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ERNDIM
Diagnostic proficiency testing
2009
Southern Europe
Lyon Centre

ANNUAL REPORT 2009

In 2009, 20 labs participated to the Proficiency Testing Scheme Southern Europe.
Organizing Centre: Dr Christine Vianey-Saban, Dr Cécile Acquaviva-Bourdain, Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal, Centre de Biologie et de Pathologie Est, Lyon.

Geographical distribution of participants

Country	Number of participants
France	9
Italy	4
Spain	4
Portugal	2
Switzerland	1
TOTAL	**20**

Logistic of the scheme

- **2 surveys** 2009-1: patient P1, P2 and P3
  2009-2: patient P4, P5 and P6

- **Origin of patients**: 4 out the 6 urine samples have been kindly provided by participants (they will get a 20% discount on the DPT fee for 2010)
- Patient P1: Mucopolysaccharidosis type III, Sanfilippo disease – Centre de Biologie Est, Lyon
- Patient P2: Sialic acid storage disease (Salla disease) – Dr B Fowler, University Children's Hospital, Basel. This sample has been sent to all labs participating to the DPT scheme in Europe
- Patient P3: No metabolic disorder, patient under anti-convulsivant treatment: levetiracetam (Keppra®) and topiramate (Epitomax®) – Centre de Biologie Est, Lyon
- Patient P4: Glutaric aciduria type I – Dr D Quelhas, Dr MR Rodrigues, Instituto de Genetica, Porto
- Patient P5: Iminodipetiduria – Dr B Merinero, Universidad Autonoma, Madrid
- Patient P6: Multiple acyl-CoA dehydrogenase deficiency – Dr E Riudor, Dr J Arranz, Hospital Maternoinfantile Vall d'Hebron, Barcelona

- Mailing: samples were sent by DHL at room temperature.

Timetable of the schemes

- April 27: shipment of samples of Survey 1 and Survey 2 by DHL and of the forms by e-mail
- May 21: deadline for result submission (Survey 1)
- June 23: analysis of samples of the second survey
- July 6: report of Survey 1 by e-mail
- July 14: deadline for result submission (Survey 2)
- August 5: report of Survey 2 by e-mail
- October 23: meeting in Basel
- March 25 2010: annual report with scoring sent by e-mail

Date of receipt of samples

Once again, DHL has been very efficient.

	Survey 1 + 2
+ 24 hours	19
+ 72 hours	1

Date of reporting

All labs sent reports, but with some delay for the two surveys despite reminders from organizers. This will no be possible next year, when submission will be on the website.

	Survey 1	**Survey 2**
	(3 weeks)	(3 weeks)
Receipt of results:	20 labs	20 labs
Before deadline	15	17
+ 1 day	2	2
+ 2 days		1
+ 5 days	2	
+ 1 week	1	
No answer	**0**	**0**

Scoring of results

The scoring system established by the International Scientific Advisory Board of ERNDIM is still the same. Three criteria are evaluated:

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

Since most of the laboratories in Southern Europe don't give therapeutic advices to the attending clinician, this criterion is not evaluated.

The **total score** is calculated as the sum of these 3 criteria without weighting. The maximum that can be achieved is 5 for one sample.

Meeting of participants

It took place in Basel on 23 October 2009 from 9.00 to 10.30, during the ERNDIM Meeting.

❖ Participants

Representatives from 10 labs were present: MH Read (Caen), S Funghini, E Pasquini (Florence), U Caruso (Genova), O. Boulat, C. Roux (Lausanne), R. Ramos, I. Tavares de Almeida (Lisbon), B. Merinero (Madrid), E. Jeannesson (Nancy), C. Ottolenghi (Necker, Paris), O. Rigal (Robert Debré, Paris), C Rizzo (Roma).

❖ Information from the Executive Board and the Scientific Advisory Board

- Certificate of participation for 2009 will be issued for participation and it will be additionally notified whether the participant has received an assistance letter. This assistance letter is sent out if the performance is less than 50%. **Caution**: the definition of poor performers will change in 2010: score < 18 / 30 or 60% (see later).
- No assistance letter has been sent in 2009.
- Reference materials provided by SKML (mix of the four samples of the scheme) are still available and can be ordered through the ERNDIM website. We encourage you to use them as internal control, but they should not be used as calibrants.
- The availability of the website reporting system has been delayed. It will be most probably available in 2010 for all centres.
- The cost of the scheme has increased in 2010 because ERNDIM will employ a scientist for accreditation and training.
- **Training**: SSIEM Academy training courses.
    - A 2 days course has been organized on Tuesday and Wednesday 20 & 21 October 2009 on organic acidurias. 42 biochemists attended this meeting. The lectures are available on

the SSIEM website.

- The next course will take place on 4 and 5 October 2010 in Manchester: the subject has to be defined. Information 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 a "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. 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:

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

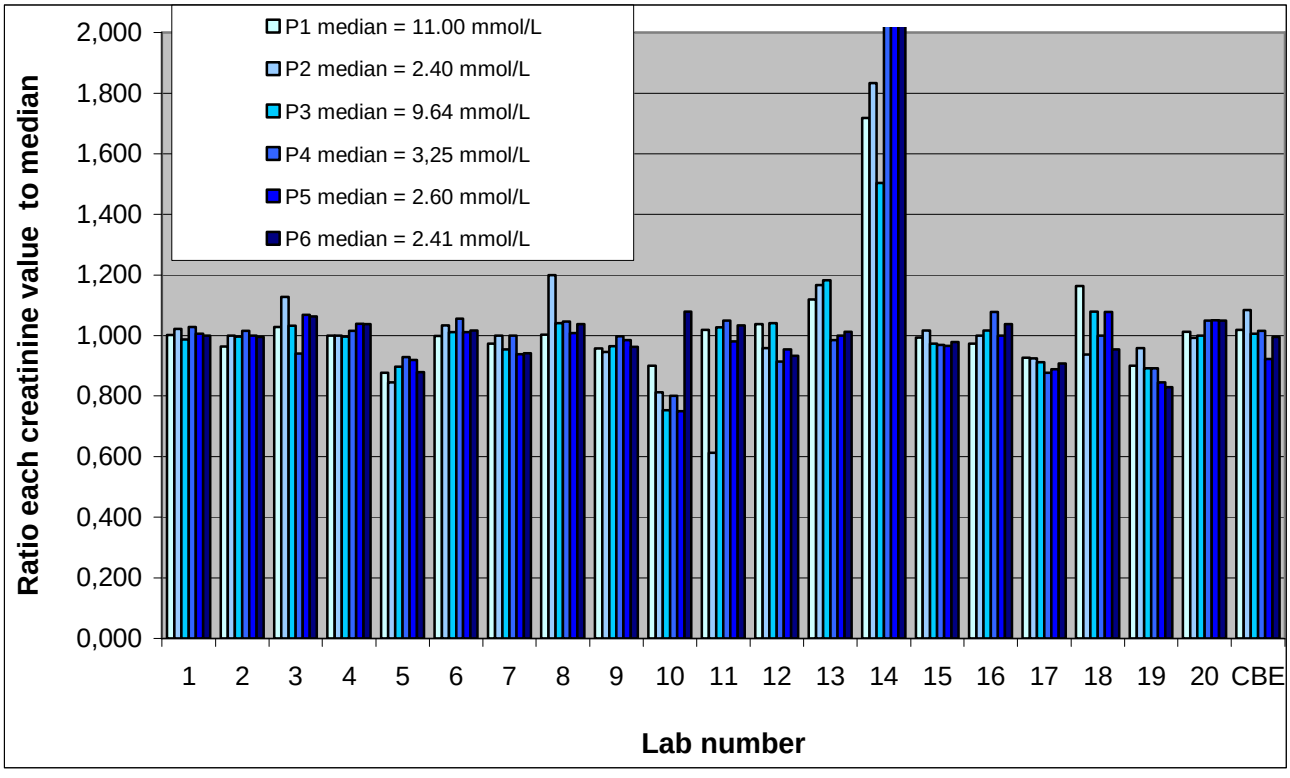
  Please send us an e-mail on the day you send the samples.

