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Diagnostic Proficiency Testing
Centre: France

Final Report 2020

prepared by
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Published: 17 February 2021[1]

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.

In 2020, 23 labs participated to the Diagnostic Proficiency Testing France Scheme.

1. Geographical distribution of participants

For the first survey, 22 participants submitted results for all samples (one participant submitted results for only one sample of this first survey) and, for the second survey, all 23 registered laboratories submitted results.

Country	Number of participants
France	10
Italia	5
Netherlands	1
Portugal	2
Spain	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 Xavier Albe and Anthony Barrozo as scheme organisers (sub-contractor on behalf of CSCQ), both appointed by and according to procedures laid down the ERNDIM Board.

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

CSCQ dispatches DPT EQA samples to the scheme participants and provides a website for on-line submission of results and access to scheme reports. 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

Origin of patients: urine samples have been provided by the Scientific Advisors, by Dr Begoňa Merinero and Dr Pedro Ruiz-Sala from Madrid, by Dr Odile Rigal from Paris and by Dr Jose Antonio Arrantz Amo from Barcelona.

Patient A: PKU This sample has been sent to all labs participating to the DPT scheme in Europe (common sample)
Patient B: Alkaptonuria
Patient C: MPS IVA
Patient D: Citrullinaemia type I
Patient E: Iminodipeptiduria
Patient F: GAMT deficiency

The samples have been heat-treated. They were pre-analysed in our institute after 14 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 were required in 2020.

4. Schedule of the scheme

- February 11, 2020: Shipment of samples of Survey 1 and Survey 2 by CSCQ
- March 9: Clinical data available on CSCQ website and start analysis of samples A, B, C (Survey 1)
- March 23: Reminder for website submission
- June 1: Deadline for result submission (Survey 1). Deadline was postponed because of COVID-19 pandemics
- June 8: Clinical data available on the CSCQ website and start analysis of samples D, E, F (Survey 2)
- June 22: Reminder for website submission
- June 29: Deadline for result submission (Survey 2)
- July 1: Interim report of Survey 1 available on CSCQ website
- July 25: Interim report of Survey 2 available on CSCQ website
- September 1: Meeting of participants by teleconference
- November 20: SAB meeting: definition of critical errors
- December 10: Annual Report with definitive scoring

5. Results

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

	Survey 1	**Survey 2**
Receipt of results	23	23
No answer	0	0

6. Web site reporting

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

- Selection of tests: **do not 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 do not give quantitative data.
  - If the profile is normal: enter “Normal profile” in “Key metabolites”.
  - **Do not 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.
  - **Do not 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 2020 have been also scored by Dr Petr Chrastina, the scientific advisor of DPT Czech Republic. At the SAB meeting on November 20th, 2020, 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 2020, the SAB decided that sample B (alkaptonuria) has to be considered as a critical error for the labs who did not perform organic acids and did not advise to do it, as well as sample C (MPS IVA) for the labs who did not perform glycosaminoglycans quantification or fractionation and did not advise to perform it/them. Sample F was considered by SAB as educational since measurement of creatine and guanidinoacetate does not belong to the required tests and because it was a difficult sample: GAMT deficiency supplemented with creatine.

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. Three performance support letters will be sent by the Scheme Advisor for 2020 for unsatisfactory performance and critical error. Any partial submitters will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.

7.1. Score for satisfactory performance

Since sample F is educational, the maximum score for 2020 is 20 points. Score for satisfactory performance in 2020 is at least 12 points from the maximum of 20 (60%).

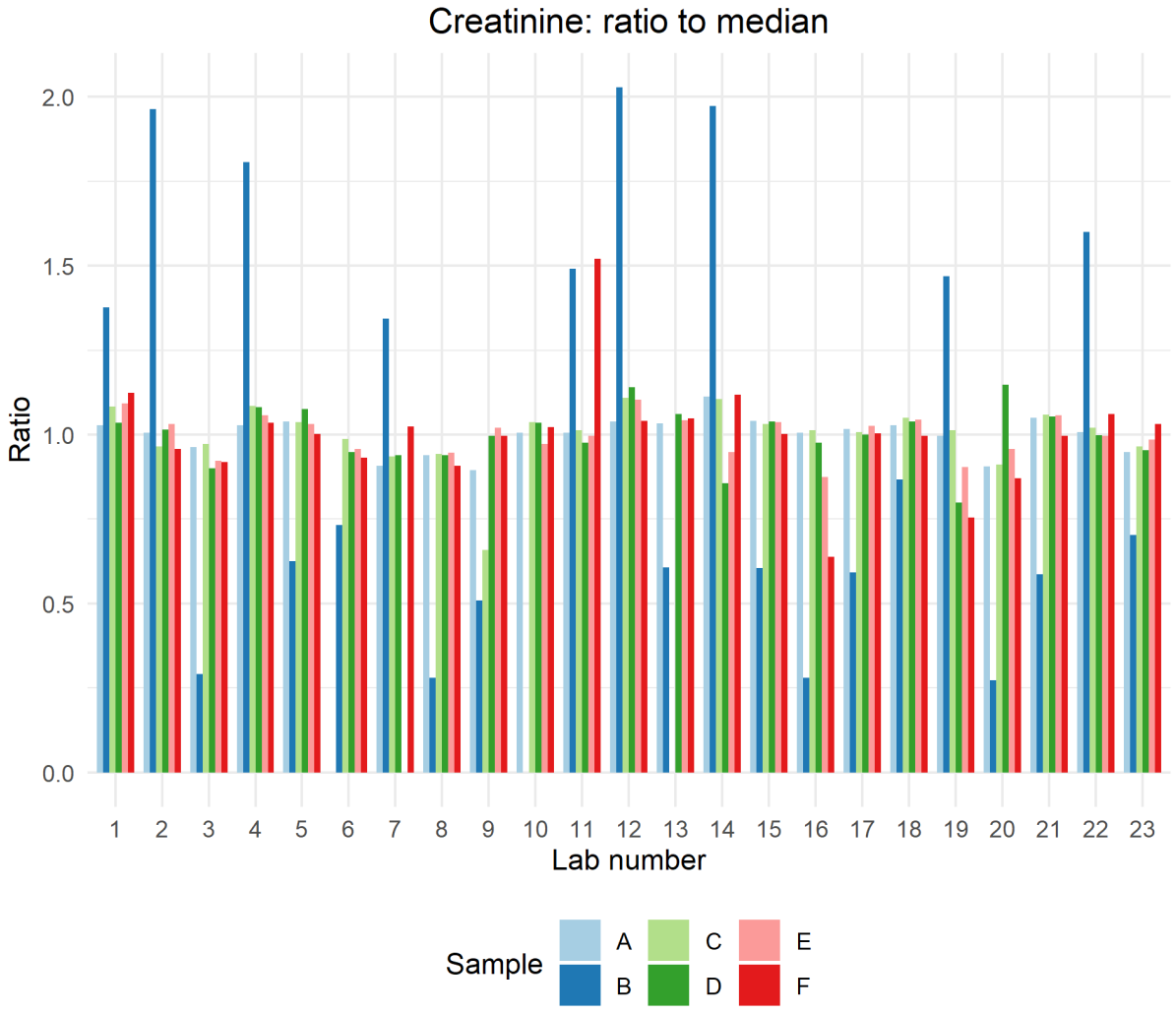
If your laboratory is assigned poor performance and you wish to appeal against this classification please email the ERNDIM Administration Office (admin@erndim.org), with full details of the reason for your appeal, within one month receiving your Performance Support Letter.

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 for sample B (alkaptonuria) and sample F (GAMT deficiency). For sample B, the dark colour of the urine interferes with the measurement of creatinine by enzymatic method. By tandem MS, the creatinine value was 5.8 mmol/L. For sample F, it is possible that the high creatine value interferes with the measurement of creatinine by enzymatic method.

Otherwise, 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 < 8.1 % for all samples (5.1 – 8.1 %), except for sample B (85.8 %) and sample F (15.5 %).



Sample	A	B	C	D	E	F
Median	4.50	2.82	4.30	5.22	8.30	7.85
SD	0.24	2.41	0.40	0.43	0.49	1.23

8.2. Patient A

Phenylketonuria (phenylalanine hydroxylase deficiency)

Patient details provided to participants

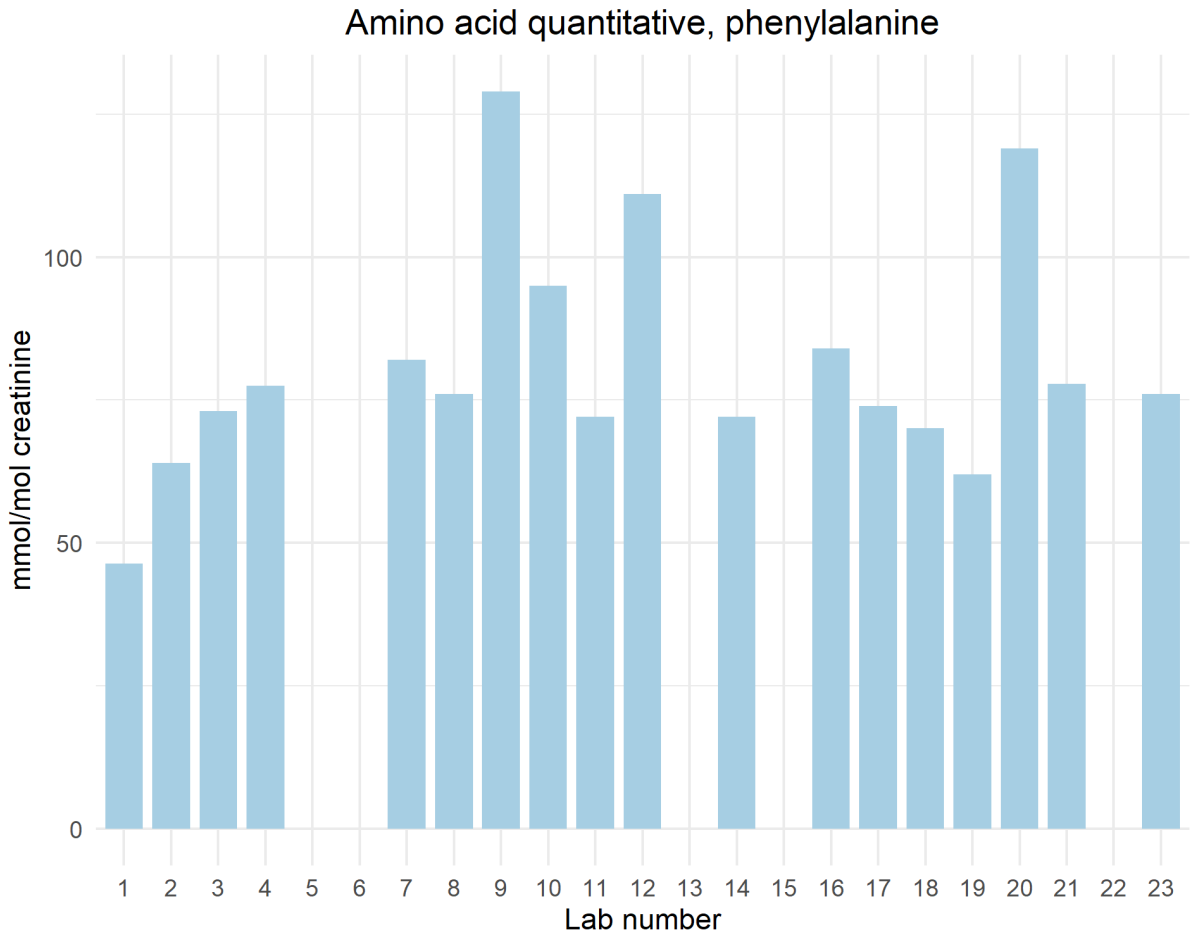
Adult patient investigated due to spastic paraparesis, leukodystrophy and hemolytic uremic syndrome

