ERNDiM

Quality Assurance in Laboratory Testing for IEM

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Diagnostic Proficiency Testing

Centre: France

Final Report 2021

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.

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 ERNDIM Privacy Policy on www.erndim.org.

In 2021, 21 labs participated to the Proficiency Testing Scheme France.

1. Geographical distribution of participants

For the first survey, 20 laboratories submitted results and 20 for the second survey.

Country	Number of participants	Country	Number of participants
Czechia	1	Portugal	2
France	8	Spain	5
Italia	5		

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 CSCQ as scheme organizer (sub-contractor on behalf of ERNDIM), 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 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

[1] If this report is not Version 1 for this scheme year, go to APPENDIX 1 for details of the changes made since the last version of this document.

Origin of patients: urine samples have been provided by the Scientific Advisors, by George Ruijter (Rotterdam), and Marie-Hélène Read (Caen).

Patient A: Alpha-mannosidosis – This sample has been sent to all labs participating to the DPT scheme in Europe
Patient B: Alpha-mannosidosis
Patient C: MAT deficiency
Patient D: CBS deficiency
Patient E: 4-hydroxybutyric aciduria
Patient F: Hyperprolinaemia type II

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 purines / pyrimidines are mandatory in 2021.

4. Schedule of the scheme

- February 9, 2021: Shipment of samples of Survey 1 and Survey 2
- March 8, 2021: Clinical data available on CSCQ website and start analysis of samples A, B, C (Survey 1)
- March 22, 2021: Reminder for website submission (Survey 1)
- March 29, 2021: Deadline for result submission (Survey 1)
- May 5, 2021: Interim report of Survey 1 available on CSCQ website
- June 7, 2021: Clinical data available on CSCQ website and start analysis of samples D, E, F (Survey 2)
- June 21, 2021: Reminder for website submission (Survey 2)
- June 28, 2021: Deadline for result submission (Survey 2)
- August 6, 2021: Interim report of Survey 2 available on CSCQ website
- September 6, 2021: Meeting of participants by teleconference
- November 25, 2021: SAB meeting; definition of critical errors
- December 20, 2021: Annual Report with definitive scoring

5. Results

Twenty of 21 participants returned results for both surveys, by the deadline.

	Survey 1	**Survey 2**
Receipt of results	21	20
No answer	0	1

6. Web site reporting

The website reporting system is compulsory for all centres. Please carefully read 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 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 advisors. The results of DPT France 2021 have been also scored by Joanne Croft, the Scientific Advisor of DPT UK. At the SAB meeting on November 25, 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 2021, the SAB decided that sample A has to be considered as a critical error for the labs who did not perform oligosaccharides and did not recommend performing this assay. The failure to detect an increase of proline in sample F, while all other participants detected it, has also been considered by the SAB as a critical error.

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 Scheme Advisor for 2021 for poor performance by score and critical error. Partial- or non- submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

On November 25, the SAB also decided that **the score for satisfactory performance will be increased from 15 points to 17 points from the maximum of 24 (70%) in 2022**, in accordance with the other qualitative schemes.

7.1. Score for satisfactory performance

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

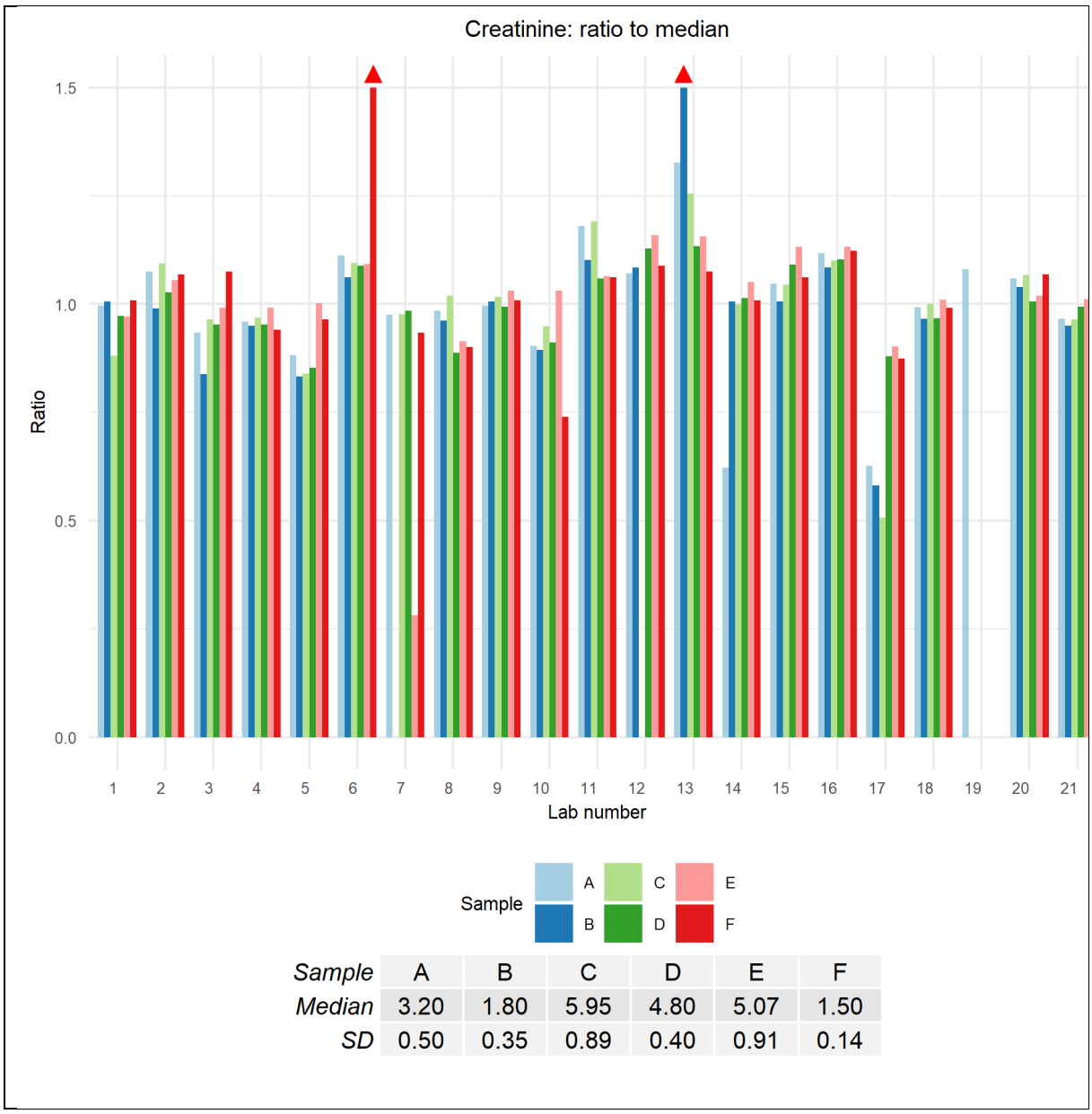
If your laboratory is assigned poor performance and you wish to appeal against this classification please email the ERNDIM Administration Office (admin@erndim.org), with full details of the reason for your appeal, within one month receiving your Performance Support Letter. Details of how to appeal poor performance are included in the Performance Support Letter sent to poor performing laboratories.

8. Results of samples and evaluation of reporting

8.1. Creatinine measurement for all samples

After exclusion of some wrong values, the CV for creatinine determination ranged from 7.1% (sample E) to 18.9% (sample B); this is higher than the interlab CV 2020 for Special Assays in Urine (5.0%, n = 127).
Creatinine values are expressed in the figure as the ratio of each measurement over the median for all labs.

Creatinine: ratio to median



Sample	A	B	C	D	E	F
Median	3.20	1.80	5.95	4.80	5.07	1.50
SD	0.50	0.35	0.89	0.40	0.91	0.14

8.2. Patient A

Alpha-mannosidosis due to alpha-mannosidase deficiency (MAN2B1 gene).

Patient details provided to participants

A 36 year old male with craniosynostosis, dysmorphic facial features, retardation and deafness.

Patient details

Activity of alpha-mannosidase in this 36-old patient was 0 mU/ml in plasma and 0 mU/mg in leucocytes. Activity in DBS was 4.72 pmol/punch/h (5% residual activity). Unfortunately, no information is available on age at diagnosis and mutations.
This is the common sample distributed to all participants of the 5 DPT schemes. Results from all centers has been presented at the ERNDIM virtual meeting on October 21st.

Analytical performance

Seventeen out of the 20 labs who submitted results performed **oligosaccharides**, and all of them reported an abnormal profile consistent with alpha-mannosidosis.
Twelve labs performed mucopolysaccharides quantification: 10 reported a normal result, and 2 an increase of GAGs. Among the 8 participants who performed mucopolysaccharides fractionation, 7 reported a normal profile, whereas one reported an increase of chondroitin and keratan sulphate.

Diagnosis / Interpretative proficiency

Most likely diagnosis	
Alpha-mannosidosis	17
No diagnosis	3
Alternative diagnosis	
Other oligosaccharidosis	1
Morquio IVA	1

Recommendations

The participants who did not perform oligosaccharides but recommended to perform this assay were scored one mark.