- **Lab identification**: since 2007, it has been accepted that the ERNDIM number is used for "in centre" communication but anonymous identification is used for the Annual Report on the website or other purposes.

❖ Discussion of results

• Creatinine measurement

Results greatly improved compared to last year, except for one lab.



Lab 14 has systematically very high values, while labs 5, 10, 17 and 19 have systematically slightly low values. If we exclude results from lab 14, the CV is < 8% for all samples except for P2 (CV = 12.8%) but it corresponds to the sample with the lowest creatinine value (creat = 2.4 mmol/L). These CV are comparable with the interlab CV 2008 for Special Assay in urine (= 7.2 %) and with the interlab CV 2008 for Quantitative organic acids (= 5.9 %).

- **Patient P1 – Mucopolysaccharidosis type III C, Sanfilippo disease**

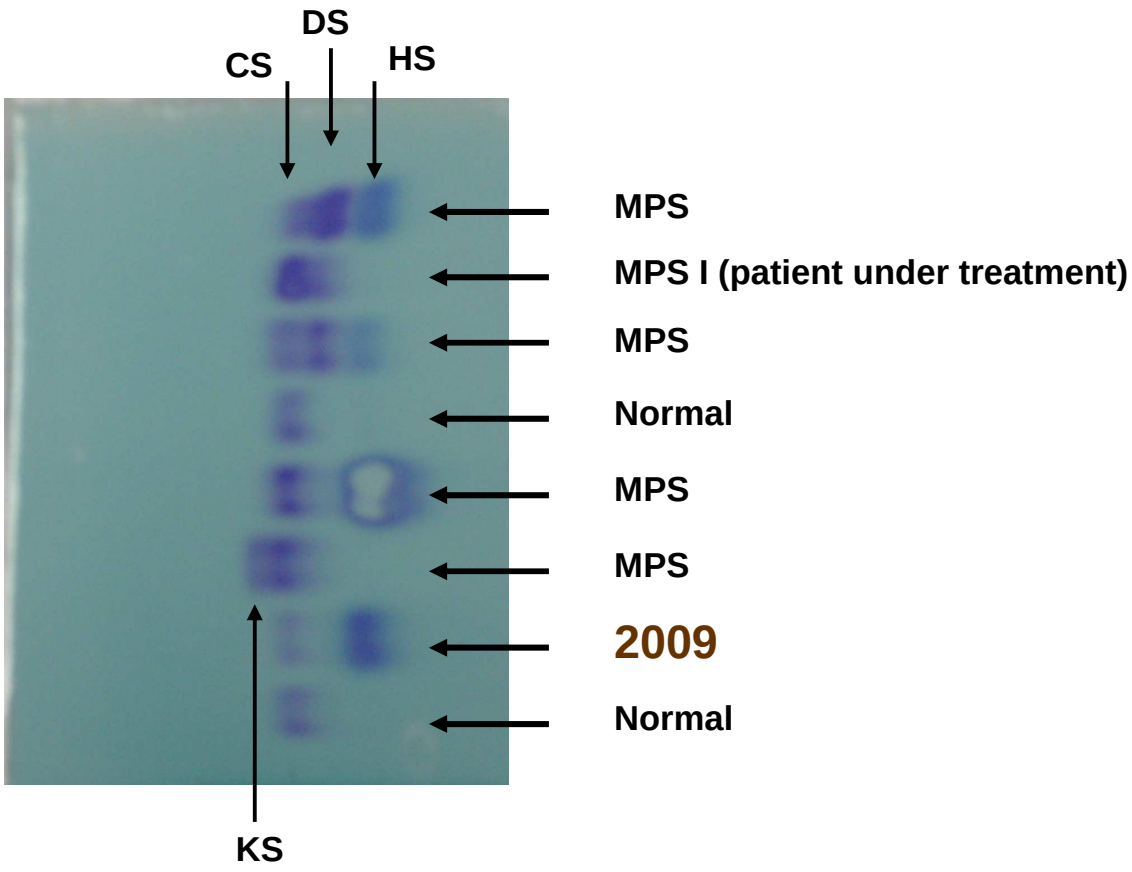
The urine sample was collected from a 11 year-old girl who presented hepatomegaly since the first months of age, with slight cytolysis. She also had digestive troubles with diarrhoea, recurrent otites but no articular signs, a normal cardiac examination, and no gingival hypertrophy. She had a progressive mental retardation with sometimes an aggressive or restless behaviour.
Diagnosis was suspected at the age of 5 years. Mucopolysaccharides were elevated (69.7 mg/g creat – controls: 6 – 23 mg/g creat) and electrophoresis revealed an increase of heparan sulphate.
Confirmation of diagnosis of MPS IIIC was performed in leukocytes (Dr R. Froissart, Lyon): activity of N-acetyl-CoA: α-glucosaminide N-acetyltransferase = 0.01 mKat/kg prot – simultaneous control = 0.35. Other enzyme activities for MPS III were in the range of controls: type IIIA: heparan-N-sulfatase, type IIIB: N-acetyl α-glucosaminidase, type IIID: N-acetyglucosamine-6-sulfatase.

Diagnosis

Sixteen labs reached the diagnosis of mucopolysaccharidosis type III and 1 lab concluded to mucopolysaccharidosis by identification of an increase of GAG's, but diagnosis of MPS III has been performed after the report.

Three labs gave no or a wrong diagnosis: one of them reported a normal quantification of GAG's, the second reported a normal electrophoretic pattern of GAG's, while the third did not investigate mucopolysaccharides at all. However, this is a slight improvement compared to last year when 5 labs missed this diagnosis.

The following figure illustrates the electrophoresis pattern we obtained for patient 2009-P1.



CS: chondroitine sulphate, DS: dermatane sulphate, HS: heparane sulphate, KS: keratane sulphate

Among the 15 labs who performed **oligosaccharide** profile, 12 reported a normal profile, 2 an abnormal profile probably related to the mucopolysaccharidosis, and one an ambiguous profile.

Advice for further investigations was OK for those who reached a correct diagnosis

Scoring

- Analytical: Increase of GAG and heparane sulphate (score 2), increase of GAG (score 1)
- Interpretation of results: MPS III (score 2), mucopolysaccharidosis (score 1).
- Recommendations: activity of the 4 enzymes possibly deficient in MPS III and/or mutation analysis of the corresponding genes and/or MPS analysis on a new urine sample (score 1).

