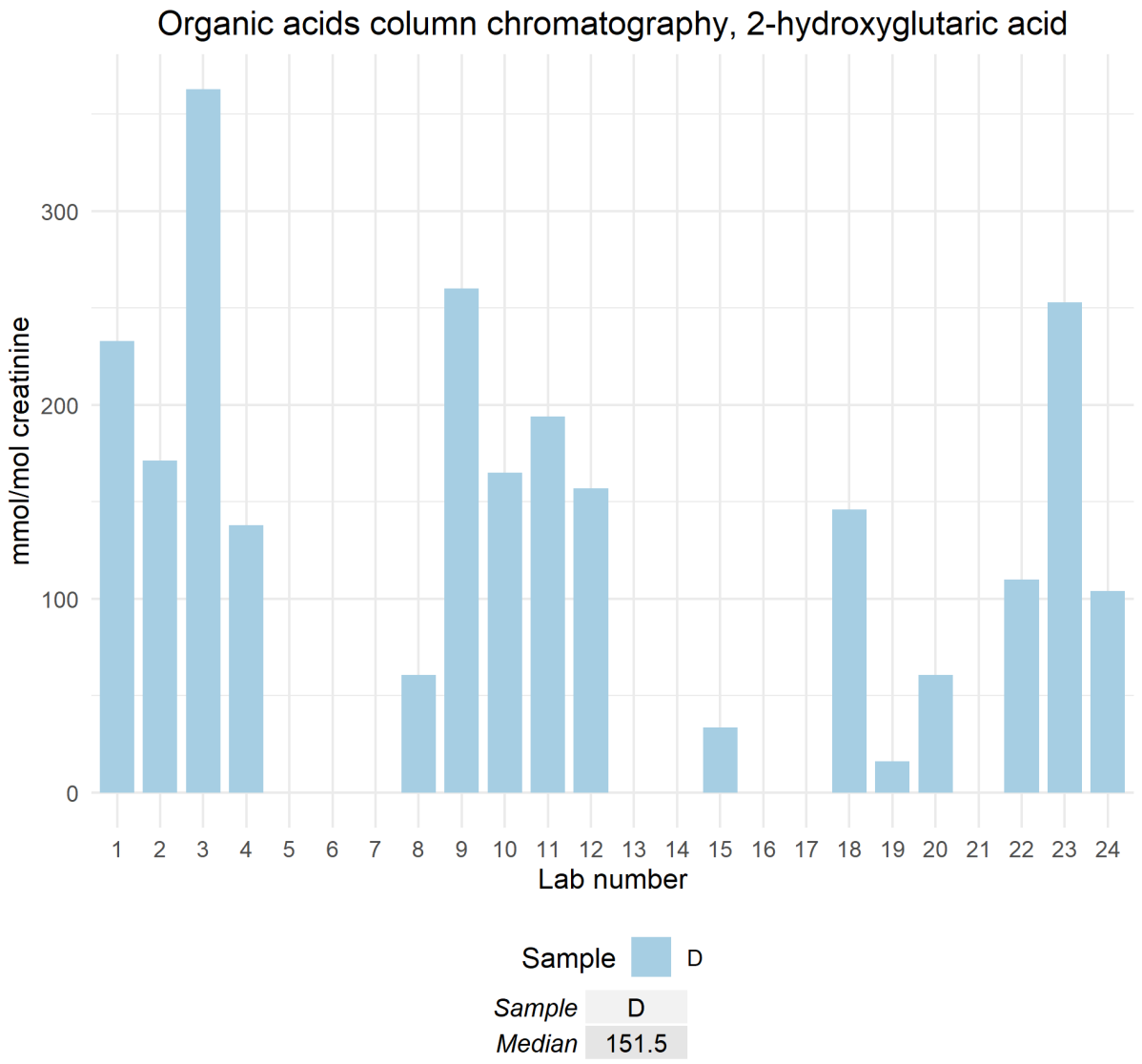
There are 11 ETF dependent dehydrogenases, involved in 5 metabolisms:

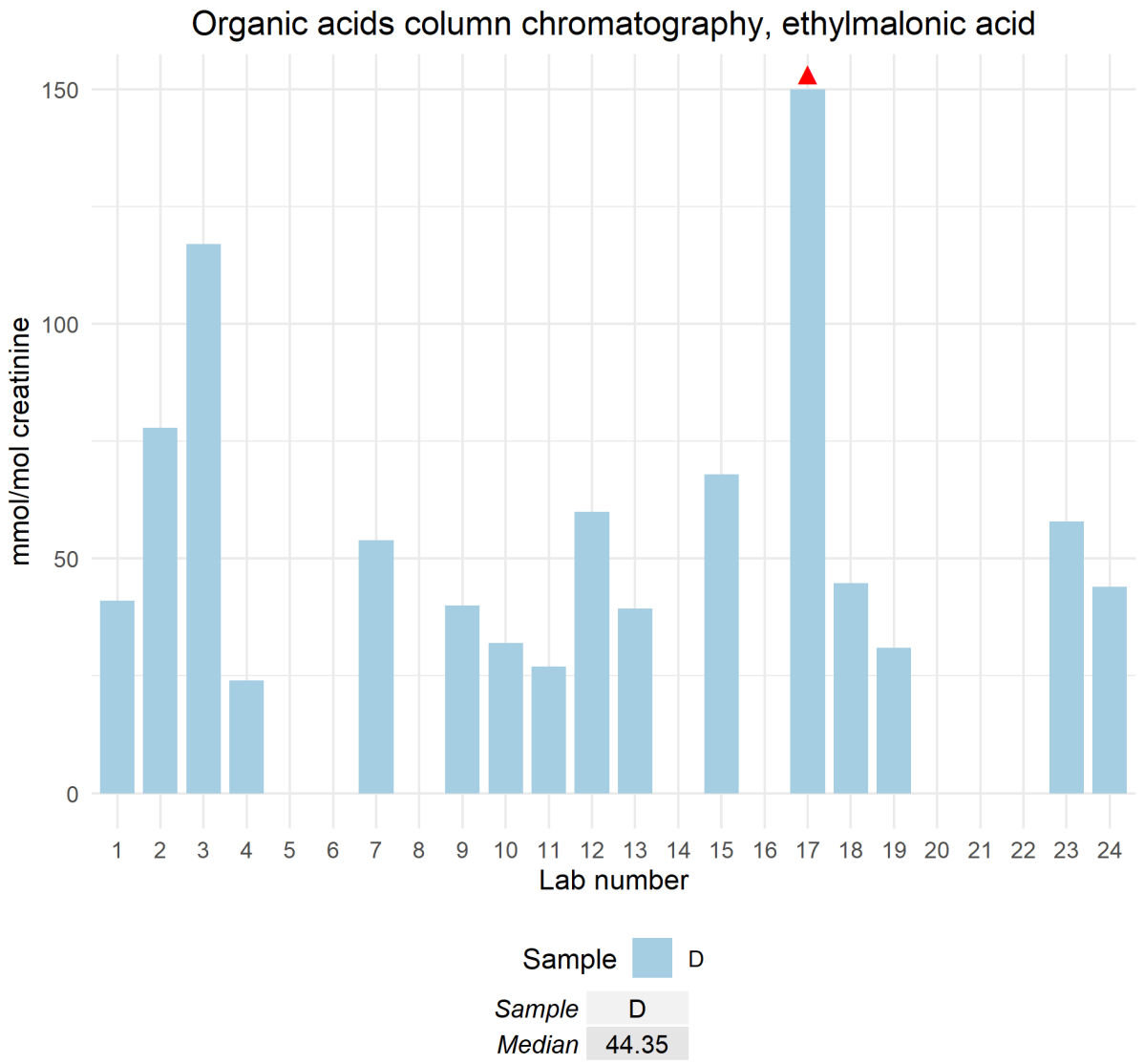
- Fatty acid oxidation: VLCAD, LCAD, MCAD, SCAD
- Branched-chain amino acid metabolism: isovaleryl-CoA dehydrogenase, isobutyryl-CoA dehydrogenase, 2-methylbutyryl-CoA dehydrogenase
- Lysine metabolism: glutaryl-CoA dehydrogenase
- Choline metabolism: sarcosine dehydrogenase, dimethylglycine dehydrogenase
- 2-hydroxyglutarate dehydrogenase

MADD is sometimes called glutaric aciduria type II: this name is **misleading and has to be abandoned.**

Analytical performance
All participants performed organic acids and identified an increase of:

- 2-hydroxyglutaric acid (n=23): median = 151.5 mmol/mol creat ; range: 16 – 363 ; n=16
- Ethylmalonic acid (n=22): median = 44 mmol/mol creat ; range: 24 – 428.7 ; n=16 (one wrong value = 428.7 excluded – error of dilution?)
- Isovalerylglycine (n=14): median = 9 mmol/mol creat ; range: 8 – 15.3 ; n=9
- Glutaric acid (n=10): median = 6.0 mmol/mol creat ; range: 5 – 10.2 ; n=10
- Hexanoylglycine (n=10): median = 1.5 mmol/mol creat ; range: 1 – 2.8 ; n=7
- Isobutyrylglycine (n=3)
- Butyrylglycine (n=3)





Five labs performed acylcarnitines and identified an increase of glutarylcarnitine (C5DC ; n=3), butyrylcarnitine (C4 ; n= 3), isovalerylcarnitine (C5 ; n=2), octanoylcarnitine (C8 ; n=2), hexanoylcarnitine (C6 ; n=1), and hexanedioylcarnitine (C6DC ; n=1) .

Using tandem mass spectrometry for amino acid analysis, the scientific advisors identified an increase of **dimethylglycine** (= 136 mmol/mol creat - controls <10), which is frequent in MADD, but not specific. Conversely, sarcosine excretion was normal (= 2 mmol/mol creat – controls <5).

Diagnosis / Interpretative proficiency

Most likely diagnosis

MADD	21
2-hydroxyglutaric aciduria	2
SCAD deficiency	1

Alternative diagnosis

Riboflavin transport and metabolism defect	8
2-hydroxyglutaric aciduria	1
Ethylmalonic encephalopathy	1
Glutaric aciduria type I	1
Isocitrate dehydrogenase deficiency	1

Scoring

- Analytical performance: Increase of ethylmalonic acid and/or 2-hydroxyglutaric acid (score 1), increase of at least one acylglycine derivative (score 1).

- Interpretation of results: MADD with recommendation to perform plasma or DBS acylcarnitines (score 2), MADD without recommendation to perform plasma or DBS acylcarnitines (score 1).

Overall impression

The overall proficiency was 91%.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2005 and 2009: the overall performance is lower but probably because of the scoring of recommendation.

	2005	2009	2019
Analytical performance	95 %	100 %	94 %
Interpretative performance	100 %	98 %	88 %
Overall performance	97 %	99 %	91 %

8.1. Patient E

Mucopolysaccharidosis type II (MPSII) due to iduronate-2-sulphatase deficency (IDS gene).

Patient details provided to participants
Male investigated at 15 months of age with hepatosplenomegaly and noisy breathing

Patient details
The diagnosis of mucopolysaccharidosis type II (MPS II) was confirmed by measurement of iduronate-2-sulphatase activity and by mutation analysis of *IDS* gene. The urine sample has been collected at diagnosis.

MPS II (Hunter syndrome) is inherited as an X-linked recessive trait. Symptomatic females are extremely rare. The clinical presentation is the same as MPS I (Hurler syndrome), except that there is no corneal clouding. It associates upper airway obstruction, short stature, hepatosplenomegaly, facial dysmorphism, cardiac disease, and progressive learning difficulties.
The biological diagnosis relies on urinary glycosaminoglycans (GAGs) quantification and fractionation, measurement of iduronate-2-sulphatase activity in leucocytes or fibroblasts, and mutation analysis of *IDS* gene.

Analytical performance
GAGs fractionation was performed by 20 participants out of 23, who all reported an abnormal profile: increase of dermatan and heparan sulphate (n=17), increase of dermatan sulphate (n=2), not specified (n=1).
Nineteen out of the 20 labs who performed GAGs quantification reported a high result, and GAGs screening was positive for 2 labs.

Diagnosis / Interpretative proficiency

Most likely diagnosis

MPS II	6
MPS I or II	7
MPS I	7
MPS I, II or VI	1
MPS VI	1
MPS	1

Alternative diagnosis

MPS II	5
MPS I	4
MPS VII	2
MPS II, VI or VII	1
MPS I or VII	1

Scoring
- Analytical performance: Increase of dermatan +/- heparan sulphate (score 2), increase of GAGs quantification without GAGs fractionation (score 1).