Scoring

- Analytical performance
  - Abnormal oligosaccharide profile consistent with alpha-mannosidosis (score 2)
- Interpretation of results
  - Alpha-mannosidosis as first or alternative diagnosis (score 2)
  - Recommendation to perform oligosaccharide analysis (score 1)

Overall impression

The overall proficiency was excellent for a lysosomal storage disease.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2008: the overall performance has greatly improved.

	2008	2021
Analytical performance	78 %	85 %
Interpretative performance	68 %	90 %
Overall performance	74 %*	87 %

(*recommendations were scored separately in 2008)

8.3. Patient B

Alpha-mannosidosis due to alpha-mannosidase deficiency (MAN2B1 gene).

Patient details provided to participants

3.5-year-old boy with psychomotor retardation, hearing impairment and immune deficiency, requiring immunoglobulin replacement therapy.

Patient details

This 3.5-year-old boy was born after a normal pregnancy and a normal delivery. At 11 months of age, a immune deficiency was identified, requiring immunoglobulin replacement therapy. At 3 years of age, psychomotor retardation, and hearing impairment were noticed.
He was investigated at 3.5 years of age: exome sequencing identified pathogenic variants in the *MAN2B* gene. The diagnosis of alpha-mannosidosis was confirmed by oligosaccharide analysis, and by measurement of alpha-mannosidase activity in leucocytes, which was severely decreased.
This was a difficult sample, because the clinical presentation was atypical, dominated by the immune deficiency, and, by bad luck, two similar samples have been distributed in the same survey: some participants were confused because of this.

Analytical performance

Only 14 participants out of 20 performed **oligosaccharides**, whereas 17 of them performed this assay for sample A. All but one concluded to an abnormal profile consistent with alpha-mannosidosis. The last one concluded to a normal profile.

Eleven participants performed mucopolysaccharides quantification: 9 reported a normal result and 2 an increase of GAGs. Nine labs performed mucopolysaccharides fractionation: 7 reported a normal profile, 1 an elevated chondroitin/dermatan sulphate ratio, and 1 an increase of keratan sulphate.

Diagnosis / Interpretative proficiency

The numerous possible diagnoses reflected the confusion of participants.

Most likely diagnosis

Alpha-mannosidosis	14
Beta-mannosidosis	1
GM1 gangliosidosis	1
Lysosomal storage disorder	1
No diagnosis	3

Alternative diagnosis

Alpha-mannosidosis	1
Morquio IVB	1
Adenylate kinase 2 deficiency?	1
PNP deficiency	1
Peroxisomal disorder, riboflavin transporter def., RC defect	1
Other IEM associated with immunodeficiency like CDG	1
Increase of pyroglutamic acid	1
Biotinidase deficiency	1
ADA deficiency under ERT?	1

Recommendations

The participants who did not perform oligosaccharides but recommended to perform this assay were scored one mark.

Scoring

The same scoring than for sample A was applied

- Analytical performance
  - Abnormal oligosaccharide profile consistent with alpha-mannosidosis (score 2)
- Interpretation of results
  - Alpha-mannosidosis as first or alternative diagnosis (score 2)
  - A lysosomal storage disease suspected on clinical information or recommendation to perform oligosaccharide analysis (score 1)

Overall impression

The overall proficiency was lower than for sample A.

Multiple distributions of similar samples

The comparison was done with sample A.

	2021 A	2021 B
Analytical performance	85 %	65 %
Interpretative performance	90 %	75 %
Overall performance	87 %	70 %

8.4. Patient C

2-methylacetoacetyl-CoA thiolase (MAT) deficiency, also called mitochondrial acetoacetyl-CoA thiolase or beta-ketothiolase or 3-oxothiolase deficiency (ACAT1 gene).

Patient details provided to participants

First child of non-consanguineous parents. He presented, during the first week of life, vomiting, tachypnea, metabolic acidosis with ketonuria, but no hypoglycaemia, and no hyperammonaemia.

Patient details

Diagnosis has been suspected on organic acid analysis, performed on the first week of age, and confirmed by measurement of enzyme activity in cultured skin fibroblasts (with and without addition of KCl to differentiate the mitochondrial isoform from the cytoplasmic one).
The urine sample has been collected at 12 years of age. He has a normal psychomotor development. His treatment consists in a low protein and a low lipid diet, and L-carnitine supplementation.

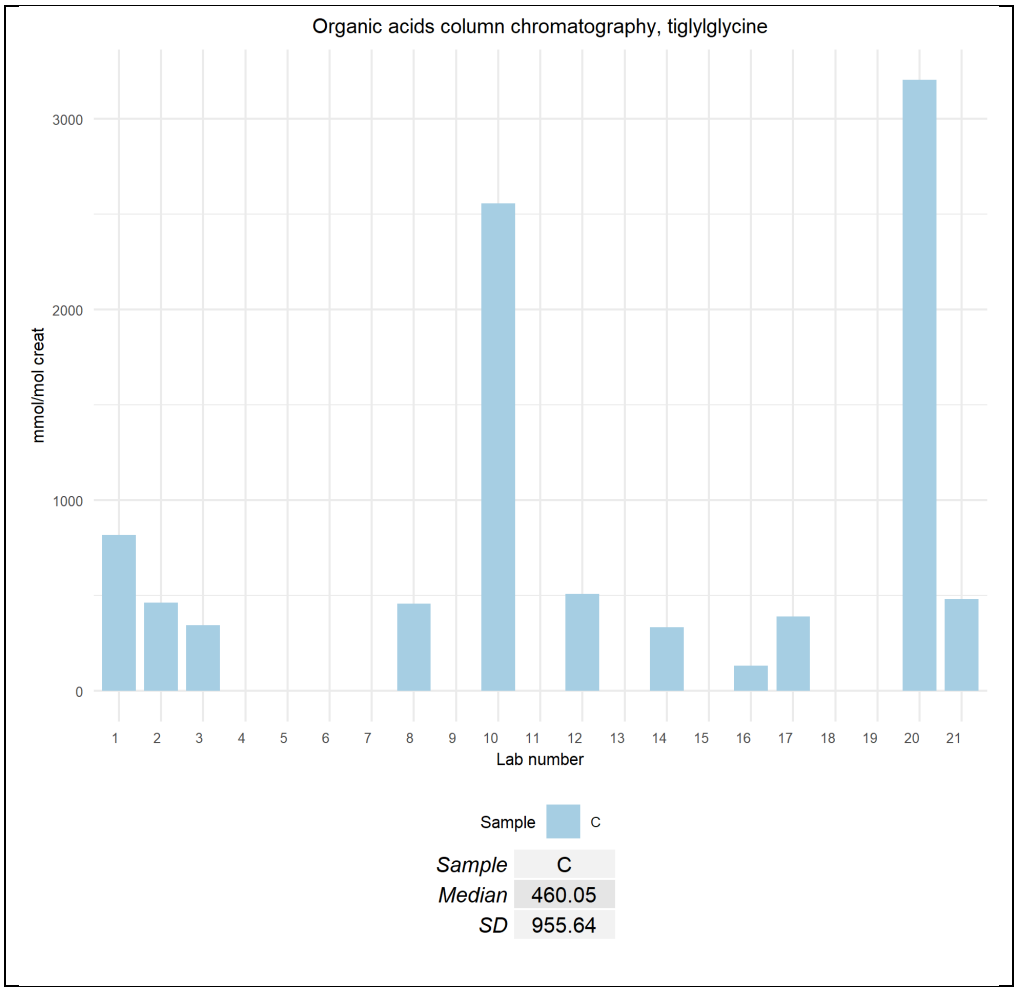
Analytical performance

All participants performed **organic acid** analysis. They reported:
An increase of:

- **Tiglylglycine** 20
  (median = 460 mmol/mol creatinine; range : 2 – 3205; n = 12)
- **2-methyl-3-hydroxybutyric acid** 19
  (median = 384 mmol/mol creatinine; range : 105 – 899; n = 10)
- 2-methylacetoacetic acid 2
  (2 mmol/mol creatinine)
- 2-methylglutaconic acid 2