Patient details

This adult patient presented with spastic paraparesis, leukodystrophy and haemolytic uremic syndrome. Phenylketonuria (phenylalanine hydroxylase deficiency) was diagnosed in adulthood since he did not undergo neonatal screening. This is the common sample distributed to all participants of the 5 DPT schemes. **Results from all DPT schemes are available on the ERNDIM website**.

Analytical performance

Twenty labs performed **amino acid** analysis and 19 of them reported an increase of phenylalanine.

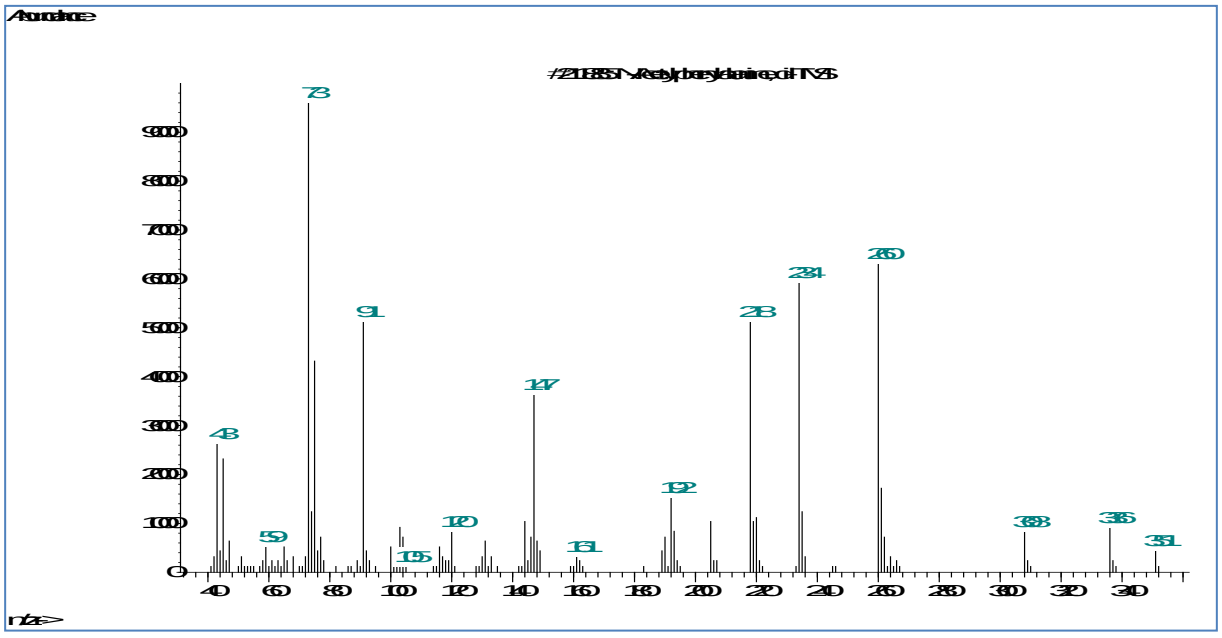


Sample	A
Median	76
SD	19.97

All participants performed **organic acid** analysis and reported metabolites of phenylalanine:

Organic acid	n	Quantitative results		
		Median (mmol/mol creat)	Range (mmol/mol creat)	n
Phenylpyruvic	17	210	172 - 308	4
Mandelic	16	-	41 ; 81	2
2-hydroxyphenylacetic	16	-	102.8 ; 133	2
Phenyllactic	15	563	220 – 979.5	4
Phenylacetic	15	-	130	1
N-acetylphenylalanine	4	-	-	-
4-hydroxyphenyllactic	14	-	30 - 138	4

N-acetylphenylalanine is eluted between azelaic and hippuric acids, and has the following mass spectrum:



Thanks to Dr Merinero and Dr Ferrer, Madrid

Four labs performed analysis of pterins, and reported normal levels for neopterins and biopterins and normal ratio, excluding a defect in biosynthesis or regeneration of BH4.

Diagnosis / Interpretative proficiency

Most likely diagnosis

Phenylketonuria	21
Hyperphenylalaninemia	1

Alternative diagnosis

Phenylketonuria	1
Hyperphenylalaninemia / biopterin deficiency	5

Scoring

- Analytical performance
  - Increase of phenylalanine (score 1)
  - Increase of at least one organic acid present in phenylketonuria (phenylpyruvic, mandelic, phenyllactic, N-acetylphenylalanine, phenylacetic, 2-hydroxyphenylacetic) (score 1)
- Interpretation of results
  - Phenylketonuria as first or alternative diagnosis (score 2)

Overall impression

The overall performance was quite satisfying.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2011: the overall performance was similar.

	2011	2020
Analytical performance	95 %	91 %
Interpretative performance	100 %	100 %
Overall performance	87 %*	95 %

(*recommendations were scored separately in 2011)

8.3. Patient B

Alkaptonuria (homogentisate 1,2-dioxygenase deficiency)

Patient details provided to participants

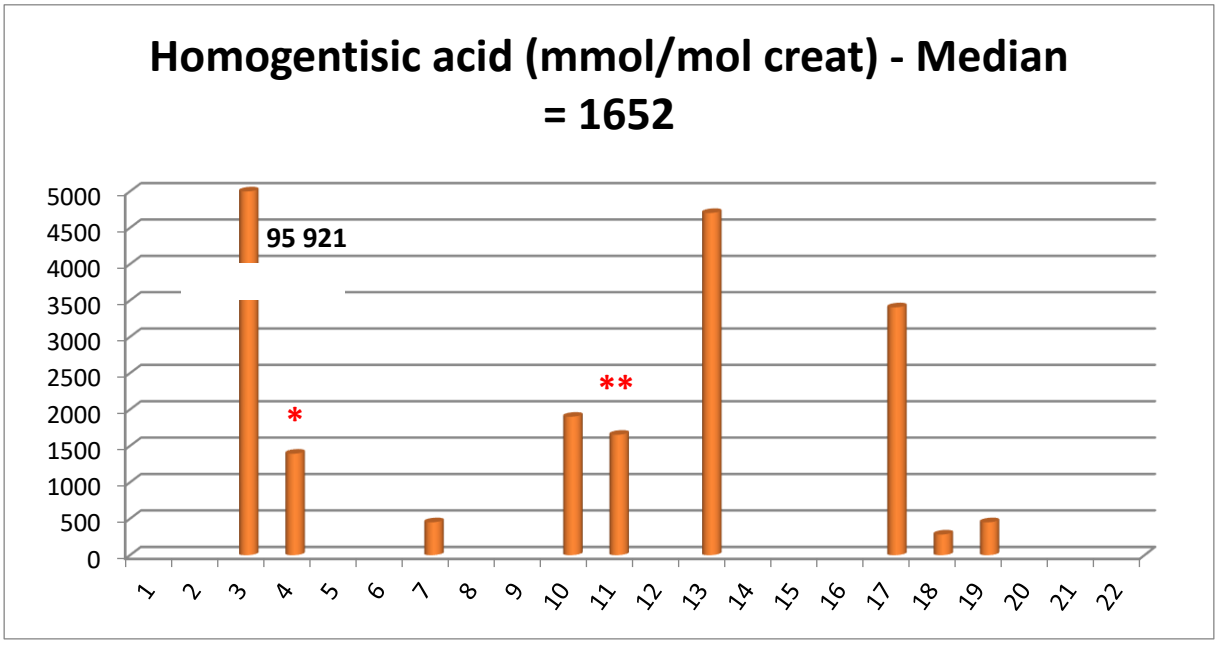
Adult patient. He was investigated because of arthritis and cardiac valve disease.

Patient details

This adult patient was investigated because of arthritis, cardiac valve dysfunction and dark urine. When urine was collected, he was treated with NTBC associated to a low protein diet. Diagnosis was established by identification of homogentisic acid in urinary organic acids.
Alkaptonuria (also called ochronosis) is due to homogentisate 1,2-dioxygenase deficiency (*HGD* gene).