- Interpretation of results: MPS II (score 2), wrong mucopolysaccharidosis or no specification of the MPS type (score 1).

Overall impression
The overall proficiency is 95% which is quite satisfying for a LSD.

Multiple distributions of similar samples
A similar urine sample has been distributed in 2006: the overall performance is better.

	2006	2019
Analytical performance	86 %	96 %
Interpretative performance	89 %	93 %
Overall performance	88 %	95 %

8.1. Patient F

Argininemia due to arginase deficiency (ARG1 gene).

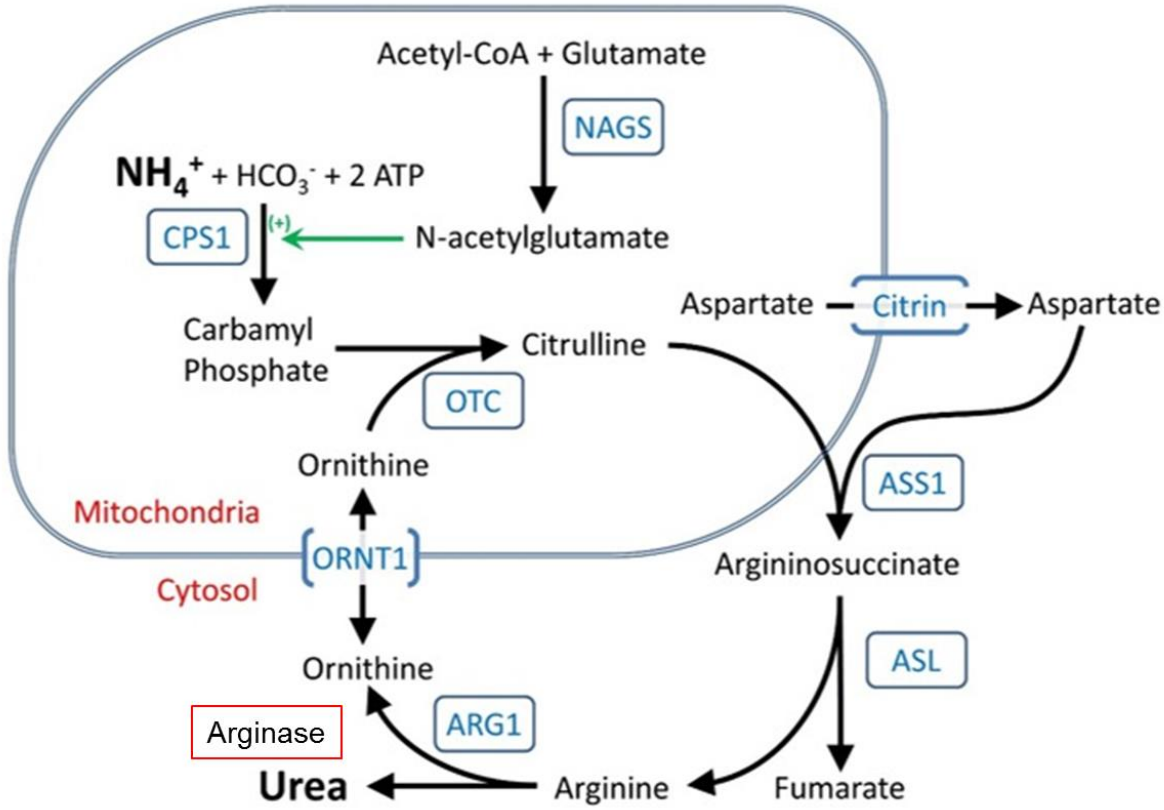
Patient details provided to participants

Male who presented at 2 years an episode of lethargy in the course of gastroenteritis. At 16 years of age, he has mental retardation and spastic diplegia.

Patient details

This 17-year-old patient presented at 2 years of age an episode of lethargy in the course of gastroenteritis, with hyperammonaemia. Argininaemia was diagnosed at that time. He received a restricted protein diet and sodium benzoate treatment. He is now 17-year-old, and present with mental retardation and spastic diplegia.

Plasma arginine level was 568 µmol/L when the urine sample was collected.