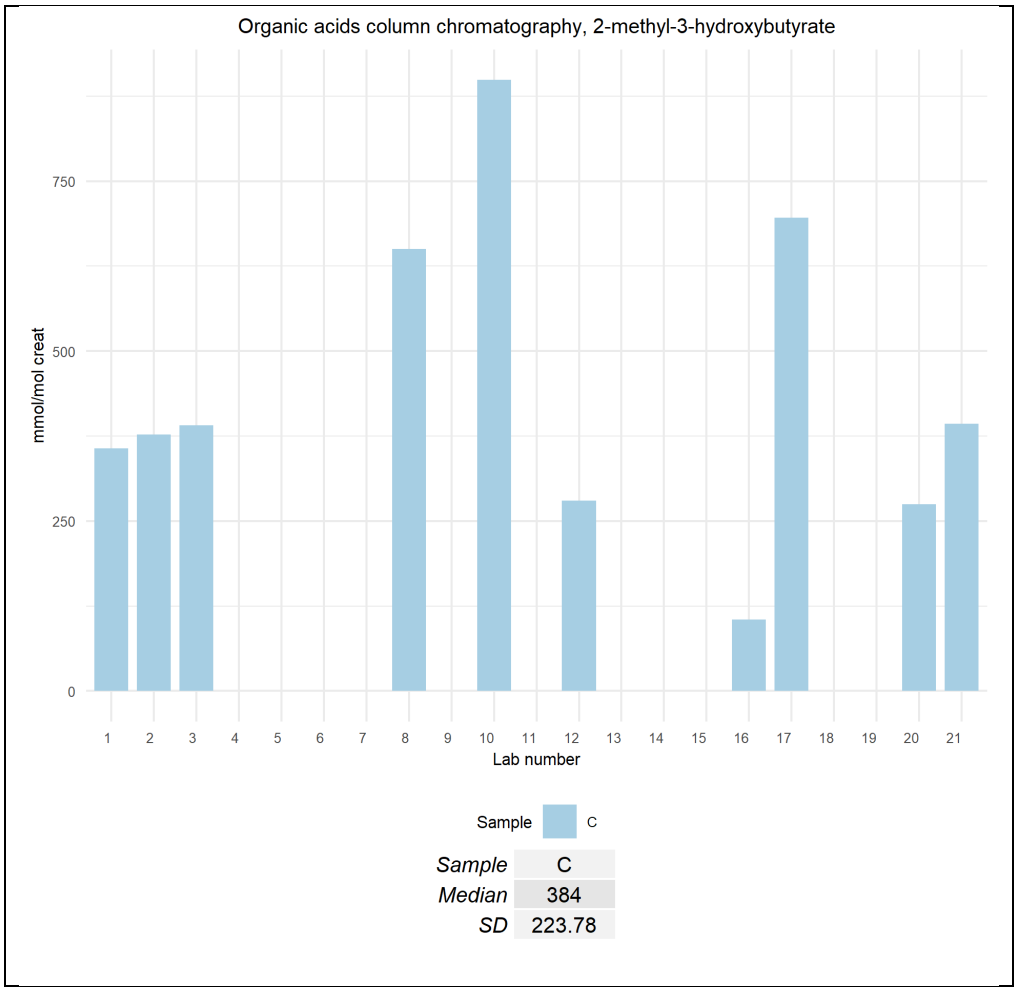
No increase of:

- 2-methylacetoacetic acid 9
- 3-hydroxybutyric acid 3



Sample	C
Median	460.05
SD	955.64

Although tiglyglycine belongs to the quantitative organic acid scheme, there was a wide range of values for this compound. Conversely, values for 2-methy-3-hydroxybutyric acid were less dispersed.



The organic acid profile performed by the Scientific Advisors did not allow to identify an increase of 2-methylacetoacetic acid: this is possibly due to the degradation of this organic acid by heat treatment or to a poor storage of the sample.

Four labs performed a urinary acylcarnitine profile. They reported:

- Increase of **tiglylcarnitine** (C5:1) 4
  (23.5 ; 33.4 mmol/mol create)
- Increase of **2-methyl-3-hydroxybutyrylcarnitine** (C5OH) 4
  (4.83 ; 14.75 mmol/mol create)
- Peak of m/z 316 (butylated derivatives) 1

The 12 participants who performed amino acids reported no significant abnormality.

Diagnosis / Interpretative proficiency

Most likely diagnosis

- Mitochondrial acetoacetyl-CoA thiolase (MAT) deficiency 15
  (3-oxothiolase, beta-ketothiolase, 3-ketothiolase, 2-methylacetoacetyl-CoA thiolase)
- 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency 5

Alternative diagnosis

- Mitochondrial acetoacetyl-CoA thiolase (MAT) deficiency 2
- 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency 9

Scoring

- Analytical performance
  - Increase of tiglylglycine (score 1)
  - Increase of 2-methyl-3-hydroxybutyric acid or tiglylcarnitine or 2-methyl-3-hydroxybutyrylcarnitine (score 1)
- Interpretation of results

- Mitochondrial acetoacetyl-CoA thiolase as first or alternative diagnosis (score 2)
- 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency as only diagnosis (score 1)

Overall impression

The overall proficiency was excellent: 96%. Some labs did not conclude to MAT deficiency because of the absence of 2-methylacetoacetic acid, but the clinical information was in agreement with this diagnosis.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2013: the overall performance has greatly improved.

	2013	2021
Analytical performance	78 %	100 %
Interpretative performance	87 %	92 %
Overall performance	83 %	96 %

8.5. Patient D

Cystathionine beta-synthase (CBS) deficiency (CBS gene)

Patient details provided to participants

34-year-old woman, with normal psychomotor development, investigated because of phlebitis at the age of 30 (under oestrogens).