- **Patient P2 – Sialic acid storage disease (Salla disease)**

The patient is a girl, born from unrelated parents with no known Finnish ancestors. At 6 months of age, hypotonia was reported but her psychomotor development was otherwise considered as normal. At 14 months, the child's gross motor development was delayed at the level of 9 m. The next two-three years, she presented a slow progression, but no deterioration with slowly increasing hypertonicity of the lower limbs. At 4.5 year, she was active and co-operative although slightly retarded. She was unable to walk unsupported, with a severe cerebellar ataxia, severe dyskinetic movements sometimes resembling myoclonic jerks. She also had signs of spastic paraparesis, brisk tendon reflexes, pseudoclonus of the ankles. She speaks only a few words. She is now 6 years, and presents a slow

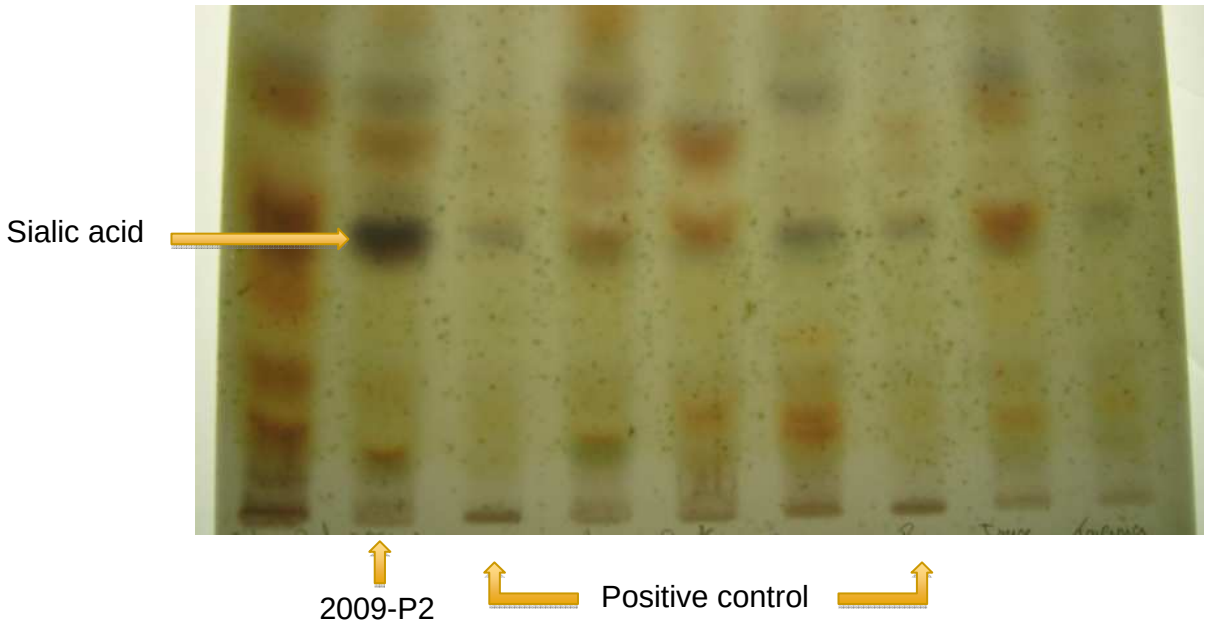
deterioration, with even greater motor disability. She has severe dysarthria and she is considered as slightly intellectually delayed. Mutation analysis *SLC17A5* gene revealed that she is homozygous for the Finnish sialin mutation p.Arg39Cys. Sialin is a lysosomal membrane protein that transport sialic acid out of lysosomes.

This sample has been sent to all DPT centers. The presentation of Brian Fowler, during the ERNDIM meeting in Basel on October 23, concerning this common sample is available on the ERNDIM website: http://www.erndim.org

Diagnosis

Only 2 labs concluded to sialic acid storage disease, whereas 18 labs gave no or a wrong diagnosis.

The method we use for the screening of free sialic acid using TLC is: Michalski et al, Clin Chim Acta, 1983;129:99-101.



Briefly, it uses:

- Silicagel plates (20 cm x 20 cm)
- Volume of urine: corresponding to 15 mg creatinine
- Solvent: n-butanol / 96 % acetic acid / water (2:1:1 v/v)
- Migration: overnight
- Staining: orcinol chlorhydric 15 minutes 150 ℃
- Interpretation is qualitative and age dependent

If the spot for sialic acid (N-acetylneuraminic acid) seems increased, quantification is performed.

The method we use for quantification of free sialic acid is the Warren method modified by Roboz et al. (Anal. Biochem 1981;110:380-388). The principle is:

- Oxidation by periodic acid into 3-formylpyruvic acid
- Reduction of periodic acid in excess by sodium arseniate
- Addition of thiobarbituric acid: the red chromophore is extracted by cyclohexanone (549 nm)

Four labs reported results for quantification of free sialic acid: 2 increased value and 2 normal results.

During the meeting of our DPT scheme in Basel, several labs informed us that they performed either TLC or quantification for sialic acid, and obtained normal result(s).

We therefore asked all labs for further details. The results are reported in the following table.

	Before the ERNDIM meeting		After the ERNDIM meeting		Sample re-analyzed in our lab	
lab	**TLC**	**Quantification ***	**TLC**	**Quantification ***	**TLC**	**Quantification ***
1	-	-				
2	-	-	+++			
3	N¤	45 (N<60)¤				
4	N¤	45 (N<60)¤				
5		82 (N<224)			+++	81 (N<50)
6						
7	N	-			+++	80 (N<50)
8						
9	-	-	-			
10	N¤	45 (N<60)¤				
11	N	-				
12						
13	-	-				
14						
15						
16	++	86 (N<50)				
17	-	-	-	91,5 (N<50)		
18	N	-				
19		119 (N<43)				
20						
CBE	+++	86 (N<50)				

* mmol/mol creat; – : not performed; empty cell: information not available; N: normal; ¤ Assays performed by the same lab

Advice for further investigations was OK for the few labs who reached a correct diagnosis

Scoring

- Analytical: Increase of sialic acid on TLC and/or quantification (score 2), doubt on oligosaccharides (score 1)
- Interpretation of results: sialic acid storage disease (score 2).
- Recommendations: mutation analysis SLC25A5 gene and/or confirm sialic acid excretion on a new urine sample and/or investigate lysosomal transport defects (score 1).

- **Patient P3 – No metabolic disorder, patient receiving anticonvulsivant treatment: levetiracetam (Keppra®) and topiramate (Epitomax®)**

This girl is born from non consanguineous parents. She presented progressive psychomotor retardation and autistic traits. She started to have seizures from the age of 6.5 years. EEG revealed slow activity with paroxystic abnormalities. Urine has been collected at 12 years of age when the patient was receiving anticonvulsivant treatment: levetiracetam (Keppra®) and topiramate (Epitomax®). All metabolic investigations were negative in urine. Plasma and CSF amino acid profiles were normal. Activity of lysosomal enzymes in leukocytes was in the control range, excluding GM1, GM2 gangliosidosis, metachromatic leukodystrophy, and β-mannosidosis. Peroxisomal investigation, including VLCFA, pristanic, phytanic and pipecolic acids, was normal. CDG was also excluded.