Analytical performance

All participants but one (22/23) performed urinary **organic acid** analysis and all identified an increase of homogentisic acid. Seven of them gave quantitative results (see below). One participant used an HPLC with a Photo Diode Array detector (PDA), and another one used an ESI-MS/MS method. The median of all results was 1652 mmol/mol creat (range: 203 - 95921 mmol/mol creat). From the information we got from participants who answered the mail we send them, no one used a stable isotope of homogentisic acid as internal standard, although $^{13}C_6$-homogentisic acid is available from different companies (Larodan, Santa Cruz Biotechnology, LGC Group, …). The huge range of quantitative data observed can be due to calibration problems but also probably to the poor storage of homogentisic acid which undergoes auto-oxidation into hydrogen peroxide, superoxide (responsible for arthritis?) and benzoquinoneacetate. In addition, 9 labs mentioned an increase of 4-hydroxyphenyllactic, most probably due to NTBC treatment.



* ESI-MS/MS ** HPLC-PDA

Amino acid analysis was performed by 13 participants: 11 of them reported an increase of tyrosine (median = 45 mmol/mol creat; range: 21 – 245 mmol/mol creat) most probably due to NTBC treatment.

Diagnosis / Interpretative proficiency

Most likely diagnosis

Alkaptonuria	22
No diagnosis	1

Alternative diagnosis

Heparin contamination	1

Scoring

- Analytical performance
  - Increase of homogentisic acid (score 2)
- Interpretation of results
  - Alkaptonuria (score 2)

Overall impression

The overall performance was satisfying since all participants except one reached the diagnosis. The SAB decided that missing the diagnosis was a **critical error**.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2004: the overall performance is slightly better in 2020.

	2004	2020
Analytical performance	84 %	96 %
Interpretative performance	95 %	96 %
Overall performance	87 %*	96 %

(*recommendations were scored separately in 2011)

8.1. Patient C

Morquio disease A (mucopolysaccharidosis type IVA, galactosamine-6-sulfate sulfatase deficiency).

Patient details provided to participants

5 year-old girl. She was investigated because of short stature and skeletal abnormalities.

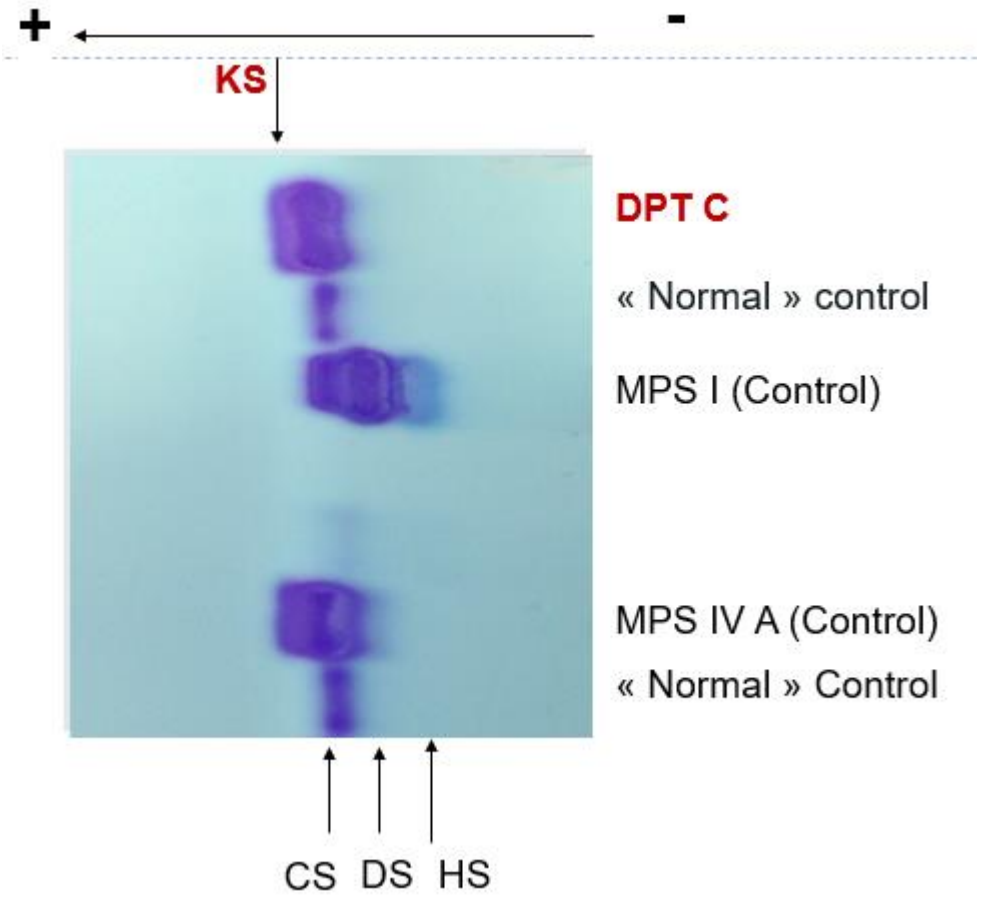
Patient details

This 5-year-old girl presented with a stop of growth from the age of 3 years, progressive chest deformation and joint pain. She has a normal intellectual development. Skeleton X-rays were characteristic of Morquio disease.

The diagnosis of mucopolysaccharidosis type IVA (Morquio A disease) was suspected by mucopolysaccharide analysis, and confirmed by measurement of galactosamine-6-sulfate sulfatase activity in leucocytes and mutation analysis of *GALNS* gene.

Analytical performance

Eighteen labs performed **glycosaminoglycans (GAGs) quantification** and all but one reported an increased concentration of GAGs. Eighteen participants also performed **GAGs fractionation**: 16 reported an increase of keratan sulphate (KS), 9 an increase of chondroitin sulphate (CS) and 1 of dermatan sulphate (DS). One lab specified that there was no increase of keratan sulphate.



Patient C: Electrophoretic pattern of MPS

Among the 9 labs who performed oligosaccharides, 8 reported a normal profile and 1 an increase of sialyloligosaccharides.

Diagnosis / Interpretative proficiency

Most likely diagnosis

Morquio A (MPS IVA)	12
Morquio A or B (MPS IVA or IVB)	4
Morquio (MPS IV)	1
Morquio B (MPS IVB)	1
MPS I, IV or IX	1
MPS VI	1
Mucopolysaccharidosis	1
No diagnosis	2

Alternative diagnosis

Morquio B (MPS IVB)	6
Mucopolysaccharidosis	2
MPS VI	1
MPS VII	1

Scoring

- Analytical performance
  - Increase of keratan sulfate (score 2)
  - Increase of GAGs quantification without GAGs fractionation (score 1)
- Interpretation of results
  - Morquio A disease IVA (MPS IVA) (score 2)
  - Morquio B (MPS IVB) as only diagnosis, mucopolysaccharidosis because of the increase of GAGs quantification or because of the clinical presentation with recommendation to perform GAGs fractionation (score 1)

Overall impression

The overall performance is satisfying for a lysosomal storage disease. However, two labs did not perform MPS analysis and did not recommend performing it. The SAB considered this as a **critical error**.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2013: the overall performance is similar in 2020.

	2013	2020
Analytical performance	80 %	78 %
Interpretative performance	83 %	83 %
Overall performance	82 %	80 %

8.1. Patient D

Citrullinaemia type I (argininosuccinate synthetase deficiency - ASS1 gene)

Patient details provided to participants

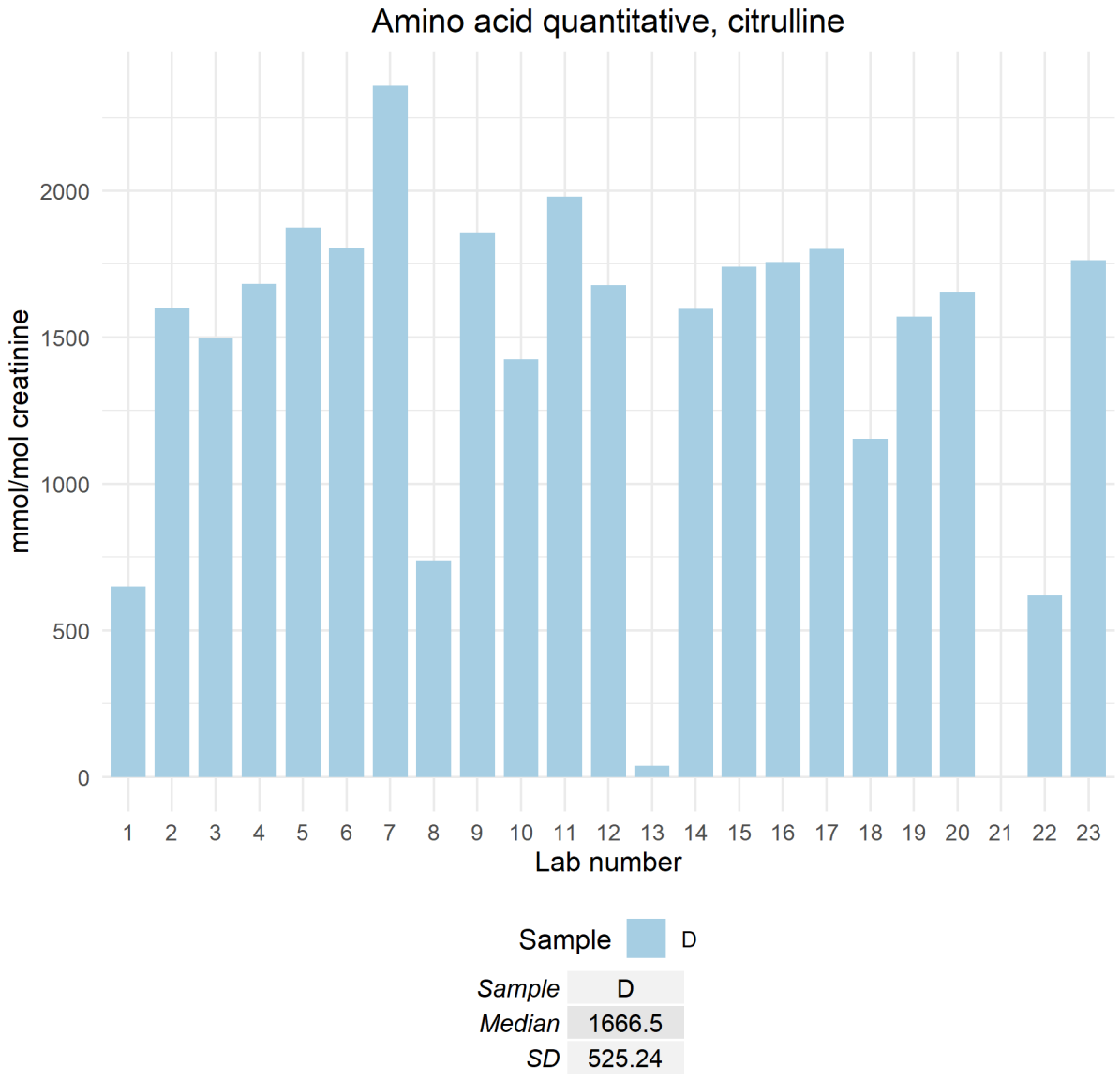
Third child of consanguineous parents. She presented at 3 days of life with hyporeactivity, grunting, which rapidly worsened. Ammonaemia was >700 µmol/L. Urines were collected when she was 10 years, under treatment. Ammonaemia was normal.