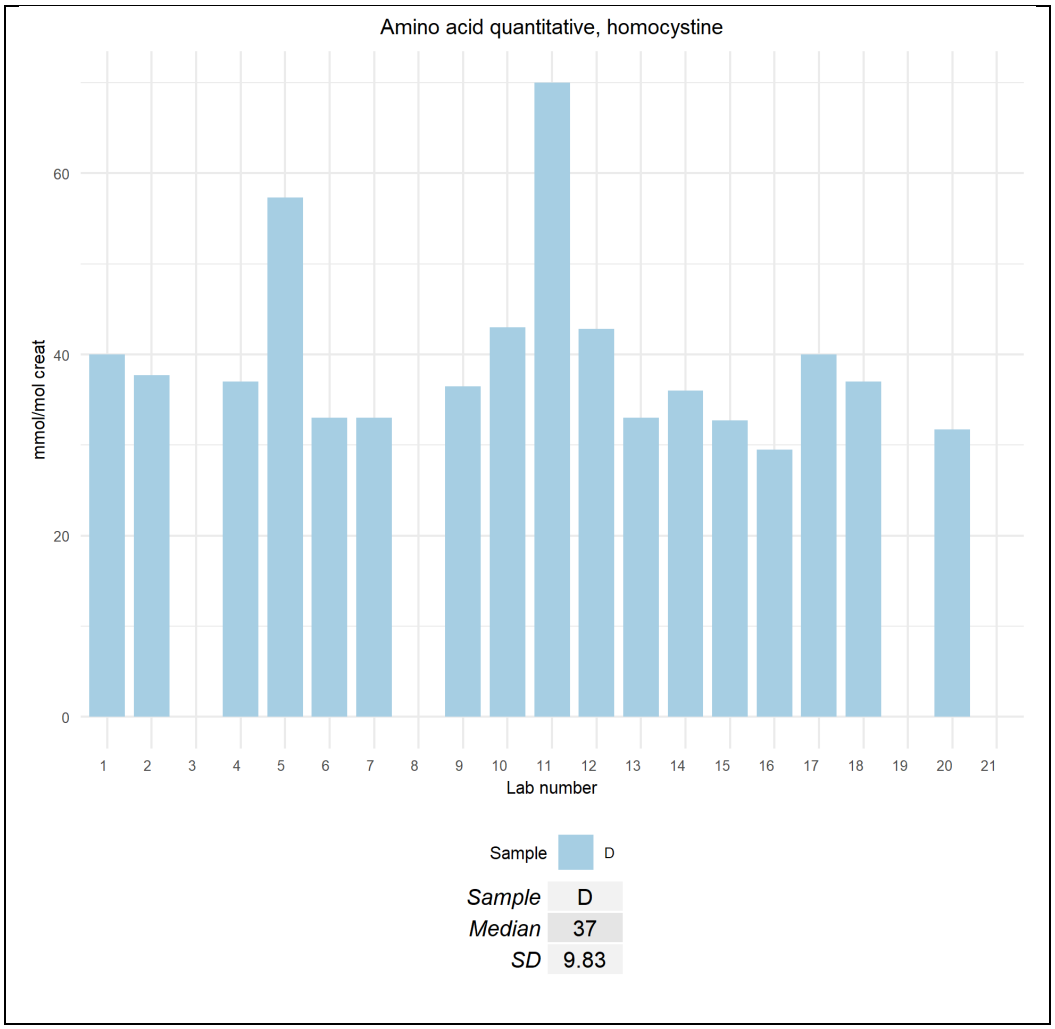
Patient details

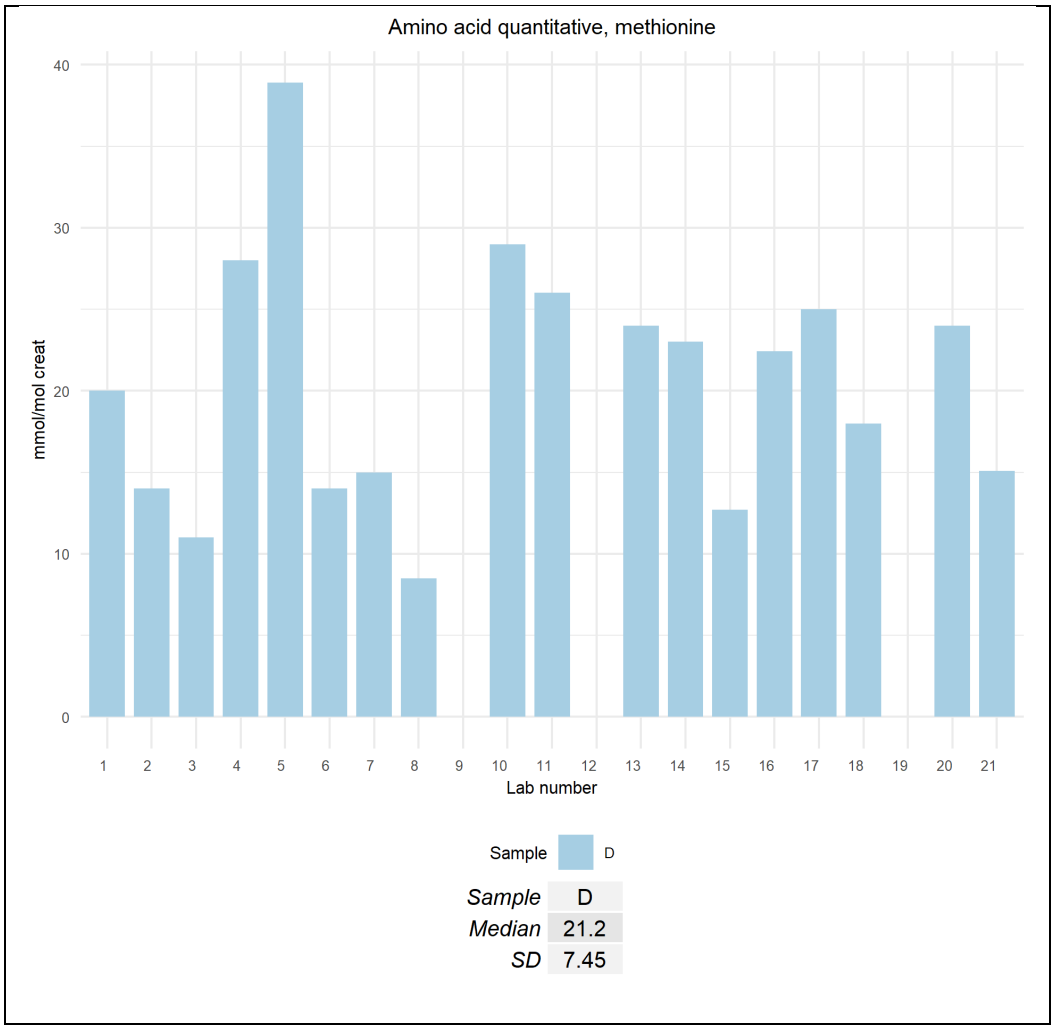
Diagnosis of cystathionine beta-synthase deficiency was suspected on results of plasma and urine amino acids. Under pyridoxine (vitamin B6) treatment, together with vitamin B9 and B12, plasma homocystine and methionine concentrations normalized. Spontaneously, the patient had a high protein diet (134 g/j). She felt much better (less anxiety and irritability) under vitamin supplementation and a normoproteic diet (80 g/j). Mutation analysis confirmed the diagnosis: she is compound heterozygote for two deleterious variants in *CBS* gene. No measurement of CBS activity has been performed.

Analytical performance

All participants performed **amino acids** analysis and reported:

- **Increase of homocystine** 18
  (median = 37 mmol/mol creat, range: 29.47 – 70 ; n=17)
- **Increase of methionine** 14
  (median = 21 mmol/mol creat, range: 8.48 – 38.9 ; n=18)
- Increase of cysteine-homocysteine mixt disulfide 6
- Decreased or normal level of cystine 4
- Normal level of methionine 4


Amino acid quantitative, homocystine
mmol/mol creat
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Lab number
Sample D
Sample D
Median 37
SD 9.83



The two figures above give the results of homocystine (CV=27%) and methionine (CV=35%) measurement.

Three participants also reported an increase of **total homocysteine** (median = 148 mmol/mol creat, range: 33.7 – 171.3).

The 15 labs who performed **organic acids** either mentionned that there was **no increase of methylmalonic acid** (n=10) or a normal profile (n=5).

Diagnosis / Interpretative proficiency

Most likely diagnosis

- CBS deficiency 19
- MTHFR deficiency or another remethylation disorder 1

Alternative diagnosis

- MTHFR deficiency (or another remethylation disorder) 7
- Transcobalamin II deficiency 1
- Folate deficiency 1
- Mild hyperhomocysteinemia 1
- CblG 1

Scoring

- Analytical performance
  - Increase of free homocystine and/or cysteine-homocysteine disulphide and/or total homocysteine (score 1)
  - Increase of methionine and/or no increase of methylmalonic acid (score 1)
- Interpretation of results
  - Cystathionine beta-synthase deficiency as first or alternative diagnosis (score 2)

- Methylene tetrahydrofolate reductase deficiency or another remethylation disorder as only diagnosis with the recommendation to perform plasma amino acids (score 1)

Overall impression

The overall proficiency was excellent: 98%.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2015: the overall performance is almost similar.

	2015	2021
Analytical performance	96 %	98 %
Interpretative performance	96 %	98 %
Overall performance	96 %	98 %

8.6. Patient E

4-hydroxybutyric aciduria (succinic semialdehyde dehydrogenase deficiency - ALDH5A1 gene)

Patient details provided to participants

17-year-old boy with psychomotor retardation, behaviour disturbance and slight failure to thrive.

Patient details

4-hydroxybutyric aciduria was diagnosed when he was 16-year-old. Diagnosis of succinic semialdehyde dehydrogenase (SSADH) deficiency has been confirmed by mutation analysis of *ALDH5A1* gene (homozygous variant).

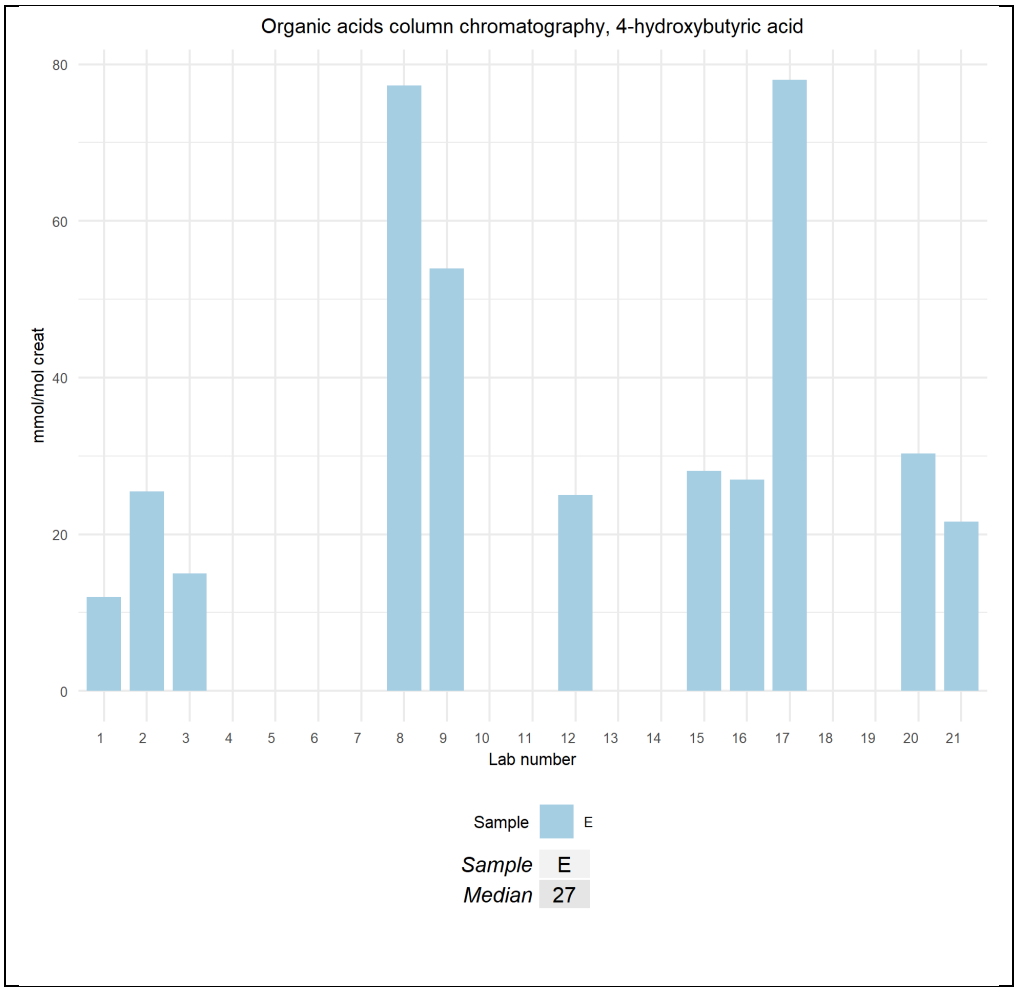
Analytical performance

All participants performed **organic acids**. They reported an increase of:

- **4-hydroxybutyric acid** 18
  (median = 27 mmol/mol creatinine; range: 12 – 78 ; n=11)
- **Erythro- and/or threo-4,5-dihydroxyhexanoic acids** 14
- 3,4-dihydroxybutyric acid 3
- 2,4-dihydroxybutyric acid 2
- 3-hydroxypropionic acid 2

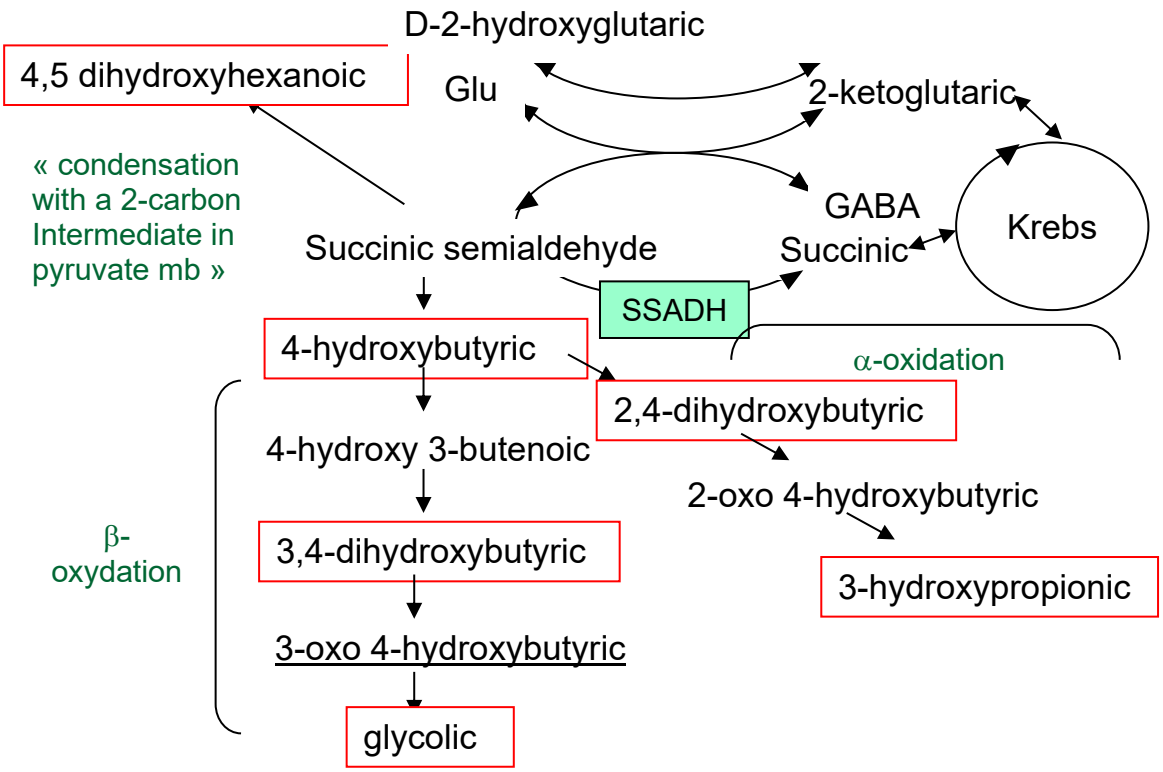
- Normal profile 2

The CV of the measurement of 4-hydroxybutyric acid was quite high (82%).

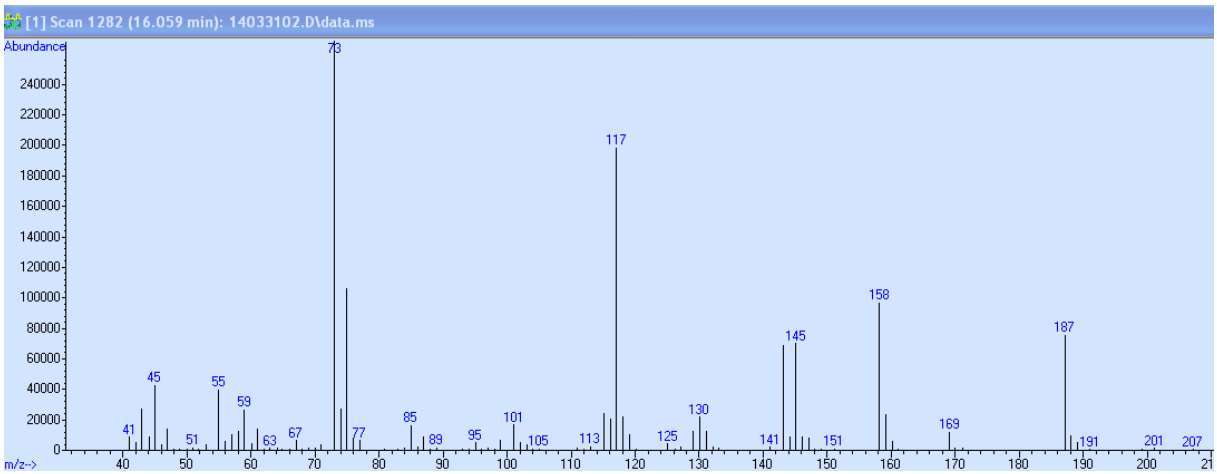


During the participant meeting, Cristiano Rizzo (Rome) mentioned that in patients with SSADH deficiency who have low excretion of 4-hydroxybutyric, like in this patient, measurement of 4-hydroxybutyric in plasma or CSF can be more informative.

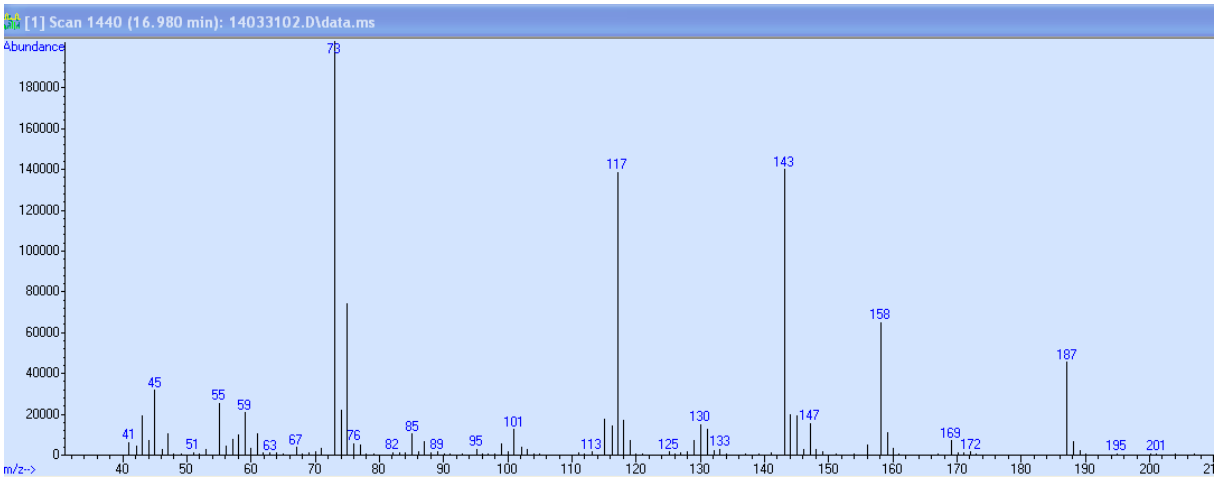
The figure below illustrates the origin of metabolites excreted in SSADH deficiency. Only 4,5-dihydroxyhexanoic acids (erythro, threo and the lactones, which are dependent upon the pH of extraction) are specific of SSADH deficiency, but other metabolites (2,4-dihydroxybutyric, 3-hydroxypropionic, 3,4-dihydroxybutyric and glycolic acids) can be elevated. In case of exogenous intake (anaesthesia with Gamma-OH®, or treatment with Xyrem®, or GBH poisoning: drug abuse), only 4-hydroxybutyric is elevated. Therefore, identification of 4,5-dihydroxyhexanoic acids is important. However, a non-specific increase of 4,5-dihydroxyhexanoic acids is sometimes observed, mainly in patients from neuropaediatry (observation from Cristiano Rizzo and scientific advisors), possibly due to anticonvulsant therapy, although no specific drug has been identified as responsible for this increase.



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



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



Diagnosis / Interpretative proficiency

Most likely diagnosis

- 4-hydroxybutyric aciduria (succinic semialdehyde dehydrogenase deficiency) 18
- No diagnosis 2

Alternative diagnosis 0

Scoring

- Analytical performance
  - Increase of 4-hydroxybutyric (score 1)
  - Increase of erythro or threo-4,5-dihydroxyhexanoic acids or lactones (score 1)
- Interpretation of results
  - 4-hydroxybutyric aciduria (score 2)

Due to the relatively low excretion of 4-hydroxybutyric acid in this patient, and due to the lack of treatment in this disorder, the SAB decided that missing the diagnosis of SSADH deficiency was harmful but was not a critical error.