Urea cycle metabolism

GeneReviews® Internet

Argininaemia is due to arginase deficiency, the last step of ureagenesis. Patients with argininaemia rarely present in the newborn period with hyperammonaemia and/or cholestasis. In children, adolescents and adults, the symptoms are developmental delay, with neurological and intellectual impairment, growth retardation and spastic tetra- or diplegia. Many patients have seizures and may even develop *status epilepticus*.

Hyperammonaemia, hyperornithinaemia, homocitrullinuria (HHH) syndrome has the same clinical presentation.

The mild and sporadic hyperammonaemia present in argininaemia does not account for the spastic diplegia and the seizures. Arginine and its metabolites (polyamines, guanidino compounds, NO, agmatine) are probably toxic compounds for the central nervous system.

The diagnostic tests involve plasma amino acids analysis (Arg > 500 µmol/L), increase of urinary orotic acid, measurement of arginase activity in erythrocytes, and sequencing of *ARG1* gene.

Analytical performance

All participants but one identified an increase of orotic acid using either organic acid profiling (12/19: median 9.0 mml/mol creat – range: 7.0 – 13.0), purine & pyrimidine analysis (5/5: median 7.4 mml/mol

creat – range: 6.4 – 21.8), or specific measurement of orotic acid (13/13: median 8.5 mml/mol creat – range: 6.6 – 17.2).

All participants but one performed amino acid analysis. The median urinary arginine concentration was in the range of controls (median 5.0 mml/mol creat – range: 4 – 59 ; n = 7).

Diagnosis / Interpretative proficiency

Most likely diagnosis

Argininaemia	7
OCT deficiency	8
HHH syndrome	4
Urea cycle disorder	2
No IEM	1
No diagnosis	1

Alternative diagnosis

Argininaemia	4
OCT deficiency	3
HHH syndrome	2
Urea cycle disorder	2
Citrullinaemia type I	1
Orotic aciduria	1
MPS III	1
Serine deficiency, Lesh-Nyhan, SCAD deficiency	1

Scoring

- Analytical performance: Increase of orotic acid (score 2).

- Interpretation of results: Argininaemia (score 2), urea cycle with the recommendation to perform plasma amino acids (score 2), urea cycle without the recommendation to perform plasma amino acids (score 1).

The SAB decided that sample F has to be considered as a critical error for the participants who did not identify an increase of orotic acid and did not recommend performing plasma amino acid analysis.

Overall impression

The overall proficiency was 87%.

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 in the leftmost column.

Detailed scores – **Round 1**

Lab n°	Patient A APRT deficiency			Patient B Beta-mannosidosis			Patient C Hyperprolinaemia type II			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	--	--	--	1	1	2	6
2	2	2	4	--	--	--	2	2	4	8
3	1	2	3	--	--	--	2	2	4	7
4	0	1	1	--	--	--	1	1	2	3
5	0	0	0	--	--	--	1	2	3	3
6	2	2	4	--	--	--	0	0	0	4
7	0	1	1	--	--	--	1	2	3	4
8	0	0	0	--	--	--	1	2	3	3
9	0	0	0	--	--	--	2	2	4	4
10	2	2	4	--	--	--	2	2	4	8
11	2	2	4	--	--	--	2	2	4	8
12	2	2	4	--	--	--	2	2	4	8
13	0	0	0	--	--	--	2	2	4	4
14	2	0	2	--	--	--	2	2	4	6
15	0	1	1	--	--	--	1	2	3	4
16	0	1	1	--	--	--	1	2	3	4
17	0	0	0	--	--	--	2	2	4	4
18	0	0	0	--	--	--	2	2	4	4
19	1	2	3	--	--	--	0	0	0	3
20	0	0	0	--	--	--	1	2	3	3
21	0	1	1	--	--	--	1	2	3	4
22	0	1	1	--	--	--	1	0	1	2
23	2	2	4	--	--	--	2	2	4	8
24	2	2	4	--	--	--	2	2	4	8