Patient details

This boy was the third child of consanguineous parents. He presented at 3 days of life with hyporeactivity, grunting, and his clinical condition rapidly worsened. He was hospitalized at 5 days of life. Ammonaemia went up to 1500 µmol/L. Proteins were stopped and he received IV sodium benzoate. He recovered within few days.
He is now 10-year-old. He is treated with low protein diet and *per os* sodium benzoate.
Diagnosis was confirmed by mutation analysis of *ASS1* gene.

Analytical performance

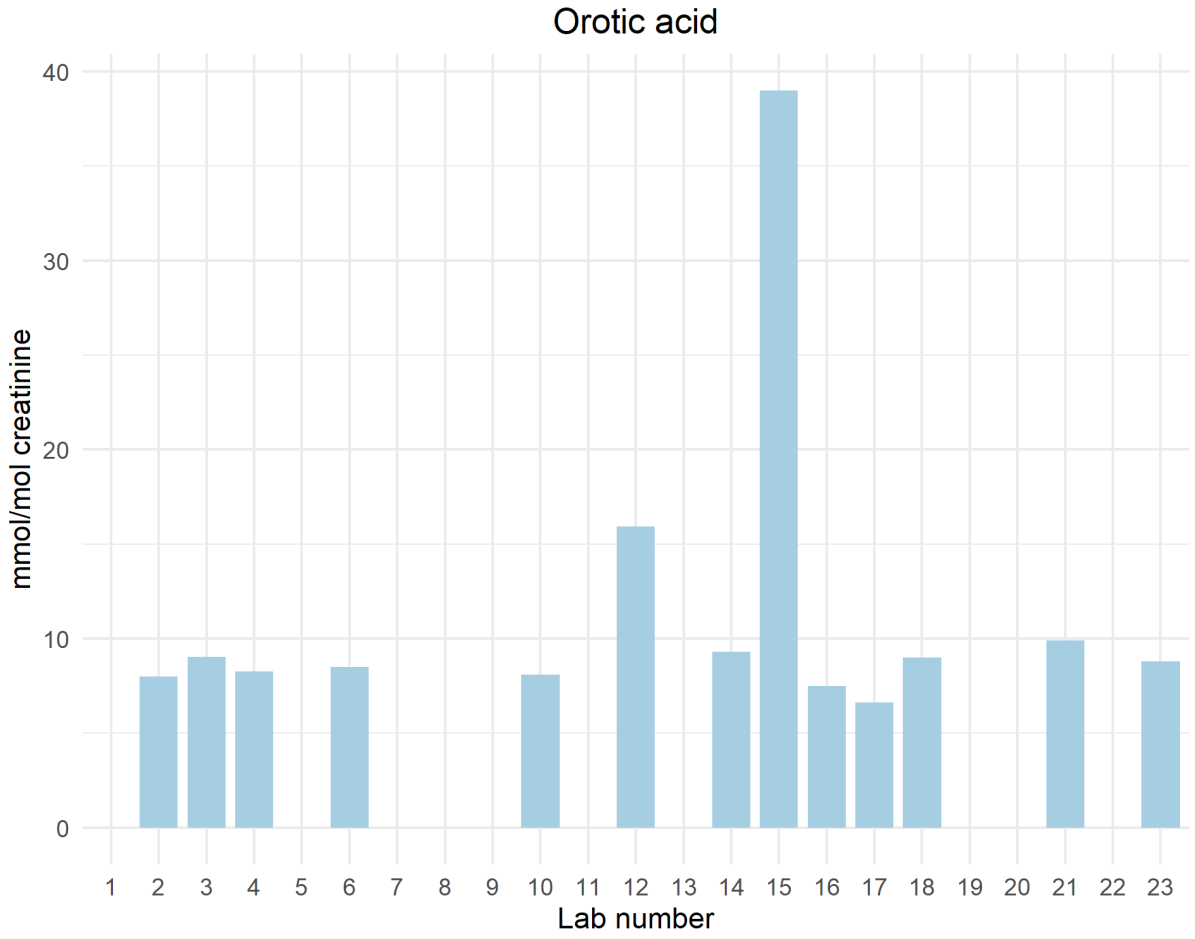
All participants but one performed **amino acid** analysis and identified an increase of citrulline. One lab reported an increase of argininosuccinic acid (212 mmol/mol creat).



Sample	D
Median	1666.5
SD	525.24

Among the 15 labs who performed a specific quantification of **orotic acid**, 13 reported an increase of

this metabolite while 2 mentioned that orotic acid concentration was below the limit de detection of their method. During the teleconference meeting, this participant specified that they would measure again orotic acid.

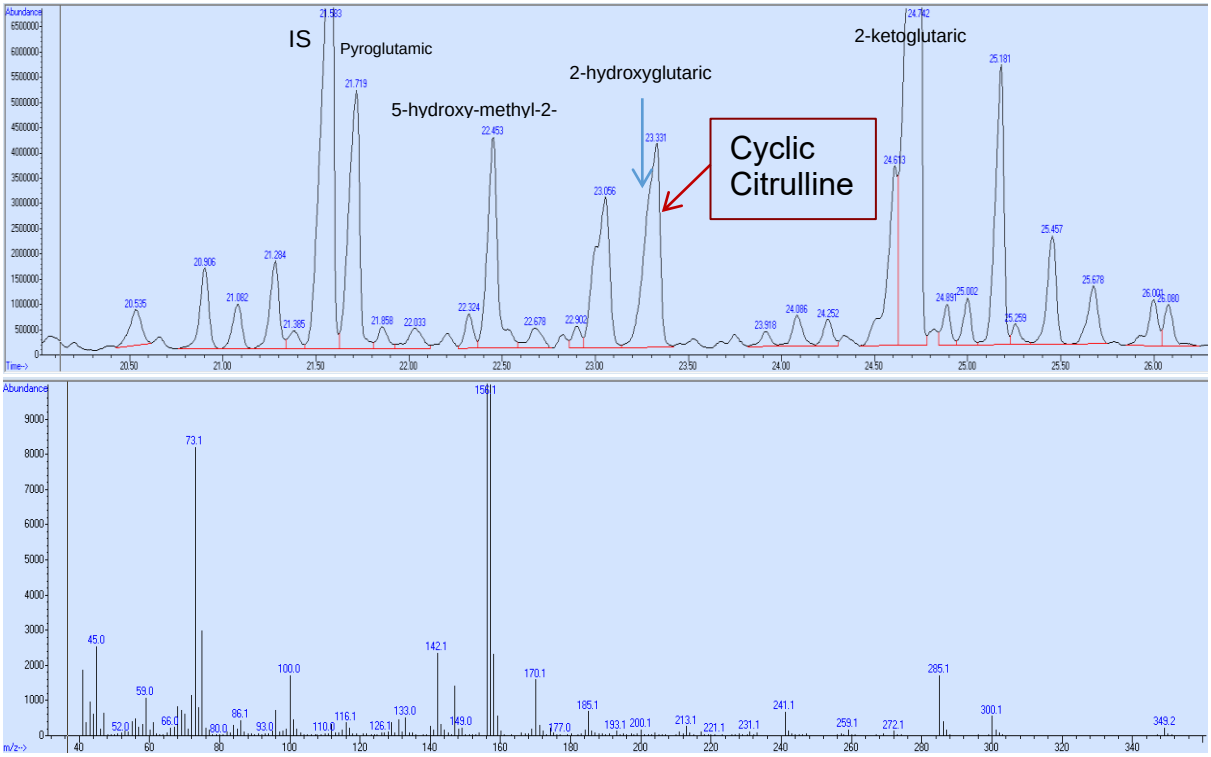


Sample	D
Median	8.8
SD	8.59

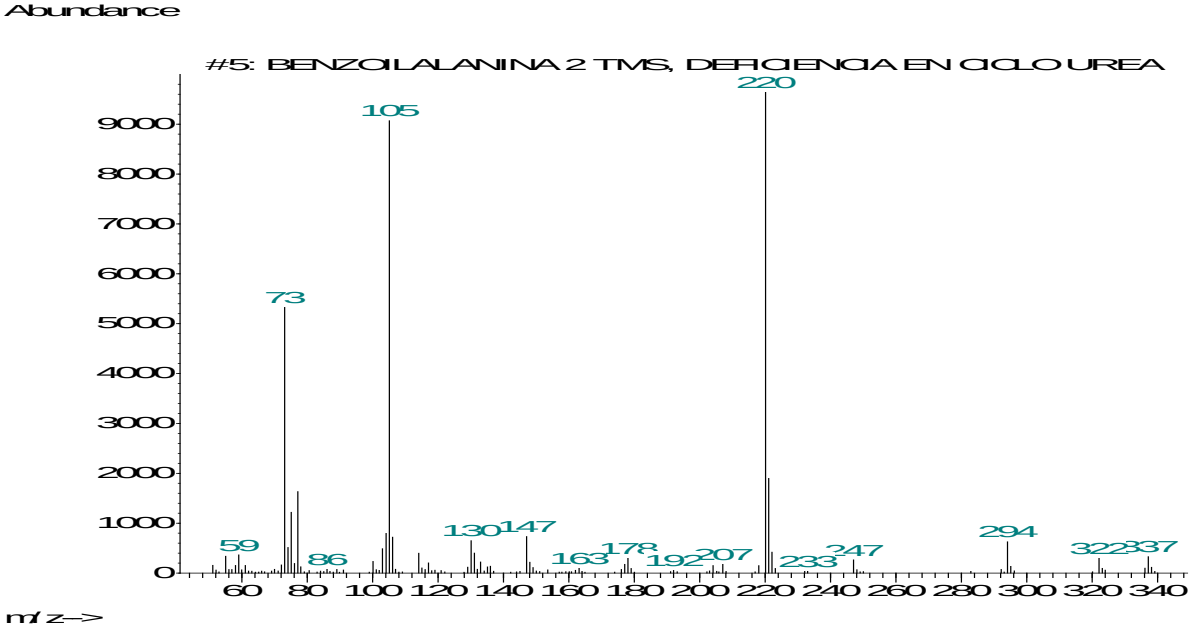
Organic acid analysis was performed by 21 labs. They reported an increase of:

Organic acid	n =	Quantitative results	
		mmol/mol creat	n =
Orotic acid	12	0.6 ; 8.8 ; 415.91	3
Hippuric acid (benzoylglycine)	16	1244 ; 1259	2
Cyclic derivative of citrulline	9		
Uracil	4	4.11 ; 10.0 ; 46.1	3
Phenylacetylglutamine	5		
Benzoylalanine	4		

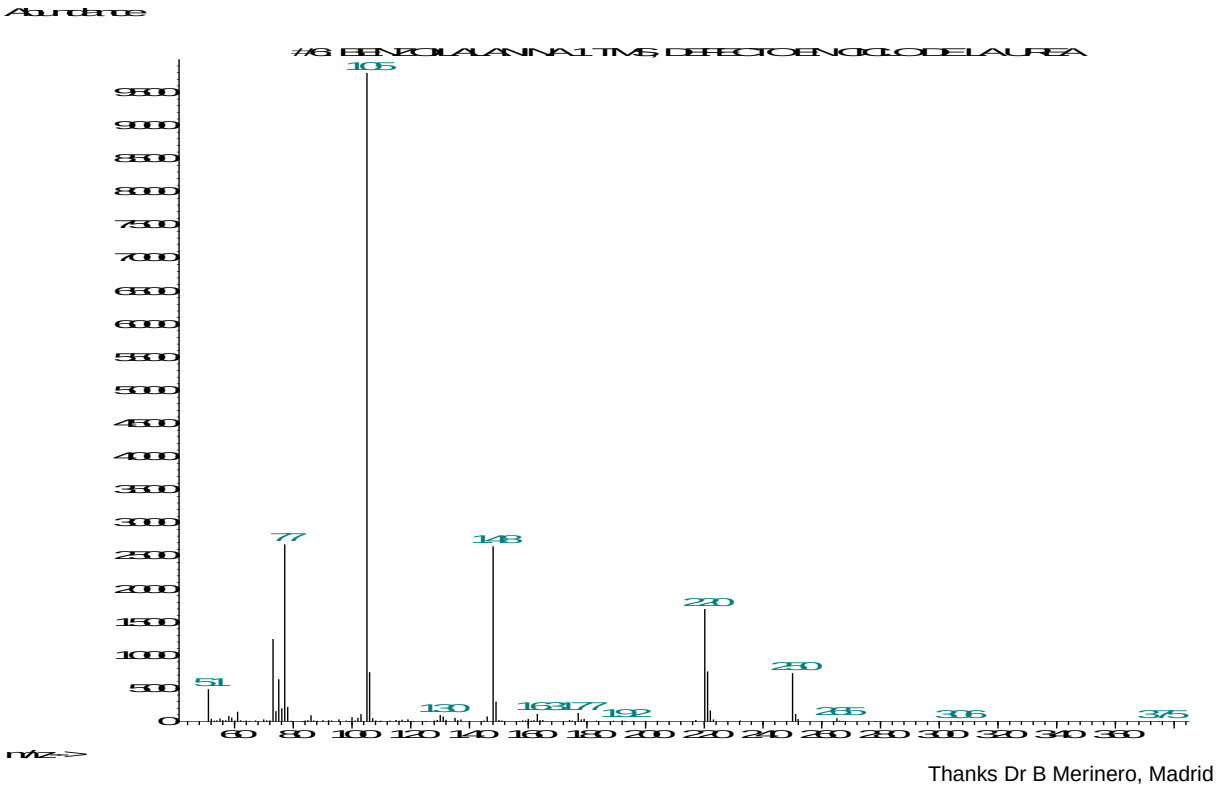
Cyclic citrulline is eluted a few seconds later than 2-hydroxyglutaric acid (see spectrum below).



Benzoylalanine di TMS is eluted between suberic acid and aconitic acid



whereas **benzoylalanine mono TMS** is eluted between homovanillic acid and hippuric acid.



Thanks Dr B Merinero, Madrid

The 2 labs who performed purines / pyrimidine analysis reported an increase of only uracil (32 ; 32 mmol/mol creat)

Diagnosis / Interpretative proficiency

Most likely diagnosis

Citrullinaemia type I	21
Argininosuccinic aciduria	1
Urea cycle deficiency (most probably OTC deficiency)	1

Alternative diagnosis

Citrullinaemia type II	2
Other urea cycle disorder	1
Argininosuccinic aciduria	1

Scoring

- Analytical performance
  - Increase of citrulline (score 1)
  - Increase of orotic acid (score 1)
- Interpretation of results
  - Citrullinaemia type I (score 2)
  - Another urea cycle defect with the recommendation to perform plasma amino acids (score 1)

Overall impression

The overall performance was quite satisfying even if two participants failed to detect an increase of orotic acid excretion. All participants suggested a urea cycle defect and recommended to perform plasma amino acids.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2011 and 2017: the overall performance is slightly lower than in 2017 when all labs diagnosed citrullinaemia type I.

	2011	2017	2020
Analytical performance	95 %	96 %	93 %
Interpretative performance	95 %	100 %	96 %
Overall performance	94 %*	98 %	95 %

(*recommendations were scored separately in 2011

8.1. Patient E

Iminodipeptiduria (prolidase deficiency - PEPD gene)

Patient details provided to participants

This patient was investigated for the first time at 27 years of age because of a slight psychomotor retardation, clubbing of fingers due to severe interstitial lung disease, and chronic eczema. The urine sample has been collected at 33 years of age.

Patient details

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

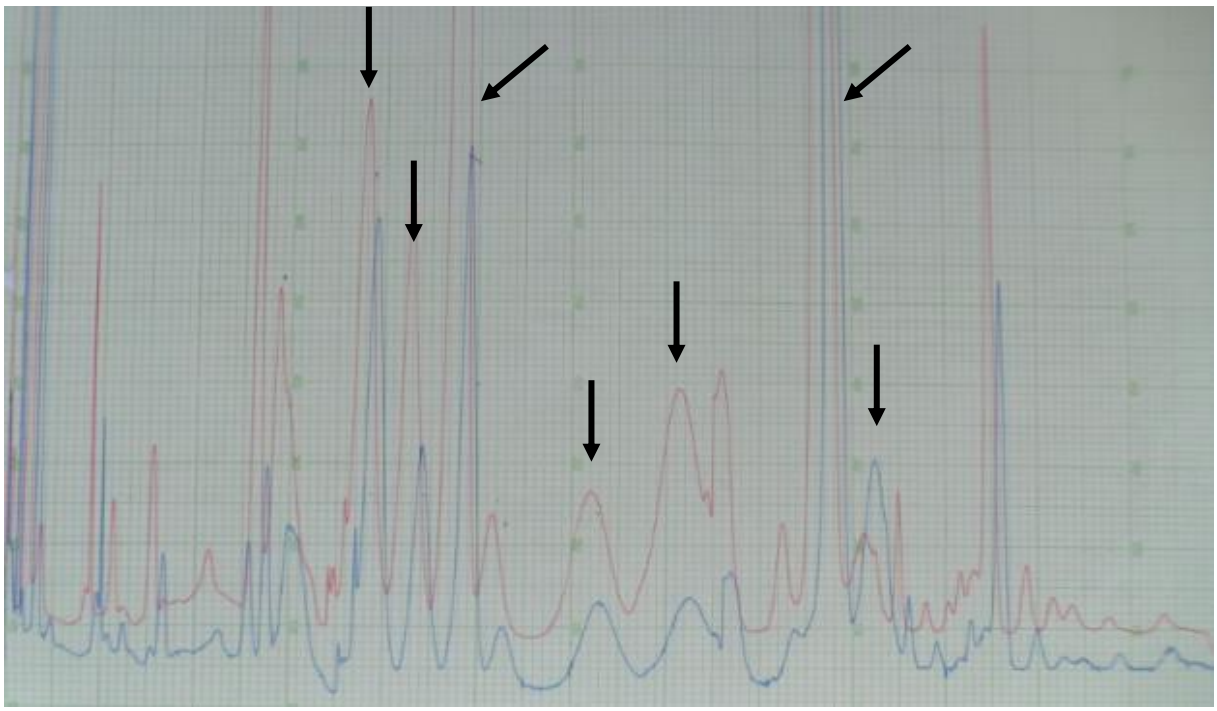
Analytical performance

All participants but one performed **amino acids**, and reported an increase of:

- iminodipeptides 9
- glycyl-proline 6
- proline after acid hydrolysis 4
- glycine after acid hydrolysis 4
- hydroxyproline after acid hydrolysis 2

whereas three of them reported a normal profile, and one an increase of Lys, Arg, Cys, Orn, and homocitrulline.

