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ERNDIM
Diagnostic proficiency testing
2005
Southern Europe

ANNUAL REPORT 2005

In 2005, 22 labs participated to the Proficiency Testing Scheme Southern Europe.
Organizing Centre: Dr Christine Vianey-Saban, Dr Cécile Acquaviva-Bourdain, Service de Biochimie Pédiatrique, Hôpital Debrousse, Lyon, in collaboration with Pr Claude Bachmann, CHUV, Lausanne, Switzerland.

Geographical distribution of participants

Country	Number of participants
France	7
Italy	5
Spain	4
Portugal	2
Belgium	1
Czech Republic	1
Swiss	1
United Kingdom	1
TOTAL	**22**

Logistic of the scheme

- 2 surveys 2005-1 : patient P1, P2 and P3

  2005-2 : patient P4, P5 and P6

- Origin of patients : 4 out the 6 urine samples have been kindly provided by participants
  - Patient P1 : isovaleric acidemia - Dr Begoña Merinero, Universidad Autonoma de Madrid
  - Patient P2 : tyrosinemia type II - Hôpital Debrousse. This sample has been sent to all labs participating to the DPT scheme in Europe
  - Patient P3 : disorder of peroxysome biogenesis - Dr Marie-Hélène Read, CHU de Caen
  - Patient P4 : MAD deficiency - Dr Marie-Hélène Read, CHU de Caen
  - Patient P5 : alpha-mannosidosis - Dr Marie-Hélène Read, CHU de Caen
  - Patient P6 : 4-hydroxybutyric aciduria - Hôpital Debrousse
- Mailing : samples were sent by DHL at room temperature.

Timetable of the schemes

- May 9 th shipment of samples of both surveys by DHL and of the forms by e-mail
- June 1st deadline for result submission (Survey 1)
- June 21st analysis of samples of the second survey
- June 28 th report of Survey 1 by e-mail
- July 11th deadline for result submission (Survey 2)
- August 3rd report of Survey 2 by e-mail
- September 6 th meeting in Paris
- January 6th 2006 annual report with scoring sent by regular mail

Date of receipt of samples

Once again, DHL has been very efficient.

	Survey 1 + 2
+ 24 hours	19
+ 48 hours	2
+ 72 hours	1

Date of reporting

All labs, except one, sent the forms in time and all labs sent an answer.

	Survey 1 (3 weeks)	**Survey 2** (3 weeks)
Receipt of results :		
Before deadline	21 / 22	21 / 22
+ 1 day	1 / 22	
+ 4 days		1 / 22
No answer	**0** / 22	**0** / 22

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 criterium 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 Paris on September 6th from 9H30 to 11h00, during the SSIEM 42st Annual Meeting.

❖ Participants

27 representatives from 21 labs were present : Barcelona Hospital Clinic (A. Ribes), Barcelona Vall d'Hebron (E. Riudor), Bordeaux (I. Redonnet-Vernhet), Caen (M.H. Read), Cagliari (F. Lilliu), Florence (S. Funghini, E. Pasquini, E. Zammarchi), Genova (U. Caruso), Grenoble (M. Ducros), Lausanne (O. Boulat, C. Bachmann), Liege (F. Boeber), Lille (G. Briand), Lisboa (I. Tavares de Almeida), Madrid (C. Perez-Cerda), Nice (M. Candito), Padova (A. Burlina), Paris Necker (C. Marsac, A. Vassault), Paris Robert Debré (O. Rigal), Porto (D. Quelhas), Prague (E. Kostalova, O. Martincova, S. Stastna), Roma (C. Rizzo), Santiago de Compostela (J. Cocho), plus 7 visitors from labs who don't participate to our DPT schemes.

❖ Informations from the Executive Board and the Scientific Advisory Board for next year

- In 2005, there were 4 centres in Europe for the DPT-scheme. A fifth centre will be created in Basel in 2006
- The scoring system will be used in all centres in 2005
- Certificate of participation for 2005 will be issued for participation and it will be contain in addition a notice if the participant has received a warning letter. This warning letter is sent out if the performance is less than 50%. Good performers are those whose performance is more than 75%.
- No warning letter has been sent in 2004, since there was no consensus about the definition of "poor performers".
- Some centres requested to submit the results via the ERNDIM website. The consequence would be that labs who are late will be considered as non responder. This is still under discussion.
- 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. 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. 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.