Overall impression

The overall proficiency was 88%.

Multiple distributions of similar samples

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

	2015	2021
Analytical performance	76 %	85 %
Interpretative performance	86 %	90 %
Overall performance	81 %	88 %

8.7. Patient F

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

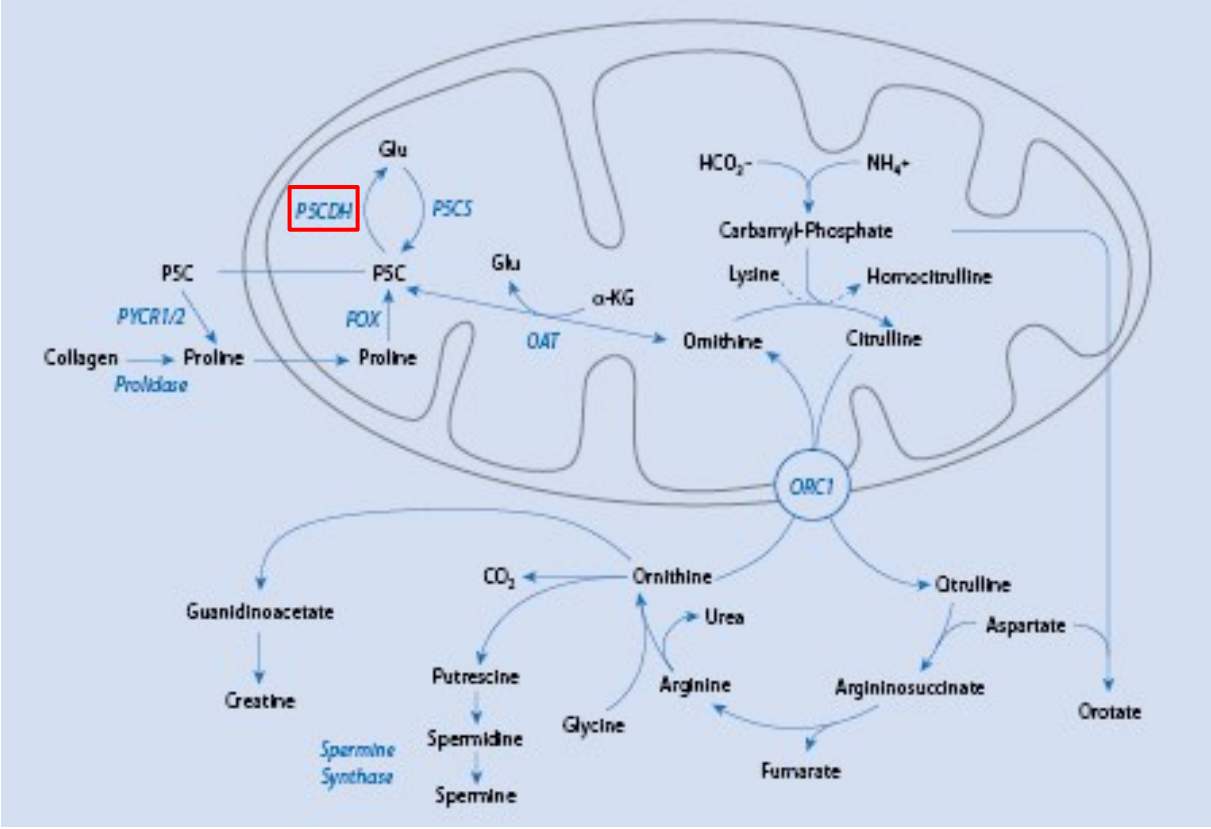
Patient details provided to participants

7-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 in this patient because of a high increase of plasma proline (2350 µmol/L), with an increase of plasma pyrroline-5-carboxylic acid (evaluated at 6 µmol/L by LC/MS-MS) at 4 years of age. Proline was also highly increased in urine (1059 mmol/mol creat), 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.

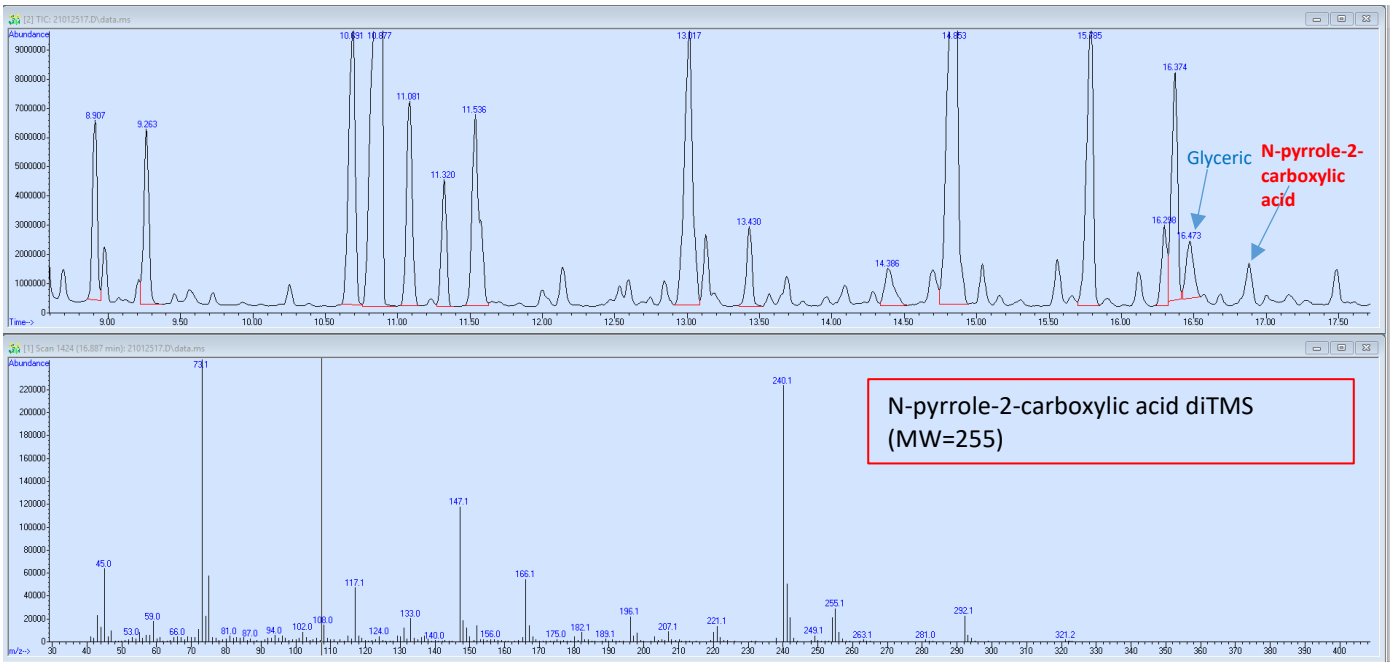
Hyperprolinaemia type II is caused by a deficiency of pyrroline-5-carboxylate dehydrogenase (P5CDH), a mitochondrial inner-membrane enzyme which converts pyrroline-5-carboxylic acid into glutamic acid.



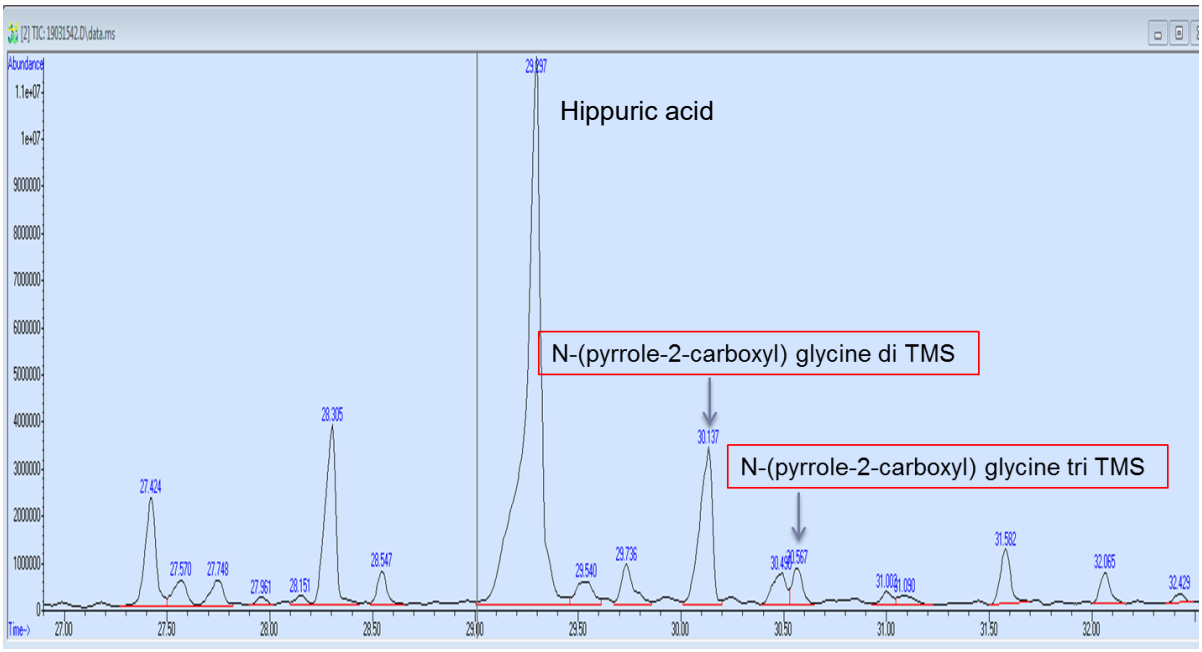
From Inborn Metabolic Diseases, Diagnosis and Treatment, Saudubray, Baumgartner, Walter Eds, Springer 2016