Diagnosis

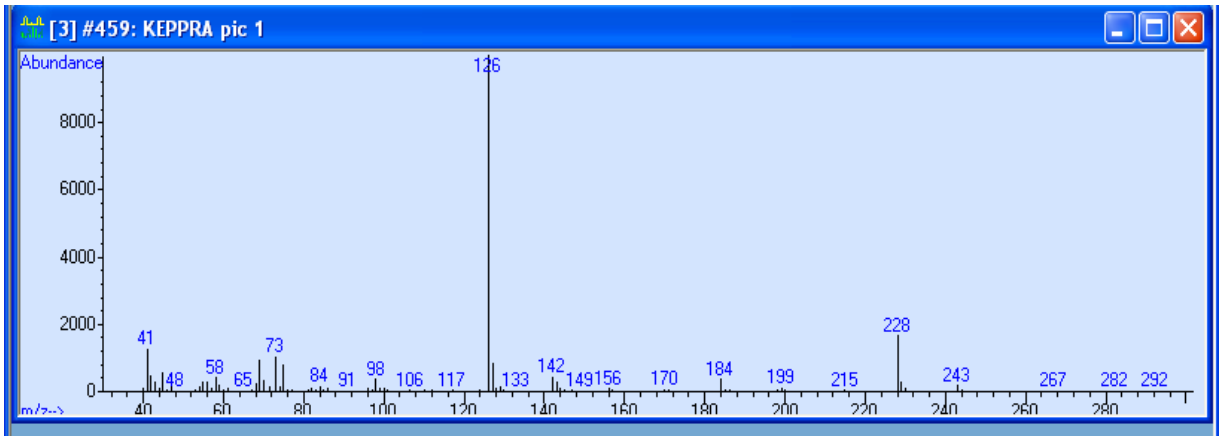
Two labs concluded to patient under anticonvulsivant treatment with topiramate, and 16 labs reported no relevant abnormalities (or can be secondary to treatment) with the performed investigations. Conversely, 2 labs gave a wrong diagnosis

All labs performed **amino acid** analysis and all reported no significant abnormality, except one who reported an increase of alpha-aminoadipic acid (69 mmol/mol creat) that we could not confirm using tandem MS (3 mmol/mol creat): this can be due to drug interference.

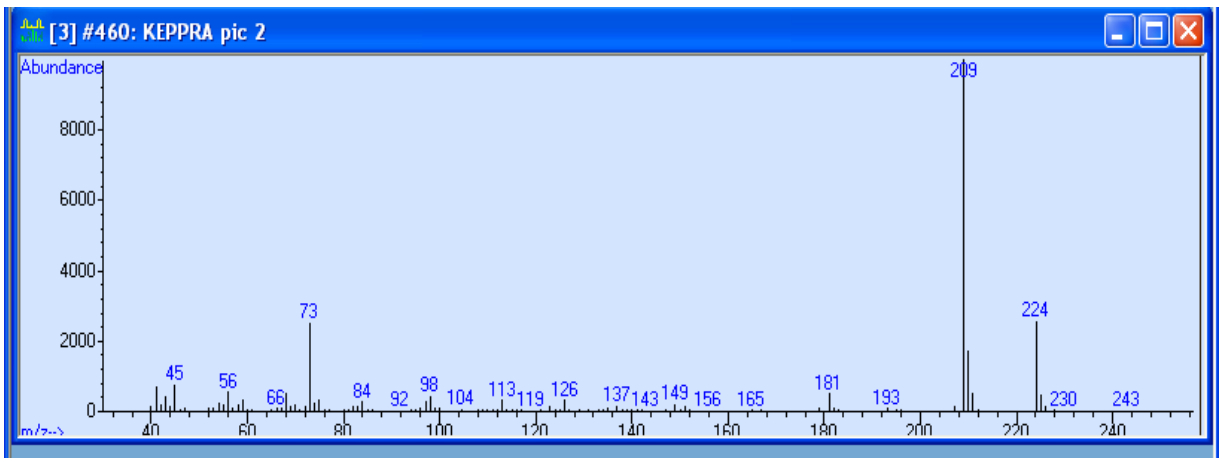
All labs also performed **organic acid** analysis but only 9 of them reported drug metabolites: paracetamol (6), levetiracetam (4), topiramate (2), and ibuprofen (1).

We obtained the following spectra for levetiracetam (Keppra®)

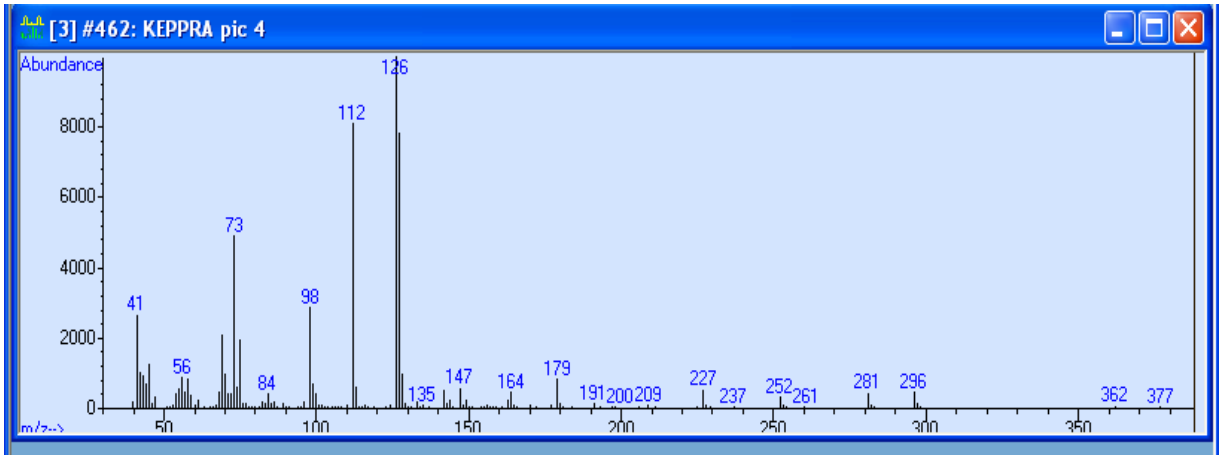
- Peak 1: eluted between 3-methyl adipic and 2-hydroxyglutaric acid