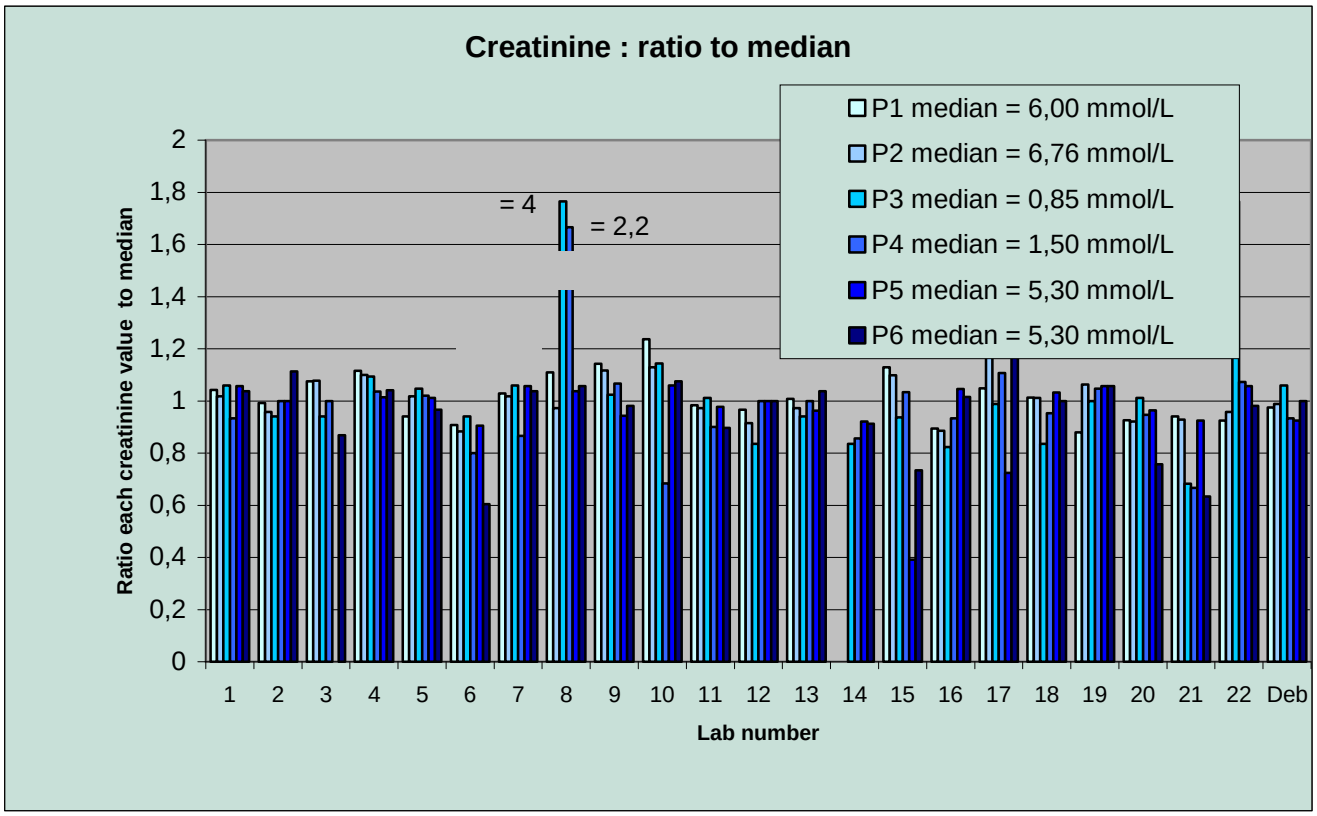
  Procedure for urine preparation and sending: as soon as possible after collection, the urine sample must be heated at 50 °C for 20 minutes. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. Then aliquot the sample in 10 ml plastic tubes (minimum 48 tubes), 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: Christine Vianey-Saban, Service de Biochimie Pédiatrique, Bâtiment D, Hôpital Debrousse, 29 Rue Sœur Bouvier, 69322 Lyon cedex 05, France. Please send me an e-mail on the day you send the samples.