Detailed scores – Round 2

Lab n°	Patient D MADD			Patient E MPSII			Patient F Argininemia			
	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	1	1	2	2	2	4	10
5	1	0	1	2	2	4	0	0	0	5
6	2	2	4	2	2	4	0	2	2	10
7	2	2	4	2	2	4	2	2	4	12
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	0	2	2	2	4	2	2	4	10
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	1	2	3	11
15	2	2	4	1	1	2	2	2	4	10
16	2	2	4	2	1	3	2	2	4	11
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	1	2	3	2	2	4	0	2	2	9
22	1	0	1	--	--	--	--	--	--	1
23	2	2	4	2	2	4	0	1	1	9
24	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	--	2	4	4	4	18	90	
2	4	--	4	4	4	4	20	100	
3	3	--	4	4	4	4	19	95	
4	1	--	2	4	2	4	13	65	
5	0	--	3	1	4	0	8	40	CE
6	4	--	0	4	4	2	14	70	CE
7	1	--	3	4	4	4	16	80	
8	0	--	3	4	4	4	15	75	
9	0	--	4	4	4	4	16	80	
10	4	--	4	4	4	4	20	100	
11	4	--	4	2	4	4	18	90	
12	4	--	4	4	4	4	20	100	
13	0	--	4	4	4	4	16	80	
14	2	--	4	4	4	3	17	85	
15	1	--	3	4	2	4	14	70	
16	1	--	3	4	3	4	15	75	
17	0	--	4	4	4	4	16	80	
18	0	--	4	4	4	4	16	80	
19	3	--	0	4	4	4	15	75	CE
20	0	--	3	4	4	4	15	75	
21	1	--	3	3	4	2	13	65	
22	1	--	1	1	--	--	3	15	
23	4	--	4	4	4	1	17	85	
24	4	--	4	4	4	4	20	100	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 60 % of adequate responses)	20	83
Unsatisfactory performers (< 60 % adequate responses and/or critical error)	4	17
Partial and non-submitters	1	4

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-FL-2019-A	APRT deficiency	42	54	48
DPT-FL-2019-B	Beta-mannosidosis	--	--	--
DPT-FL-2019-C	Hyperprolinaemia type II	71	83	77
DPT-FL-2019-D	MADD	94	88	91
DPT-FL-2019-E	MPSII	96	93	95
DPT-FL-2019-F	Argininemia	80	93	87

10. Annual meeting of participants

It took place in Rotterdam on September 3, 2019 from 9.00 to 10.30, before the SSIEM Meeting.

Participants

Representatives from 12 labs were present: JA Arranz (Barcelona), A Ribes (Barcelona), MA Donati, S Funghini (Florence), O Boulat, O Braissant, C Roux (Lausanne), P Alcaide Alonso, P Ruiz-Sala (Madrid), M Gastaldi (Marseille), A Imbard (Paris, Robert Debré), G Polo (Padova), D Quelhas (Porto), S Bekri (Rouen), C Colón (Santiago de Compostella), C Rizzo (Roma).

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 material. 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
- An educational panel for **oligosaccharides** is available from www.erndim.nl "Educational panels". β-mannosidosis is included in this kit. An educational panel for purines & pyrimidines is under preparation.
- **Training**: SSIEM Academy training courses.
  - A 2 days course will be been organized on Monday and Tuesday 20 and 21 April 2020 in Amsterdam airport. The program for biochemists includes:
    - Hyperammonaemia and urea cycle disorders
    - Aminoacidopathies
    - Hyperhomocystinaemias & remethylation defects
  - 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 we already sent.

  As soon as possible after collection, the urine sample must be heated at 50 °C for 20 minutes. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. Then aliquot the sample in 10 ml plastic tubes (minimum 48 tubes), add stoppers and freeze. Be careful to constantly homogenize the urine while aliquoting the sample. Send 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 91 - Fax 33 4 72 12 97 20
  e-mail: christine.vianeysaban@gmail.com
  cecile.acquaviva-bourdain@chu-lyon.fr

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

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 2020

Sample distribution	February 11, 2020
Start of analysis of Survey 2019/1 Website open	March 9
Survey 2019/1 - Results submission	March 30

Survey 2019/1 - Reports	April
Start of analysis of Survey 2019/2	June 8
Survey 2019/2 - Results submission	June 29
Survey 2019/2 - Reports	July
Annual meeting of participants	September 1, SSIEM Freiburg
Annual Report 2020	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, 2020-03-02
Name and signature of Scientific Advisor

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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
• 2018:1	A	DPD deficiency (common sample)
	B	MPS VII
	C	SCHAD deficiency
• 2018:2	D	Glutaric aciduria type I (low excretor)
	E	OAT deficiency
	F	Dihydropyrimidine dehydrogenase (DPD) deficiency