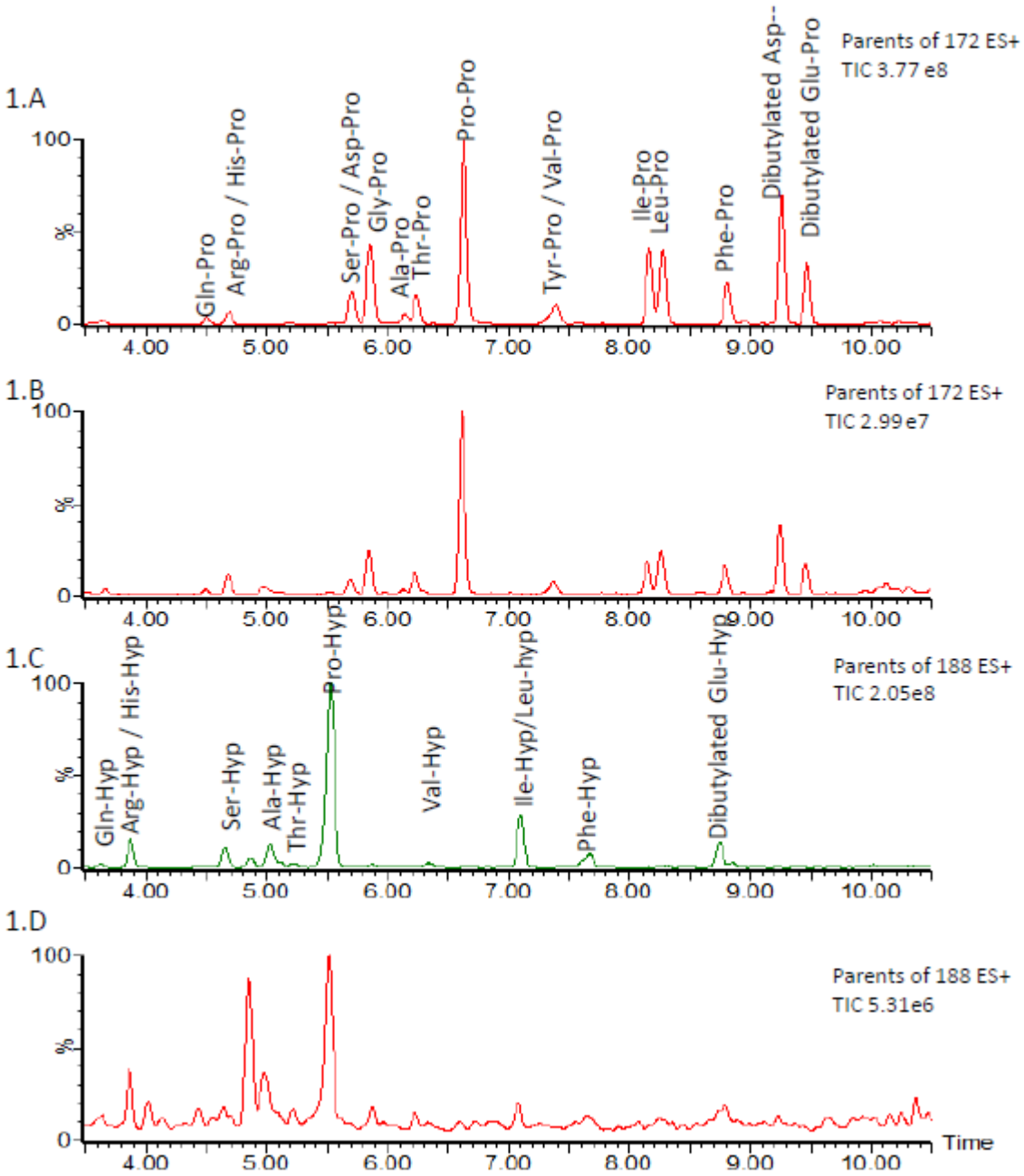
Using ion exchange chromatography, the urinary amino acid profile of prolidase deficiency is characterized by many unknown large peaks, as illustrated in the figure below. Arrows indicate dipeptides.



Tandem mass spectrometry allows the identification of glycyl-proline and eventually other iminodipetides in urine but also in plasma.

Using a HSS T3 column (Varian) and a gradient of ammonium formate 0.1%: acetonitrile (98:2 to 10:90), the group of Robert Debré in Paris (Jean-François Benoist & Apolline Imbard) developed an assay for separation of proline and hydroxyproline dipeptides in the precursor ion scan mode of either

m/z=172 for butylated proline or m/z=188 for butylated hydroxyproline. They obtained the following profile:



Chromatogram of proline- and hydroxyproline dipeptides by LC-MS/MS of their butylated derivatives

The following table gives the m/z ratios of butylated X-Pro and X-Hyp dipeptides, their retention time and product ions:

Precursor	m/z of butylated $[M+H]^+$ *	Retention time	Product ions
Prec 172 (X-Pro iminodipeptides)			
Gln-Pro	300	4,5	84
Arg-Pro	328	4,68	70/112/158
His-Pro	309	4,68	110/93
Ser-Pro	259	5,7	60/87
Asp-Pro	287	5,7	88
Dibutylated Asp-Pro	343	9,26	144
Gly-Pro	229	5,86	70/116/127
Ala-Pro	243	6,14	44
Thr-Pro	273	6,24	74/57
Pro-Pro	269	6,63	70
Tyr-Pro	335	7,39	
Val-Pro	271	7,39	72
Ile-Pro	285	8,16	85
Leu-Pro	285	8,27	85
Phe-Pro	319	8,8	120
Dibutylated Glu-Pro	357	9,45	84/102/158
Prec 188 (X-Hyp iminodipeptides)			
Gln-Hyp	316	3.63	84
Arg-Hyp	344	3.87	70/112
His-Hyp	325	3.87	110
Ser-Hyp	275	4.68	60
Ala-Hyp	259	5.03	NF
Thr-Hyp	289	5.22	74
Pro-Hyp	286	5.53	70
Val-Hyp	288	6.32	72
Ile/leu-Hyp	301	7.10	86
Phe-Hyp	336	7.67	120
Dibutylated Glu-Hyp	374	8.74	84/102/158

m/z ratio, retention time and MS2 product ions of X-Pro and X-Hyp iminodipetides.
***monobutylated ions when non specified**

For the analysis of underivatized Gly-Pro by LC-MS/MS used by the SAs in Lyon (Piraud et al, Rapid Commun Mass Spectrom 2005;19:3287), the monitored transition is m/z 173>116 (declustering potential = 15V, collision energy = 16 eV, internal standard: deuterated 4,4,5,5-D4 DL Lys).

Diagnosis / Interpretative proficiency

Most likely diagnosis

Iminodipeptiduria	18
No diagnosis	3
Lysinuric protein intolerance	1
Possibly Niemann-Pick (more probably type B)	1

Alternative diagnosis

Niemann-Pick type C or Gaucher	1

Scoring

- Analytical performance
  - Increase of iminodipeptides, or prolyl-glycine, or increase of proline, glycine or hydroxyproline after acid hydrolysis (score 2)
- Interpretation of results
  - Iminodipeptiduria (score 2)

Overall impression

The overall performance was somewhat disappointing since, among the 22 participants who performed amino acid analysis, 4 missed the diagnosis. Moreover, 2 of these 4 participants had reached a correct diagnosis in 2017.

Multiple distributions of similar samples

A similar urine sample has been distributed in 2009 and the same urine sample in 2017: the overall performance is better than in 2009 but like 2017.

	2009	2017	2020
Analytical performance	70 %	76 %	78 %
Interpretative performance	65 %	76 %	78 %
Overall performance	63 %*	76 %	78 %

(*recommendations were scored separately in 2009)

8.1. Patient F

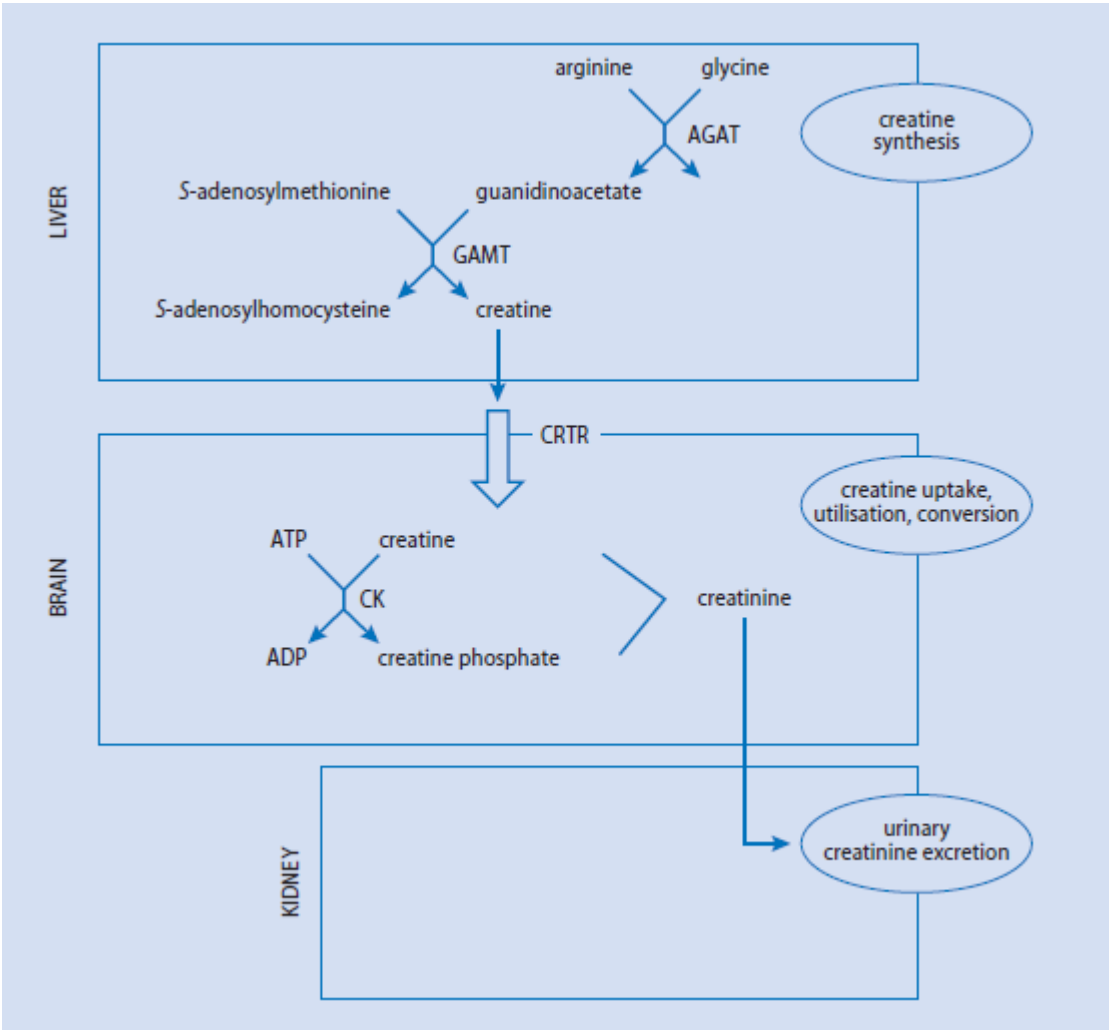
Guanidinoacetate methyltransferase (GAMT) deficiency (GAMT gene)

Patient details provided to participants

7 year-old boy, born from consanguineous parents. At 2 years, he showed language acquisition delay. He presented generalized clonic crises at 3 years and had brain NMR with spectroscopy. Under treatment, crises disappeared but he has altered behavior and intellectual disability.