❖ Discussion of results

- **Creatinine measurement**

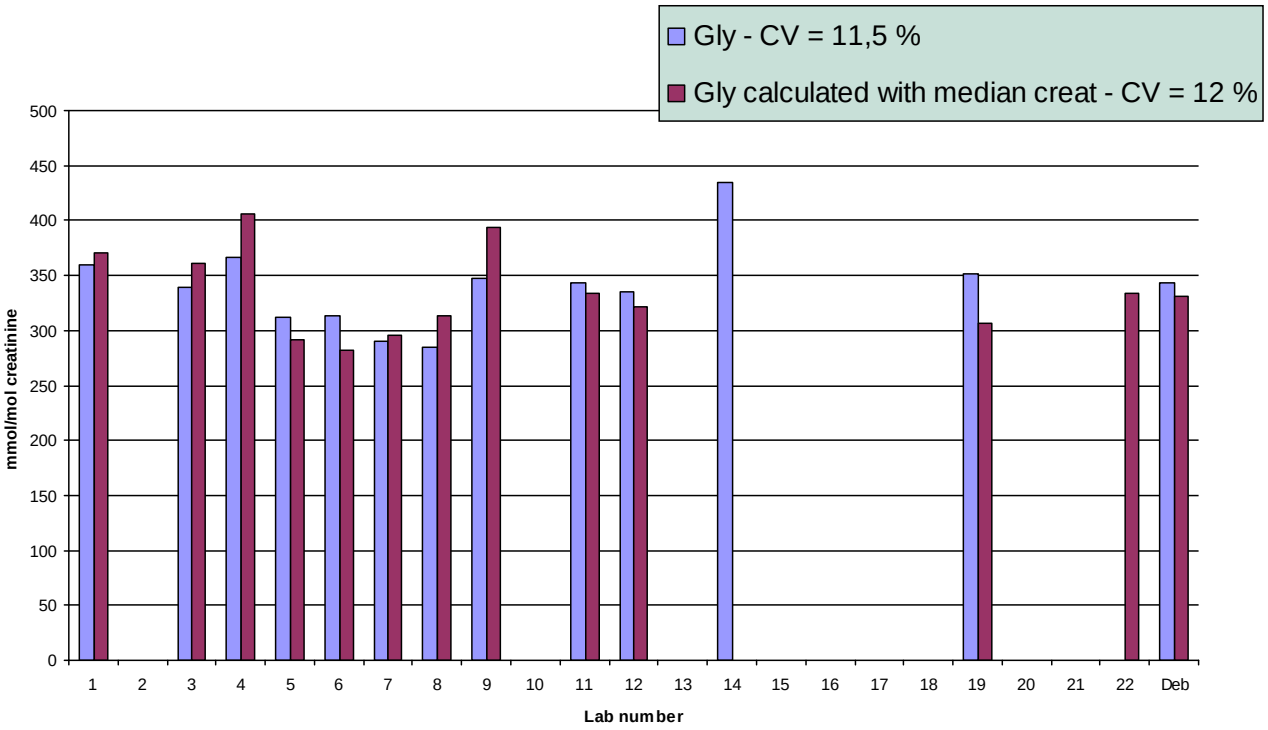
Results significantly improved compared to last year.
Lab 8, 15 and 21 still have imprecise results and some labs have one wrong value.



- **Patient P1 – isovaleric acidemia: isovaleryl-CoA dehydrogenase deficiency**

- The urine sample was collected from a 12 year old boy. Diagnosis was achieved when he was 22 month old. He had two episodes of dehydration and vomiting, with metabolic acidosis, hyperglycemia and ketonuria. The urine sample was collected while the patient was under treatment. He has a normal physical and neurological development. Isovaleryl-CoA dehydrogenase deficiency was confirmed in fibroblasts. His maternal aunt had similar episodes and also has isovaleric acidemia.

- All labs gave a correct diagnosis.
- Only one lab checked for odor which was negative. Remark : the urine should be acidified to reveal the characteristic odor of “sweaty feet”.
- 18 labs performed aminoacid analysis: 8 reported an increase of glycine, while 10 reported a normal profile including glycine. Glycine levels ranged from 285 to 434 mmol/mol creatinine (median = 341) which in our hands is abnormal (age-matched control range for Debrousse and Lausanne: < 200 mmol/mol creatinine). Therefore the question is: which control value do you use? Quantitative results were satisfactory and coefficients of variation were identical for glycine calculated according to creatinine measured by each participant and glycine calculated with the median value for creatinine. This clearly illustrates that the improvement observed on creatinine measurement has a direct impact on quantitative data.