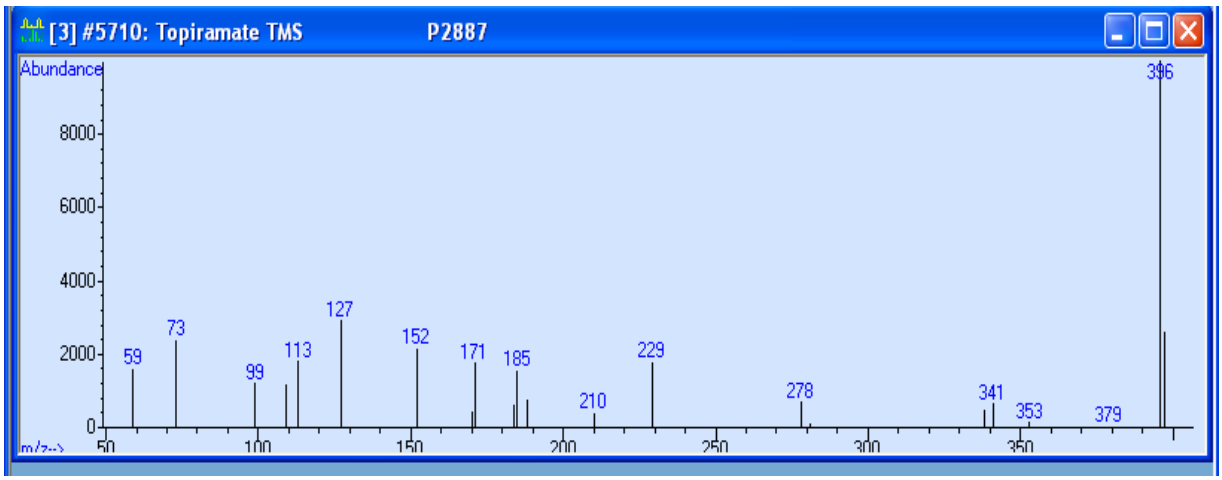
- Peak 2: eluted less than 1 minute after peak 1



- Peak 4: eluted just before 4-hydroxyphenyl acetic acid



And for topiramate (Epitomax®): eluted between 2-ketoglutaric and N-acetylaspartic acids



Mucopolysaccharide quantification was performed by 15 labs; all reported normal values, except one who advised to perform GAG identification.

Also 15 labs performed **oligosaccharides**: all reported a normal profile except one who concluded to a possible fucosidosis.

Advice for further investigations: various!

Scoring

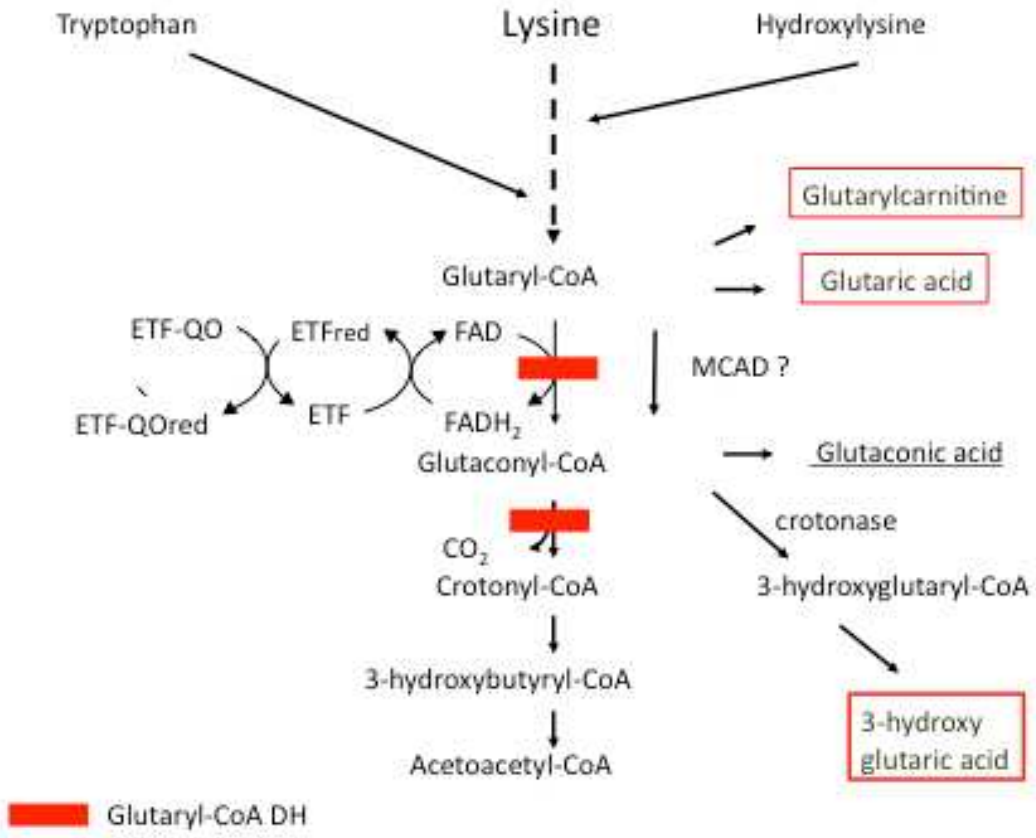
- Analytical performance: all investigations (amino acids, organic acids, oligosaccharides, mucopolysaccharides): no significant abnormalities (score 2), all but one investigations: no significant abnormalities (score 1).

- Interpretation of results: patient under anti-convulsivant treatment (score 2), no significant abnormality with the performed investigations (score 1)

- Advice for further investigations: exclude disorders (metabolic or not) which can be responsible for seizures (score 1)

- **Patient P4 – glutaric aciduria type I (glutaryl-CoA dehydrogenase deficiency)**

The urine sample was collected from a 42-year-old female with slight psychomotor delay. From the age of 39 years, she presented dystonic dyskinetic movement disorder. MRI exhibited frontal lobe atrophy. Urinary organic acids revealed a high increase of glutaric acid (2552 mmol/mol creat – controls: <2.6) and, to a lower extent, of 3-hydroxyglutaric acid (69 mmol/mol creat – controls: ND). Lysine was elevated in plasma (249 µmol/L – controls : 40 – 162) and urine (77 mmol/mol creat – controls : 7– 58). Glutarylcarnitine level in plasma was 0.51 µmol/L (controls: < 0.08). Mutation analysis of *GCDH* gene revealed homozygosis for the c.1204C>T (p.Arg402Trp) mutation. This mutation has been demonstrated to be deleterious, leading to the synthesis of an inactive enzyme, which is rapidly degraded in mitochondria, and with impairment of the formation of homotetrameric enzyme (Keyser et al, Hum Molec Genet. 2008;17:3854). Glutaric aciduria type I is due to glutaryl-CoA dehydrogenase deficiency, an ETF dependent bifunctional enzyme of mitochondrial matrix. Pathophysiology is due to high concentrations of glutaric and 3-hydroxyglutaric acids in the brain (5 times higher than in liver) due to an *in situ* production and to the poor permeability of the blood brain barrier for dicarboxylic acids.