Patient details

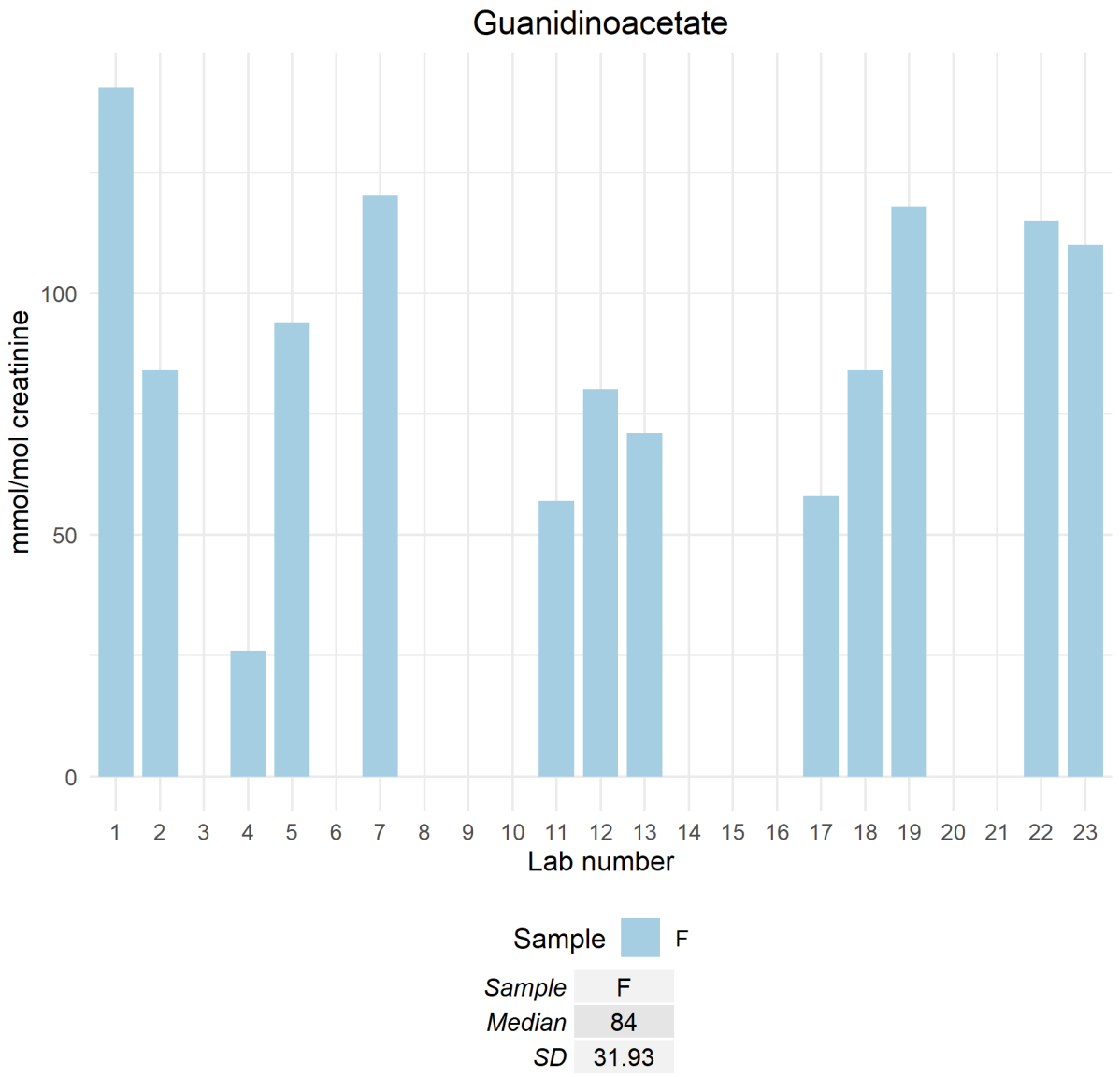
This 7-year-old boy was born from consanguineous parents. At 2 years of age, he showed language acquisition delay. At 3 years of age, he presented generalized clonic crises. Valproate treatment was initiated. Brain NMR with spectroscopy showed creatine deficiency, so valproate was stopped. Diagnosis was assessed by measurement of creatine and guanidinoacetate, and confirmed by mutation analysis of *GAMT* gene. Under creatine and ornithine supplementation, crises disappeared but he has altered behavior and intellectual disability.
Because of the treatment, creatine was grossly elevated, whereas guanidinoacetate was almost normal.



Metabolic pathway of creatine disorders (from Inborn Metabolic Diseases - Diagnosis and Treatment 6th edition)

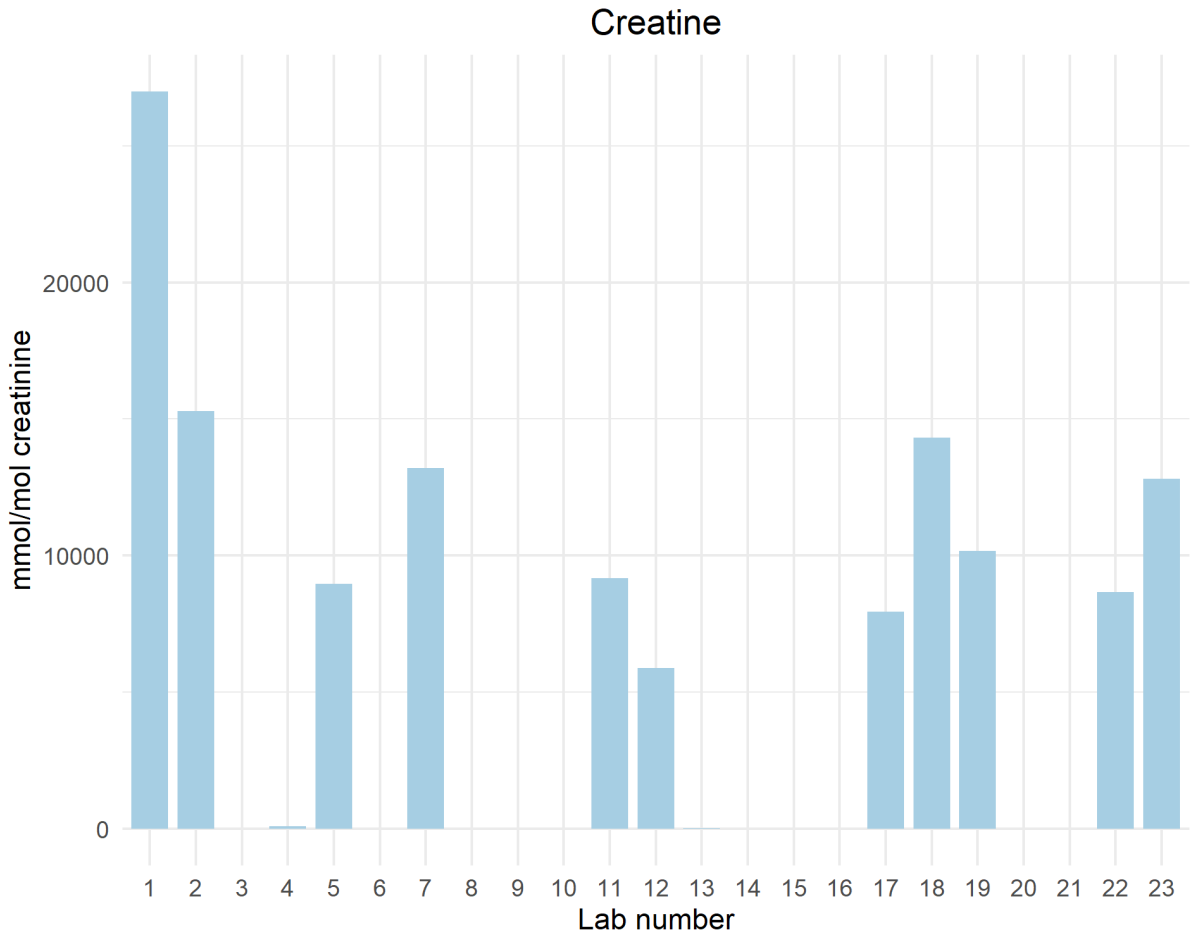
Analytical performance

Fourteen out of the 23 participants performed measurement of **guanidinoacetate (GAA) and creatine**. Three labs mentioned an increase of GAA, but, due to treatment, GAA level was almost normal.



Sample	F
Median	84
SD	31.93

Conversely creatine excretion was quite high due to creatine supplementation. Surprisingly, 2 labs reported a normal excretion of creatine.