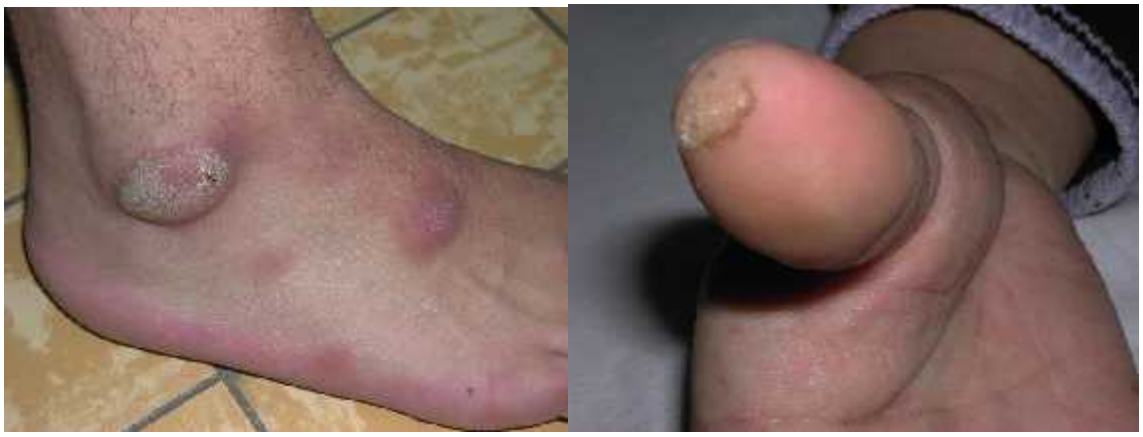


- All labs performed organic acid analysis. There was a very high variability in quantitative results for isovalerylglycine. The problem is most likely that there is no commercial standard available. H. Ten Brink (Free University, Amsterdam: HJ.tenBrink@VUmc.nl) synthetized isovalerylglycine and [d9] isovalerylglycine. Ten labs reported a normal excretion of 3-hydroxyisovaleric acid, because the patient was under treatment. H. Ten Brink can also provide 3-hydroxyisovaleric acid.
- Interpretation of results and recommendations were correct.
- Scoring
    - Analytical : increase of isovalerylglycine and glycine (score 2), wrong creatinine, normal glycine excretion (score 1)
    - Interpretation : isovaleric acidemia as first diagnosis (score 2),

- o Recommendations : plasma acylcarnitines, isovaleryl-CoA dehydrogenase activity or mutation analysis (score 1)

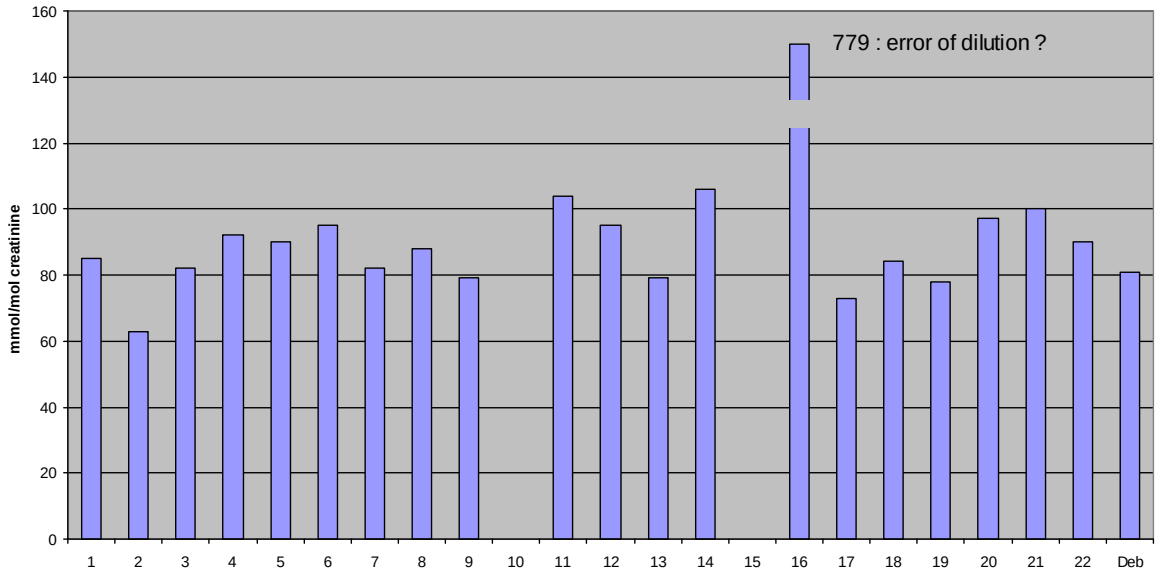
- **Patient P2 - tyrosinemia type II: tyrosine aminotransferase deficiency**