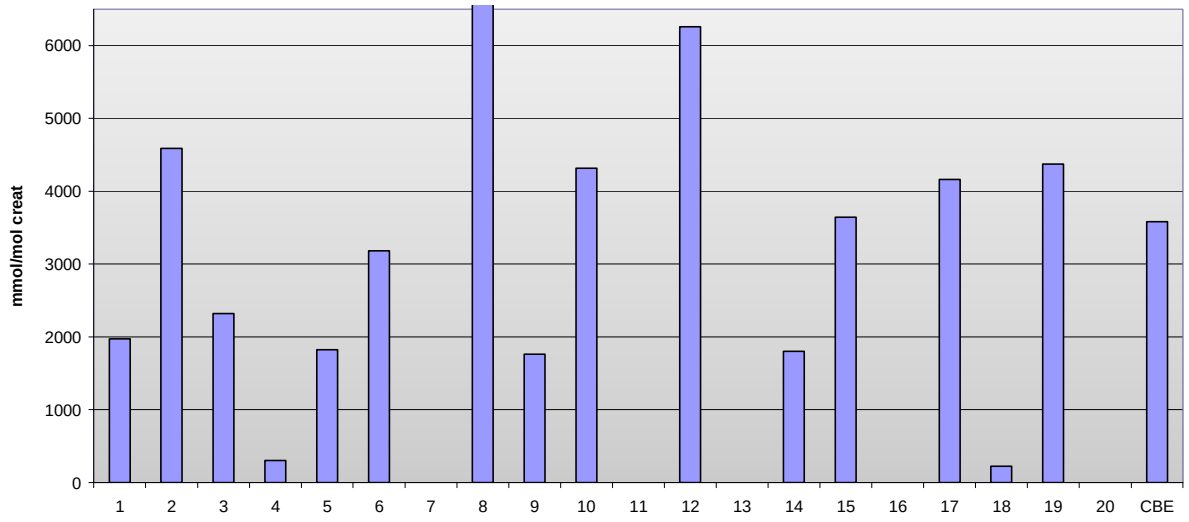


Diagnosis

All labs concluded to glutaric aciduria type I (or to possibly glutaric aciduria type III because of the low excretion of 3-hydroxyglutaric acid for one lab).

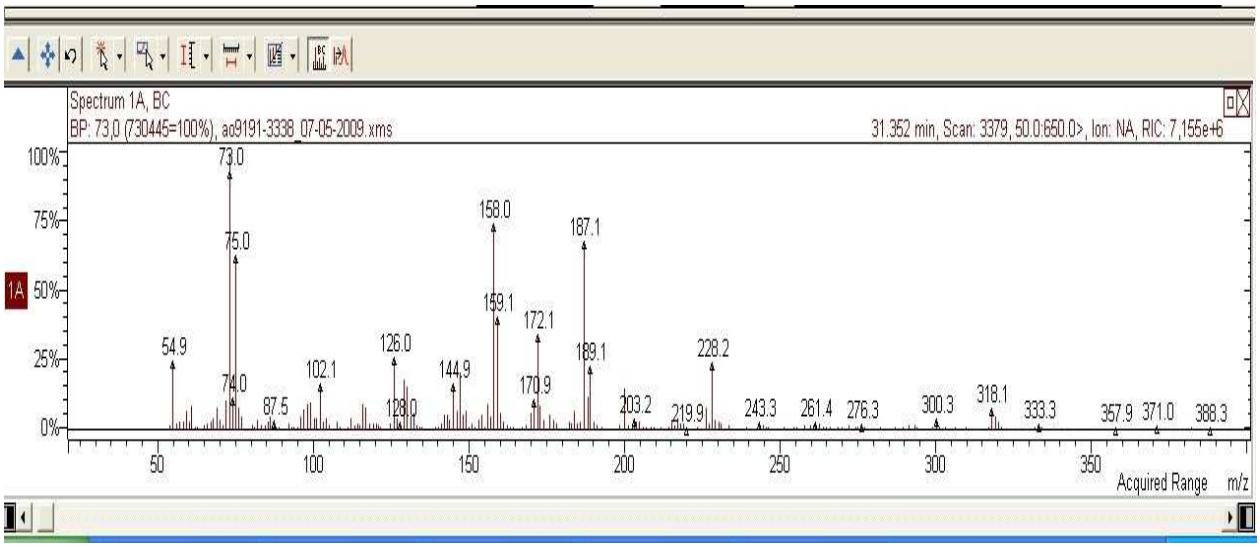
All labs reported an increase of glutaric acid, but there was a very wide range of results (229 to 6 257 mmol/mol of creatinine) and therefore a coefficient of variation of 53% (a value of 101 262 mmol/mol creat was even excluded!) which is much higher than the CV observed for the quantitative organic acids ERNDIM scheme 2008 (CV = 26 %).

Glutaric acid - median = 3 381mmol/mol creat - CV = 53% (lab 8 excluded)



All labs also reported an increase of 3-hydroxyglutaric acid, but the range of results was also very wide: 21 – 4 934 mmol/mol creat (CV = 81 %). Unfortunately, no standard is available for 3-hydroxyglutaric acid quantification.

Interestingly, two labs reported an increase of glutarylglycine: one by tandem MS and the second by GC/MS. With GC/MS, glutarylglycine is eluted 1 minute later than vanilmandelic acid. Its spectrum has been kindly provided by Dr Fontaine and Dr Briand from Lille.



Interpretation and recommendations were appropriate.

Scoring

- Analytical : increase of glutaric acid (score 1), increase of 3-hydroxyglutaric acid (score 1)
- Interpretation of results: glutaric aciduria type I (score 2)
- Advice for further investigations: mutation analysis *GCDH* gene and/or glutaryl-CoA dehydrogenase activity and/or blood acylcarnitine profile (score 1)

- **Patient P5 – Iminodipeptiduria (prolidase deficiency)**

The urine sample was collected from a 17 month-old boy, born from consanguineous parents. He presents a coarse face, hepatomegaly (5 cm), splenomegaly (3.5 cm) and a psychomotor delay. Biochemical investigation revealed a persistent microcytic anaemia (haemoglobin = 9.8 g/dl, hematocrite = 31.5 %, VCM = 71) and increased LDH (1500 – 1700 U/L). He also had a mild pruritus in legs. The patient is now 26 months old. He presents a severe neutropenia which does not respond to conventional therapy, and recurrent infections, but no skin lesions nor hepatosplenomegaly.
Amino acid profile at the time of diagnosis was:

- Plasma: Gly-Pro = 25.6 µmol/L – controls: ND
- Urine: Gly-Pro = 7928 mmol/mol creat – controls: ND
  Pro = 9.5 mmol/mol creat – controls: <13
  Hyp = ND

After acid hydrolysis of urine, proline and hydroxyproline were highly elevated (4441 and 287 mmol/mol creat, respectively).
Prolidase activity in cultured skin fibroblasts was decreased: 13.4 % of controls (Dr Forlino, Pavia).
Analysis of *PEPD* gene revealed that the patient is homozygous for the change p.Leu435Phe in exon 14 (Dr Forlino, Pavia).

Diagnosis

Only 13 labs concluded to iminodipeptiduria.

All labs except one performed **amino acids**. The presence of abnormal peaks, probably Pro dipeptides, and/or an increase of Gly-Pro dipeptide was reported by 12 of them. Two labs reported the presence of unknown peaks. But 3 labs reported no significant abnormality: one of these labs obviously inversed this urine sample with the urine from P6! One lab reported an increase of lysine, saccharopine and cystine leading to the wrong diagnosis of familial hyperlysinemia type I.

Height labs confirmed the abnormal excretion of Pro dipeptides by performing amino acid analysis after acidic hydrolysis. Two labs reported an increased concentration of Gly-Pro using tandem MS.

Organic acids were analysed by 17 labs: all reported a normal or not diagnostic profile.