Sample	F
Median	9167
SD	6928.21

Diagnosis / Interpretative proficiency

Most likely diagnosis

GAMT deficiency	4
Creatine transporter defect	8
GAMT or creatine transporter defect	1
Creatine disorder	1
AGAT deficiency	1
No IEM	7
Hydroxylysinuria	1

Alternative diagnosis

GAMT deficiency	1
Creatine synthesis defect	1
Creatine transporter defect	1
Hydroxylysinuria	1

Tentative scoring

- Analytical performance
    - Increase of creatine with or without an increase of guanidinoacetate (score 2)
- Interpretation of results
    - GAMT deficiency or creatine transporter deficiency or creatine disorder (score 2)
    - AGAT deficiency as only diagnosis or recommendation to perform plasma or urine creatine and guanidinoacetate measurement (score 1)

Overall impression

Although creatine and GAA measurement does not belong to the required tests for participation to DPT scheme, the overall performance was 64%. Some labs, who did not perform the test, recommended to perform it, leading to an interpretative performance of 72%. However, the SAB considered this sample as **educational** because measurement of creatine and GAA does not belong to the required tests and because it was a difficult sample. Therefore, **it will not be scored**.
The urine sample from an untreated patient with GAMT deficiency was distributed in 2011 (common sample). The SAB also considered it as educational, and it was not scored.

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 PKU			Patient B Alkaptonuria			Patient C MPS IVA			
	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	1	1	2	10
4	1	2	3	2	2	4	2	2	4	11
5	1	2	3	2	2	4	2	2	4	11
6	--	--	--	0	0	0	2	2	4	4
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	2	4	2	2	4	1	2	3	11
12	2	2	4	2	2	4	1	2	3	11
13	1	2	3	2	2	4	2	1	3	10
14	2	2	4	2	2	4	1	1	2	10
15	1	2	3	2	2	4	0	0	0	7
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	0	1	1	9
21	2	2	4	2	2	4	0	0	0	8
22	2	2	4	2	2	4	2	2	4	12
23	2	2	4	2	2	4	2	2	4	12

Detailed scores – Round 2

Lab n°	Patient D Citrullinaemia type I			Patient E Iminodipeptiduria			Patient F GAMT deficiency			
	A	I	Total	A	I	Total	A	I	Total	Total
1	2	2	4	2	2	4	--	--	--	8
2	2	1	3	2	2	4	--	--	--	7
3	2	2	4	2	2	4	--	--	--	8
4	2	2	4	2	2	4	--	--	--	8
5	2	2	4	0	0	0	--	--	--	4
6	2	2	4	0	0	0	--	--	--	4
7	1	2	3	2	2	4	--	--	--	7
8	2	2	4	2	2	4	--	--	--	8
9	2	2	4	2	2	4	--	--	--	8
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	2	2	4	0	0	0	--	--	--	4
14	2	2	4	2	2	4	--	--	--	8
15	2	2	4	0	0	0	--	--	--	4
16	2	2	4	2	2	4	--	--	--	8
17	2	2	4	2	2	4	--	--	--	8
18	2	2	4	2	2	4	--	--	--	8
19	1	2	3	2	2	4	--	--	--	7
20	2	2	4	2	2	4	--	--	--	8
21	1	1	2	0	0	0	--	--	--	2
22	2	2	4	2	2	4	--	--	--	8
23	2	2	4	2	2	4	--	--	--	8

Total scores

Lab n°	A	B	C	D	E	F	Cumulative score	Cumulative score (%)	Critical error
1	4	4	4	4	4	--	20	100	
2	4	4	4	3	4	--	19	95	
3	4	4	2	4	4	--	18	90	
4	3	4	4	4	4	--	19	95	
5	3	4	4	4	0	--	15	75	
6	--	0	4	4	0	--	8	40	CE
7	4	4	4	3	4	--	19	95	
8	4	4	4	4	4	--	20	100	
9	4	4	4	4	4	--	20	100	
10	4	4	4	4	4	--	20	100	
11	4	4	3	4	4	--	19	95	
12	4	4	3	4	4	--	19	95	
13	3	4	3	4	0	--	14	70	
14	4	4	2	4	4	--	18	90	
15	3	4	0	4	0	--	11	55	CE
16	4	4	4	4	4	--	20	100	
17	4	4	4	4	4	--	20	100	
18	4	4	4	4	4	--	20	100	
19	4	4	4	3	4	--	19	95	
20	4	4	1	4	4	--	17	85	
21	4	4	0	2	0	--	10	50	CE
22	4	4	4	4	4	--	20	100	
23	4	4	4	4	4	--	20	100	

Performance

	Number of labs	% total labs
Satisfactory performers (≥ 60 % of adequate responses)	20	87
Unsatisfactory performers (< 60 % adequate responses and/or critical error)	3	13
Partial and non-submitters	0	0

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
DPT-FL-2020-A	PKU	91	100	95
DPT-FL-2020-B	Alkaptonuria	96	96	96
DPT-FL-2020-C	MPS IVA	78	83	80
DPT-FL-2020-D	Citrullinaemia type I	93	96	95
DPT-FL-2020-E	Iminodipeptiduria	78	78	78
DPT-FL-2020-F	GAMT deficiency	--	--	--

10. Annual meeting of participants

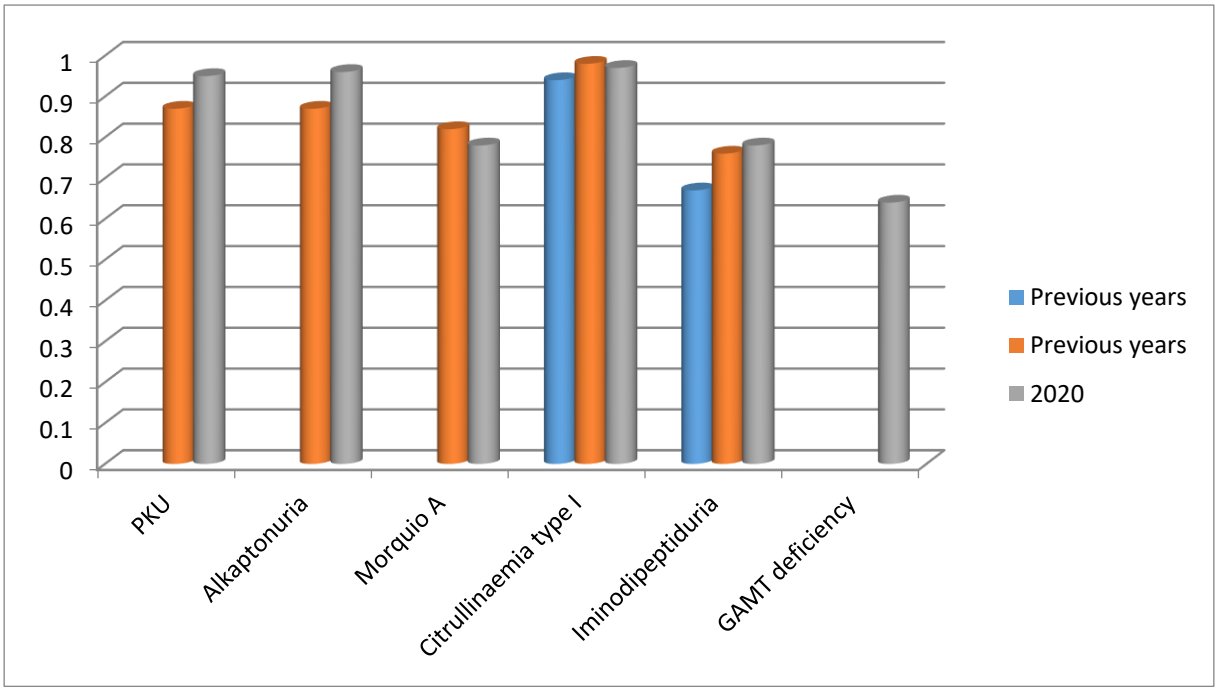
Due to COVID-19 pandemics the meeting took place as a teleconference on September 1st, 2020, from 15:00 to 16:30.

Participants

Representatives from 18 labs were present: Judit Garcia-Villoria (Hospital Clinic, Barcelona), Jose Antonio Arranz Amo (Vall d'Hebron, Barcelona), Arantza Arza Ruesga, Tiziana Gomiero, Maria Unceta Suarez (Bizkaia), Sylvia Funghini (Firenze), Laetitia Van Noolen (Grenoble), Cristina Florindo (Lisboa), Isaac Ferrer, Pedro Ruiz Sala (Madrid), Marguerite Gastaldi (Marseille), Paola Gaia, Laura Saielli (Milano), Elise Jeannesson-Thivisol (Nancy), Michela Bertan, Giulia Polo (Padova), Jean-François Benoist (Robert Debré, Paris), Chris Ottolenghi, Clément Pontoizeau (Necker, Paris), Dulce Quelhas (Porto), Cristiano Rizzo (Roma), Willem Onkenhout (Rotterdam), Bénédicte Sudrie-Arnaud (Rouen), Maria Dolores Bòveda, Daisy Castiñeiras (Santiago de Compostella).

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. The following figure illustrates the improvement for DPT France this year.

Improvement DPT France



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

- Since there will not be a SSIEM meeting in 2021 (International meeting ICIEM in Sidney), the next **Annual Meeting of participants of DPT France** will take place in **Rome** on **October 21st and 22nd, 2021**. Further details will follow.
- **Reference materials** are 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.
- **Training**: SSIEM Academy training courses: due to COVID-19 pandemics, the 2020 and 2021 courses have been cancelled. The next SSIEM Academy will take place in 2022, probably in Amsterdam. Further details will follow.
- **Urine samples**: we remind you that every year, each participant must provide to the scheme organizer at least 200 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 at least 1000 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 (do not send urine spiked with pathological compounds). Please do not 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 (do not 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 2021

Sample distribution	February 9, 2021
Start of analysis of Survey 2021/1 Website open	March 8
Survey 2021/1 - Results submission	March 29
Survey 2021/1 - Reports	April
Start of analysis of Survey 2021/2	June 7
Survey 2021/2 - Results submission	June 28
Survey 2021/2 - Reports	July
Annual meeting of participants	October 21-22 Rome
Annual Report 2021	December

14. ERNDIM certificates 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, 17 February 2021
Name and signature of Scientific Advisor

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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
• 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

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

Version Number	Published	Amendments
1	18 January 2021	2020 annual report published
2	8th February 2021	Page 5, Poor Performance Policy, information for appeal of poor performance added.

END