- This 20 year old patient, from unrelated parents, presents a slight mental retardation. He stopped school at 12. From the age of 17, he presented ophtalmological symptoms ascribed to allergy, and from the age of 18, palmar and plantar keratosis ascribed to verruca. The urine sample has been collected one month after starting a restricted protein diet. Plasma tyrosine level was 885 µmol/L when the urine sample was collected. Mutation analysis of tyrosine aminotransferase gene is under investigation. This sample has been sent to all DPT centers. The two pictures below illustrate keratosis.



- All labs gave a correct diagnosis.
- All labs performed aminoacids, and all labs, except one, reported an increase of tyrosine. Quantification of tyrosine was satisfactory.

Tyrosine - median = 86 mmol/mol creat - CV = 12 %



- All labs performed organic acids, and identified an increase of 4-hydroxyphenyllactic acid. Eleven labs also reported an increase of 4-hydroxyphenylpyruvic acid. There was a great variability in the quantification of metabolites: problem of oximation ?
- Advice for further investigations was correct, except that some labs advised to measure tyrosine aminotransferase activity in liver, which is quite invasive when mutation analysis is available.
- Scoring
    - Analytical : increase of tyrosine, and 4-hydroxyphenyllactic (score 2), normal tyrosine level, wrong tyrosine concentration, wrong creatinine (score 1)
    - Interpretation : tyrosinemia type II (score 2)
    - Recommendations : plasma aminoacids, mutation analysis tyrosine aminotransferase gene (score 1), tyrosine aminotransferase activity in liver as only advice (score 0)

- **Patient P3 : disorder of peroxysome biogenesis**

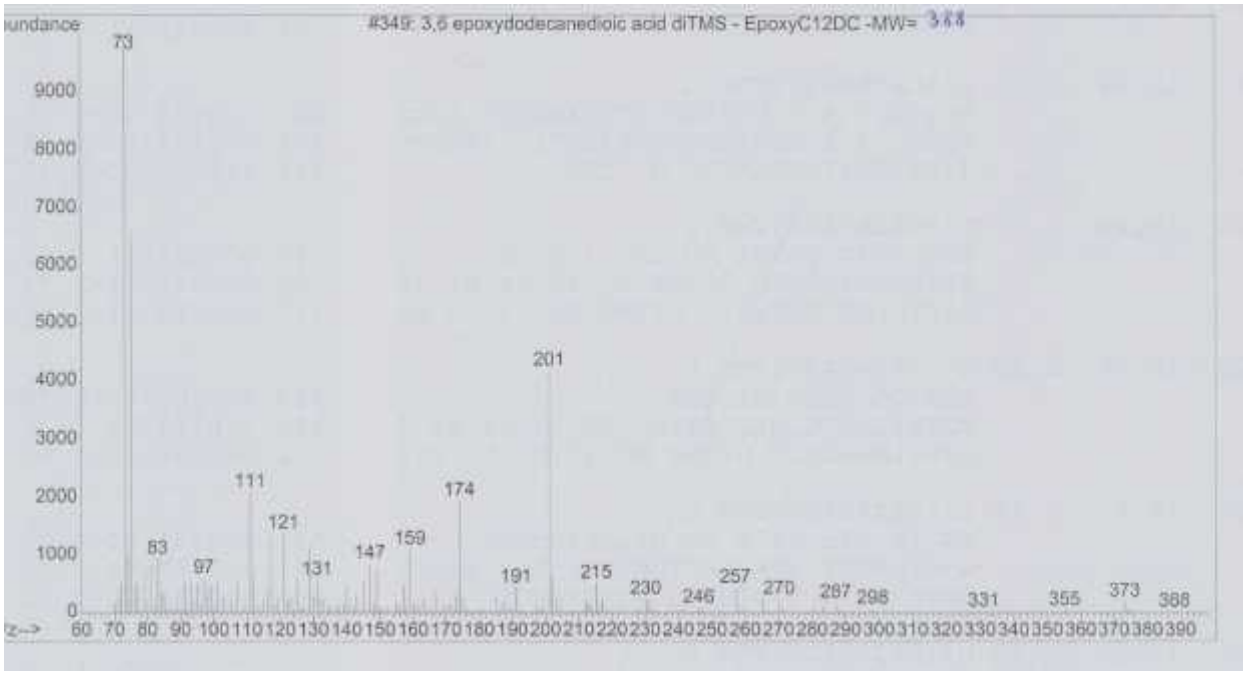
- This 1 month old girl, first child of consanguineous parents, was born after cesarian delivery because of intrauterine growth failure and foetal distress. She had dysmorphia (hypertelorism, domed forehead), hypotonia, and hepatomegaly. Biochemical investigation revealed an increase of ALAT and ASAT, and cholestasis. On X-ray of bones, she had epiphyseal calcific stippling. Acylcarnitine profiling in plasma showed an increase of C16 and C18 dicarboxylylacylcarnitines. A disorder of peroxysome biogenesis was confirmed in fibroblasts: DHAP-AT activity and VLCFA oxidation were defective. This was a challenging sample. Urine is most probably not the best biological fluid to diagnose peroxysomal abnormalities, but in this case, the organic acid profile (3,6 epoxydicarboxylic acids) allowed to suspect a peroxysomal disorder.