Fourteen labs performed **GAG quantification** and/or **electrophoresis** pattern: only one lab concluded to the wrong diagnosis of mucolipidosis type I, due to a slight increase of GAGs.

Among the 15 labs who performed **oligosaccharide profile,** one reported a profile consistent with aspartyglucosaminuria, another with galactosialidosis and a third one, a profile similar to Pompe disease.

Advice for further investigations was correct for those who reached the right diagnosis.

Scoring

- Analytical: increase of Pro dipeptides or Gly-Pro dipeptide on amino acids profile or by tandem MS (score 2), unknown peaks on amino acid profile (score 1)
- Interpretation: iminodipeptiduria (score 2)
- Recommendations: prolidase activity (hemolysates, leukocytes, or fibroblasts) and / or mutation analysis *PEPD* gene and / or amino acid analysis after acid hydrolysis (score 1)

- **Patient P6 - multiple acyl-CoA dehydrogenase (MAD) deficiency**

The patient, a girl, was born prematurely (35 weeks). She was the product of a bi-chorial, bi-amniotic gestation. She previously presented two episodes of pneumonia treated at home. At 3.5 years, during the course of chickenpox plus pneumonia requiring admission to intensive care unit, she presented a septic shock by pseudomonas aeruginosa. Diffuse lesions in the myelin were observed. At 4 years, she was admitted with fever and partial food rejection without vomiting, with metabolic acidosis partially compensated. She improved quickly. Neurological follow up revealed, in addition to the already described CNS lesions, discrete hypotonia, moderate attention deficit and clumsiness. A metabolic work up was performed which revealed a strikingly abnormal organic acid profile.

mmol/mol creatinine	Patient	Control
EMA	59	<11
Glutarate	33.7	<5
2-methylsuccinate	21.4	<5
2-hydroxyglutarate	134.8	<25
Isobutyrylglycine	51.7	ND
Isovaleryglycine	10.4	ND
Hexanoylglycine	82.1	ND
Suberylglycine	75.3	ND

Sarcosine was increased in plasma (133.4 µmol/L). Riboflavine and carnitine treatment led to an improvement in attention and muscular strength. Palmitate oxidation in fibroblasts was decreased but mutation analysis of *ETFDH*, *ETFA* and *ETFB* genes is still under investigation. Her twin has a normal development and normal organic acids. The urine sample has been collected at home at 6.5 years of age.

Diagnosis

All labs concluded to multiple acyl-CoA dehydrogenase deficiency, but one concluded to MAD deficiency associated to prolidase deficiency!

All labs performed **organic acid** analysis. The results are summarized in the following table.

Organic acids: all labs	Median mmol/mol creat (control range)	Number of labs
Ethylmalonic acid	80 (<11)	20
Glutaric acid	74.5 (<5)	18
Isovalerylglycine	29 (ND)	18
Isobutyrylglycine	42 (ND)	18
Hexanoylglycine	39 (ND)	17
Suberylglycine	8.5 (ND)	15
2-hydroxyglutaric acid	165 (<25)	15
Methylsuccinic acid	22 (<5)	7
Butyrylglycine	+	5

Although ethylmalonic acid, glutaric acid and hexanoylglycine are included in the ERNDIM quantitative organic acid scheme, the CV for these compounds was higher than the CV for the QAP: EMA 38% versus 31% (one value was excluded), glutaric acid 35% versus 26%, hexanoylglycine 64% versus 58%. The CV for isovalerylglycine is 71%. We remind you that hexanoylglycine and isovalerylglycine, as well as their deuterated internal standards are available from Dr Herman Ten Brink in Amsterdam: HJ.tenBrink@vumc.nl. Moreover, some labs identified an increase of 2-methylbutyrylglycine (n = 4), adipic acid (n = 4), suberic acid (n = 3), octanoic, decanoic, and 2-octenedioic acids (n = 1).

Amino acid analysis was performed by 17 labs. Only 5 of them identified an increase of sarcosine (median = 171 mmol/mol creat). By tandem MS, we also identified a clear increase of dimethylglycine (442 mmol/mol creat – controls: <18).

Four lab performed **acylcarnitine profile** and identified an increase of C4, C5, C5DC (4 labs), C2, C8 (3 labs), C6 (2 labs), and C10, C0, C5OH (1 lab).

Advice for further investigation was OK far all labs. From our experience with more than 20 ETF and 50 ETF-QO deficient patients, we first perform plasma / blood acylcarnitine profile (and eventually medium chain fatty acids): if the profile is suggestive of MAD, we repeat it after riboflavine supplementation. If the profile is normal but with a severely decreased signal for free carnitine, we repeat it after carnitine supplementation. We then go directly to mutation analysis: *ETFDH* gene first since more than 2/3 of patients have mutations in this gene, and then *ETFA* and *ETFB* gene if no alteration or if non sense mutation not already described are identified in *ETFDH* gene. We eventually perform "*in vitro* probe" in fibroblasts by analyzing the acylcarnitine profile in cells and supernatant after 72h incubation with ^{13}C palmitate and carnitine (Roe and Roe, Mol Genet Metab 1999;68:243).

Scoring

- Analytical: increase of EMA, glutaric acid and 2-hydroxyglutaric (at least 2 of them) (score 1), increase of acylglycine derivatives (score 1)

- Interpretation: MAD deficiency (score 2), MAD deficiency associated to prolidase deficiency (score 1)

- Recommendations: plasma / blood acylcarnitines and/or mutation analysis *ETFDH, ETFA* and *ETFB* genes and/or ETF-QO and ETF activity and/or fatty acid oxidation study (fibroblasts, lymphocytes) (score 1)

Scores of participants

- **Survey 2009-1**

Lab n°	Patient P1 MPS III				Patient P2 Salla disease				Patient P3 Non metabolic disease, anticonvulsivant treatment			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	2	1	5	0	0	1	1	1	1	0	2
2	2	2	1	5	0	0	0	0	1	1	1	3
3	2	2	1	5	0	0	1	1	2	1	1	4
4	2	2	1	5	0	0	0	0	2	1	0	3
5	0	0	0	0	2	0	0	2	1	1	0	2
6	2	2	1	5	0	0	0	0	2	1	1	4
7	1	2	1	4	0	0	0	0	2	1	1	4
8	1	1	1	3	0	0	0	0	1	0	1	2
9	2	2	1	5	0	0	1	1	0	0	0	0
10	2	2	1	5	0	0	0	0	2	1	1	4
11	2	2	1	5	1	0	1	2	2	1	0	3
12	2	2	1	5	0	0	0	0	2	1	0	3
13	1	0	0	1	0	0	0	0	2	1	1	4
14	0	0	0	0	0	0	0	0	1	1	1	3
15	2	2	1	5	1	0	1	2	2	1	1	4
16	2	2	1	5	2	2	1	5	2	1	1	4
17	2	2	1	5	0	0	0	0	2	2	1	5
18	2	2	1	5	0	0	0	0	1	1	1	3
19	2	2	1	5	2	2	1	5	2	1	1	4
20	2	2	1	5	0	0	1	1	2	2	0	4