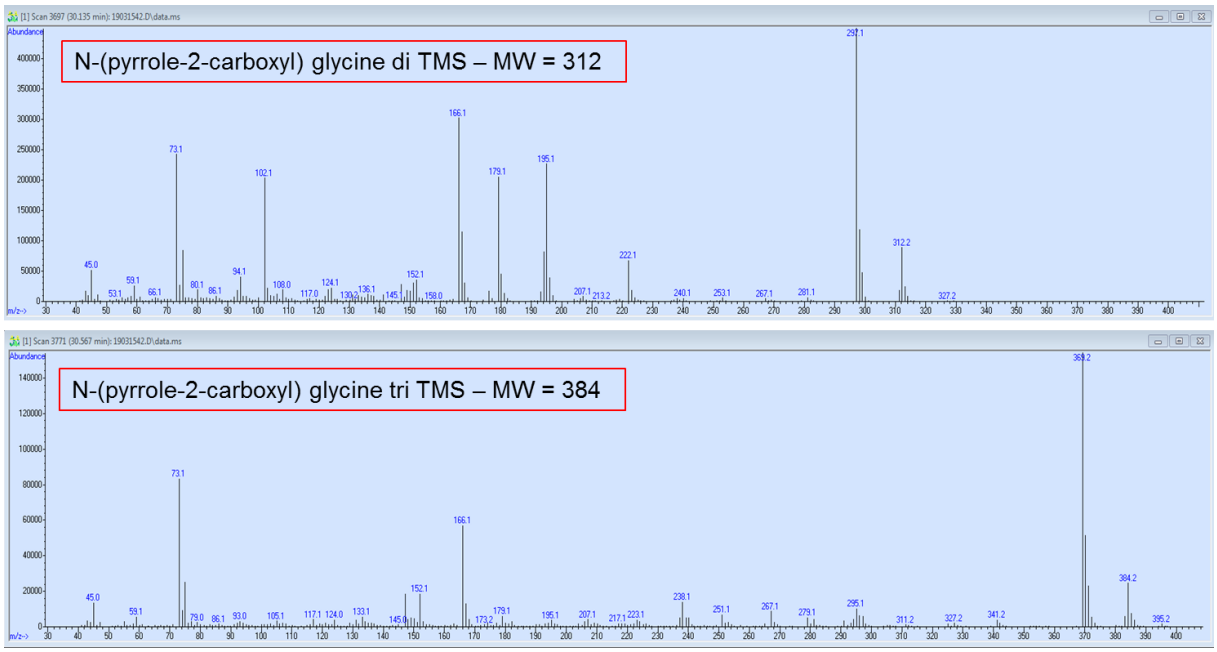
Hyperprolinaemia type II is generally associated to epilepsy and mental retardation, but asymptomatic patients have been described.
Pyrroline-5-carboxylic acid (P5C), which accumulates in this disease, is an antagonist of vitamin B6 (pyridoxine), and seizures can be due in part to B6 inactivation. Seizures are B6 responsive.
Identification of P5C by tandem MS allows differentiating between type II and type I hyperprolinaemia, as well as identification of N-(pyrroline-2carboxyl) glycine or of N-pyrroline-2-carboxylic acid 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-carboxylic acid is eluted less than 1 minute later than glyceric acid. N-(pyrroline-2carboxyl) glycine is eluted 1 minute later than hippuric acid.

N-pyrrole-2-carboxylic acid



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



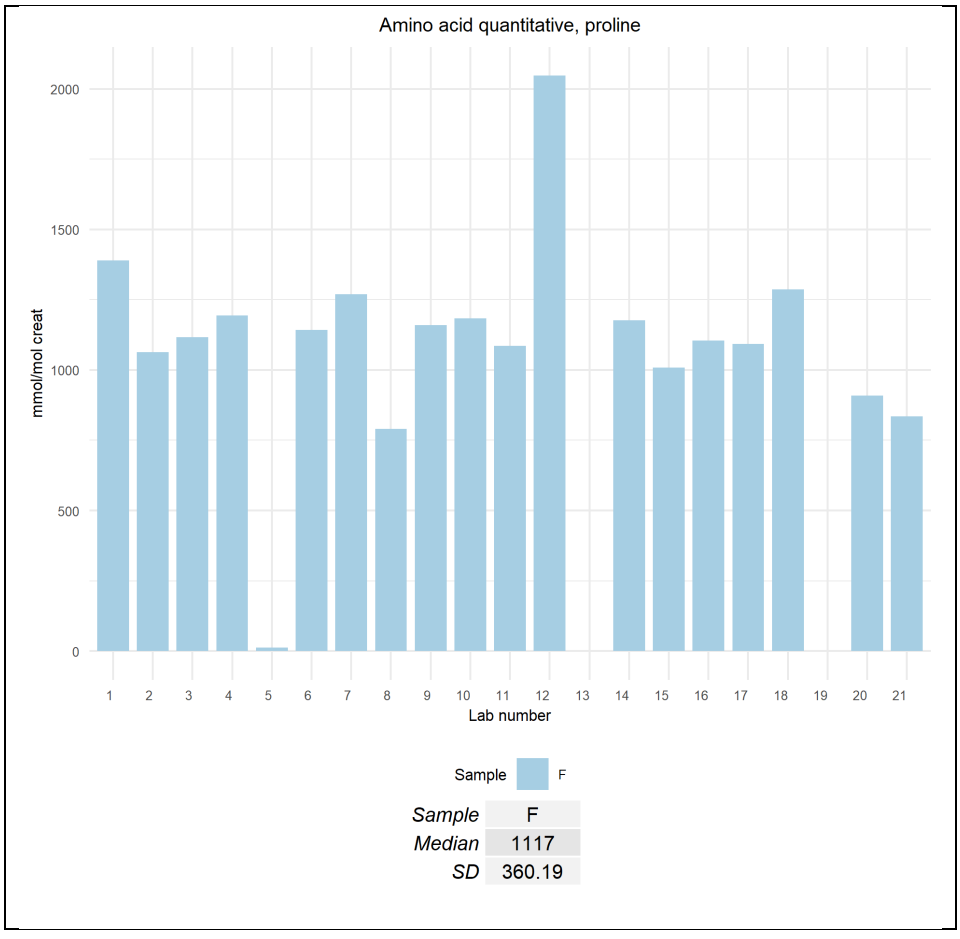


Analytical performance

All participants performed amino acids analysis and reported:

- Increase of **proline**: median = 1117 mmol/mol creat ; range: 12.7 – 2047 ; n=19
- Increase of glycine: median = 1529 mmol/mol creat ; range: 801 – 1727 ; n=19
- Increase of hydroxyproline: median = 104 mmol/mol creat ; range: 54 – 204 ; n=14

But no lab identified pyrroline-5-carboxylic acid and one lab reported a normal profile.



The CV for proline determination was 32.2 %.

All participants also performed organic acids, but only 11 of them reported an increase of **N-(pyrrole-2-carboxyl) glycine,** and 3 an increase of **N-pyrrole-2-carboxylic acid.** One participant noticed that he could not detect N-(pyrrole-2-carboxyl) glycine.

Diagnosis / Interpretative proficiency

Most likely diagnosis

- Hyperprolinaemia type II 13
- Hyperprolinaemia type I 4
- Hyperoxaluria type II 2
- Prolidase deficiency 1

Alternative diagnosis

- Hyperprolinaemia type II 3
- Hyperprolinaemia type I 2
- Iminoglycinuria 1
- Hyperoxaluria type I 1

Scoring

- Analytical performance
  - Increase of proline (score 1)
  - Increase of N-(pyrrole-2-carboxyl) glycine or N-pyrrole-2-carboxylic acid or pyrroline-5-carboxylic acid (score 1)
- Interpretation of results
  - Hyperprolinaemia type II as first diagnosis or hyperprolinaemia type II as alternative diagnosis with recommendation to perform measurement of pyrrole-5-carboxylic acid in plasma or urine (score 2)
  - Hyperprolinaemia type I as first diagnosis without recommendation to perform measurement of pyrrole-5-carboxylic acid in plasma or urine and/or mutation of *ALDH4A1* gene (score 1)

The SAB has considered as a critical error the failure to detect an increase of proline in this sample, because all other participants detected it and seizures in this disorder are B6 responsive.

Overall impression

The overall proficiency was 78%.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2019: unforntunately the overall performance has not significantly improved.

	2019	2021
Analytical performance	69 %	75 %
Interpretative performance	83 %	80 %
Overall performance	76 %	78 %

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 Alpha-mannosidosis			Patient B Alpha-mannosidosis			Patient C MAT deficiency			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	0	0	0	2	2	4	8
2	2	2	4	2	2	4	2	2	4	12
3	2	2	4	2	2	4	2	2	4	12
4	0	1	1	0	1	1	2	2	4	6
5	0	0	0	0	1	1	2	2	4	5
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	1	3	11
9	2	2	4	0	1	1	2	2	4	9
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	0	1	1	0	1	1	2	2	4	6
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	1	3	11
18	2	2	4	2	2	4	2	1	3	11
19	0	0	0	0	0	0	0	0	0	0
20	2	2	4	2	2	4	2	2	4	12
21	2	2	4	0	0	0	2	2	4	8

Detailed scores – Round 2

Lab n°	Patient D CBS deficiency			Patient E 4-hydroxybutyric aciduria			Patient F Hyperprolinaemia type II			
	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	1	0	1	9
5	2	2	4	0	0	0	1	2	3	7
6	2	2	4	2	2	4	2	2	4	12
7	2	2	4	2	2	4	1	1	2	10
8	2	2	4	1	2	3	2	2	4	11
9	2	2	4	2	2	4	2	2	4	12
10	2	2	4	2	2	4	1	1	2	10
11	2	2	4	2	2	4	2	2	4	12
12	1	2	3	2	2	4	1	2	3	10
13	2	2	4	2	2	4	0	0	0	8
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	1	2	3	2	2	4	11
18	2	1	3	0	0	0	1	2	3	6
19	--	--	--	--	--	--	--	--	--	0
20	2	2	4	2	2	4	1	0	1	9
21	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	0	4	4	4	4	20	83	
2	4	4	4	4	4	4	24	100	
3	4	4	4	4	4	4	24	100	
4	1	1	4	4	4	1	15	62	
5	0	1	4	4	0	3	12	50	CE
6	4	4	4	4	4	4	24	100	
7	4	0	4	4	4	2	18	75	
8	4	4	3	4	3	4	22	92	
9	4	1	4	4	4	4	21	88	
10	4	4	4	4	4	2	22	92	
11	4	4	4	4	4	4	24	100	
12	4	4	4	3	4	3	22	92	
13	1	1	4	4	4	0	14	58	CE
14	4	4	4	4	4	4	24	100	
15	4	4	4	4	4	4	24	100	
16	4	4	4	4	4	4	24	100	
17	4	4	3	4	3	4	22	92	
18	4	4	3	3	0	3	17	71	
19	0	0	0	--	--	--	0	0	
20	4	4	4	4	4	1	21	88	
21	4	0	4	4	4	4	20	83	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 60 % of adequate responses)	18	86
Unsatisfactory performers (< 60 % adequate responses and/or critical error)	3	14
Partial and non-submitters	1	5

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-FL-2021-A	Alpha-mannosidosis	81	86	83
DPT-FL-2021-B	Alpha-mannosidosis	62	71	67
DPT-FL-2021-C	MAT deficiency	95	88	92
DPT-FL-2021-D	CBS deficiency	98	98	98
DPT-FL-2021-E	4-hydroxybutyric aciduria	85	90	88
DPT-FL-2021-F	Hyperprolinaemia type II	78	80	79

10. Annual meeting of participants

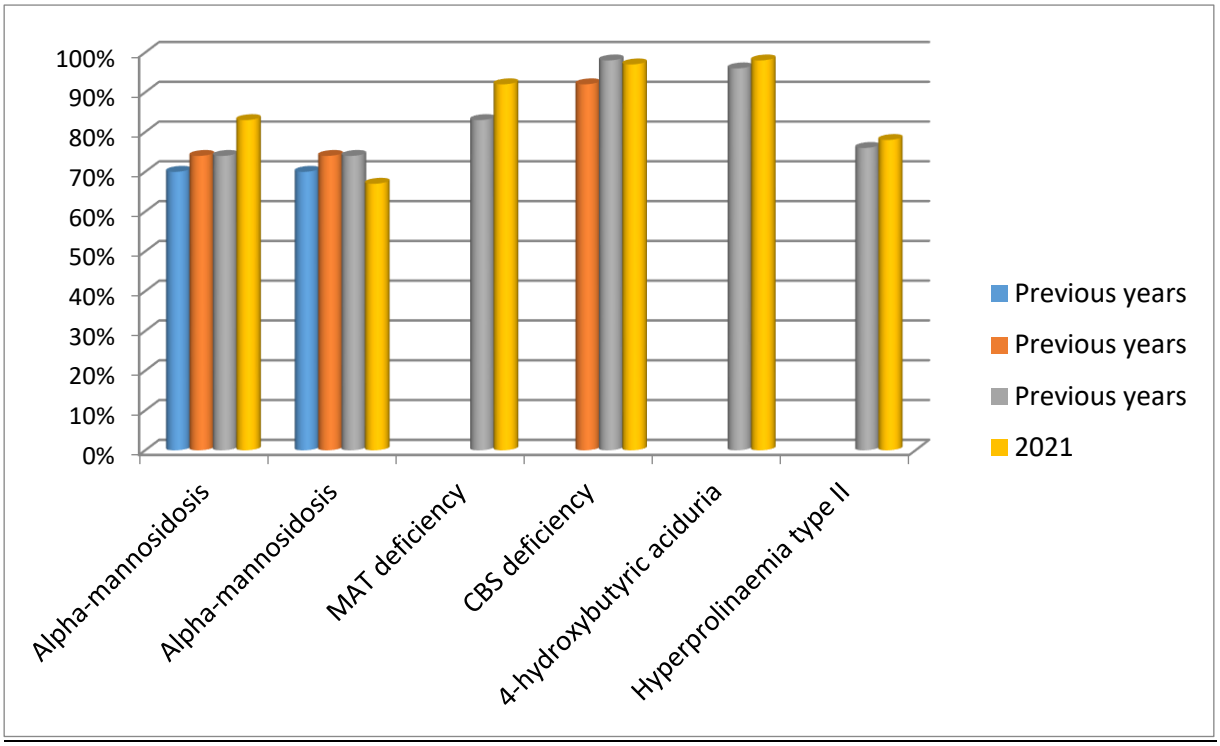
This took place by teleconference on September 6th 2021 from 10.00 to 12.00.

Participants

Representatives from 15 labs were present (4 participants joined without name: sorry if they are not included): Barcelone (Hospital Clinic), Barcelone (Vall d'Hebron), Bizkaia, Caen, Florence, Grenoble, Lille, Lisbon, Madrid, Nancy, Paris, Porto, Rome, Rouen.

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.

Improvement of DPT France 2021



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

- **New scoring policy for DPT scheme in 2022**: the score for satisfactory performance will be increased from 15 points to **at least 17 points from the maximum of 24 (70%) in 2022**, in accordance with the other qualitative schemes.
- **Reference materials** are provided by SKML: they are not related to EQA samples. There are two concentration levels for each group of analytes. The most suitable low and high concentration levels have been 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 (www.erndimqa.nl). 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 standards** has been developed by Amsterdam UMC (University Medical Center), following request and advice from ERNDIM. The product is currently available at: organic.synthesis.lab@amsterdamumc.nl
- **Training**: SSIEM Academy training courses.
  - A 2-day course will be organized on Monday and Tuesday 27 and 28 April 2022 in Amsterdam. The program for biochemists includes:
    - Aminoacidopathies
    - Hyperammonaemia
    - Urea cycle defects
- **Urine samples**: we remind you that every year, each participant must provide to the scheme organizer at least 250 ml of urine from a patient affected with an established inborn error of metabolism or a “normal” urine, together with a short clinical report. If possible, please collect at least 1200 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. 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.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 in 2022

Sample distribution	2 February 2022
Start of analysis of Survey 2022/1 Website open	March 14
Survey 2022/1 - Results submission	April 4
Survey 2022/1 - Reports	End April – beginning May
Start of analysis of Survey 2022/2	June 6
Survey 2022/2 – Results submission	June 28
Survey 2022/2 - Reports	July
Annual meeting of participants	August 30 Freiburg SSIEM
Annual Report 2022	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, 2021-12-20

Name and signature of Scientific Advisor

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APPENDIX 1. Change log (changes since the last version)

Version Number	Published	Amendments
1	26 April 2022	2021 annual report published

APPENDIX 2

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
• 2019:1	A	APRT deficiency (common sample)
	B	Beta-mannosidosis
	C	Hyperprolinaemia type II
• 2019:2	D	Multiple acyl-CoA dehydrogenase deficiency (MADD)
	E	MPS II
	F	Argininaemia
• 2020:1	A	PKU (common sample)
	B	Alcaptonuria
	C	MPS IVA
• 2020:2	D	Citrullinaemia type I
	E	Iminodipeptiduria
	F	GAMT deficiency

END