❖ **Survey 2009-2**

Lab n°	Patient P4 Glutaric aciduria type I				Patient P5 Iminodipeptiduria				Patient P6 MAD deficiency			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	2	1	5	2	2	1	5	2	2	1	5
2	2	2	1	5	2	2	1	5	2	2	1	5
3	2	2	1	5	2	2	1	5	2	2	1	5
4	2	2	1	5	1	0	0	1	2	2	1	5
5	2	2	1	5	2	2	1	5	2	2	1	5
6	2	2	1	5	2	2	1	5	2	2	1	5
7	2	2	1	5	2	2	1	5	2	2	1	5
8	2	2	1	5	0	0	0	0	2	2	1	5
9	2	2	1	5	2	2	1	5	2	2	1	5
10	2	2	1	5	2	2	1	5	2	2	1	5
11	2	2	1	5	2	2	1	5	2	2	1	5
12	2	2	1	5	0	0	0	0	2	2	1	5
13	2	2	1	5	0	0	0	0	2	2	1	5
14	2	2	1	5	2	2	1	5	2	2	1	5
15	2	2	1	5	0	0	0	0	2	2	1	5
16	2	2	1	5	1	0	0	1	2	2	1	5
17	2	2	1	5	2	2	1	5	2	2	1	5
18	2	2	1	5	2	2	1	5	2	2	1	5
19	2	2	1	5	0	0	0	0	2	1	1	4
20	2	2	1	5	2	2	1	5	2	2	1	5

❖ **Total scores**

Lab number	Survey 2009-1	Survey 2009-2	Cumulative score	Cumulative score (%)
1	8	15	23	77%
2	8	15	23	77%
3	10	15	25	83%
4	8	11	19	63%
5	4	15	19	63%
6	9	15	24	80%
7	8	15	23	77%
8	5	10	15	50%
9	6	15	21	70%
10	9	15	24	80%
11	10	15	25	83%
12	8	10	18	60%
13	5	10	15	50%
14	3	15	18	60%
15	11	10	21	70%
16	14	11	25	83%
17	10	15	25	83%
18	8	15	23	77%
19	14	9	23	77%
20	10	15	25	83%

Performance

	Number of labs	% total labs
Excellent performers (100 % of good responses)	0	0 %
Good performers (> 75 % good responses)	12	60 %
Poor performers (< 50 % good responses)	0	0 %

❖ **Summary of scores**

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Recommen-dations (%)	Total (%)
Patient P1	MPS III	**83%**	**83%**	**85%**	**83%**
Patient P2	Salla disease	**20%**	**10%**	**40%**	**20%**
Patient P3	Non metabolic	**80%**	**50%**	**65%**	**65%**
Patient P4	Glutaric ac. type I	**100%**	**100%**	**100%**	**100%**
Patient P5	Iminodipeptiduria	**70%**	**65%**	**65%**	**67%**
Patient P6	MADD deficiency	**100%**	**98%**	**100%**	**99%**

DPT-scheme in 2010

- Two surveys of 3 urines, including “normal” patients
- Results have to be sent within 3 weeks
- **The website system** developed by CSCQ (Centre Suisse de Contrôle de Qualité) should be used as pilot, adjacent to old system for all participants in 2010 samples. But overall organization of schemes in 2010 is premature.
- **Poor performers**: during the Scientific Advisory Board meeting, there was much discussion as to how satisfactory performance should be defined. Previously for the DPT schemes a score of less than 15 would indicate poor performance (<50%). The main concerns with this cut off is that ERNDIM is giving false reassurance about how well a laboratory is doing even though that laboratory may be missing a significant number of important metabolites/interpretation of results. It was felt by the group that the limits for poor performance should be higher. After some discussion, it was decided that, **from 2010, any laboratory with a score of below 18 (<60%) is now deemed unsatisfactory**.
- **Scoring**: from 2010, scoring will be performed by two different scheme organizers. For the Lyon centre, this will also be done by Viktor Kozich from Prague.

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

- Amino acids
- Organic acids
- Oligosaccharides
- Mucopolysaccharides

If you are not performing one of these assays, 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, if possible, for organic acids.

Meeting in 2010

It will take place during the SSIEM meeting in Istanbul **Tuesday 31 August 2010**, at 9.00 am.

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

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ANNEX 1

PROFICIENCY TESTING – SOUTHERN EUROPE, LYON CENTER

URINE SAMPLES ALREADY SENT

• **1998 : 1**	**A**	**OCT**	
	B	**Propionic**	
• **1999 : 1**	**C**	**MPS I or II**	
	E	**Cystinuria**	SKZL
• **1999 : 2**	**D**	**CblC**	
	F	**HMG-CoA lyase**	
• **2000 : 1**	**G**	**Iminodipeptiduria**	SKZL
	H	**Glutathion synthetase**	
• **2001 : 1**	**P1**	**Mevalonate kinase**	
	P2	**L-2-OH glutaric**	
• **2001 : 2**	**P3**	**Methylmalonic**	SKZL
	P4	**MPS IIIA San Fillippo**	
• **2002 : 1**	**P1**	**LCHAD**	
	P2	**Sulphite oxidase**	
• **2002 : 2**	**P3**	**Biotinidase**	SKZL
	P4	**MPS I**	

• **2003:1**	**P1**	**Tyrosinemia type I**
	P2	**SC-BCAD deficiency**
	P3	**Argininosuccinic aciduria**
• **2003:2**	**P4**	**MCC deficiency**
	P5	**Sialidosis** SKZL
	P6	**MSUD**
• **2004:1**	**P1**	**Tyrosinemia type I, treated patient**
	P2	**Propionic acidemia**
	P3	**Non metabolic disease, septic shock**
• **2004:2**	**P4**	**Mevalonic aciduria (common sample)**
	P5	**Fucosidosis**
	P6	**Alkaptonuria**
• **2005:1**	**P1**	**Isovaleric acidemia**
	P2	**Tyrosinemia type II (common sample)**
	P3	**Disorder of peroxysome biogenesis**
• **2005:2**	**P4**	**Multiple acyl-CoA dehydrogenase deficiency**
	P5	**Alpha-mannosidosis**
	P6	**4-hydroxybutyric aciduria**
• **2006:1**	**P1**	**Aromatic amino acid decarboxylase deficiency**
	P2	**Hyperoxaluria type I**
	P3	**Mucopolysaccharidosis type VI**
• **2006:2**	**P4**	**Hypophosphatasia (common sample)**
	P5	**Lysinuric protein intolerance**
	P6	**MCAD deficiency**
• **2007:1**	**P1**	**Mitochondrial acetoacetyl-CoA thiolase (MAT)**
	P2	**Homocystinuria due to CBS deficiency**
	P3	**Hyperlysinemia (common sample)**
• **2007:2**	**P4**	**Aspartylglucosaminuria**
	P5	**Phenylketonuria**
	P6	**SCAD deficiency**

• **2008:1**	**P1**	**Cbl C/D**
	P2	**Mucopolysaccharidosis type III (common sample)**
	P3	**2-hydroxyglutaric aciduria**
• **2008:2**	**P4**	**Glycerol kinase deficiency**
	P5	**α-mannosidosis**
	P6	**3-methylcrotonyglycinuria**