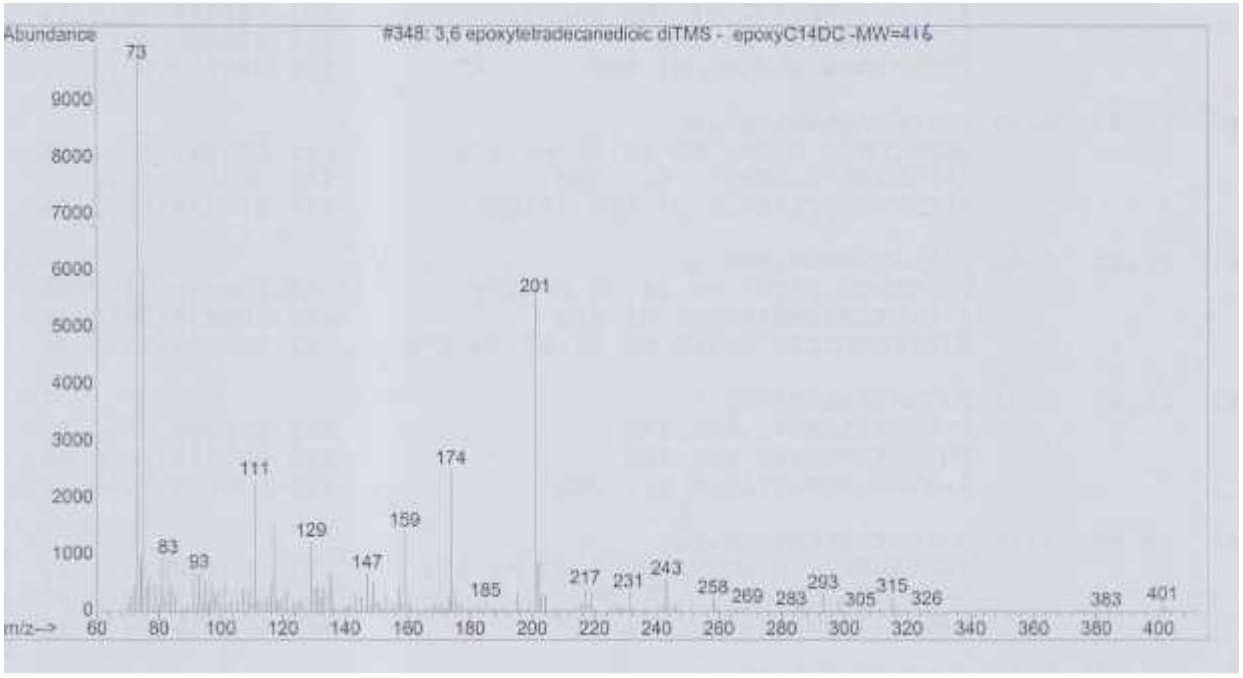
- Fifteen labs gave a correct diagnosis, whereas 7 labs concluded to a lysosomal storage disease.

- Eighteen labs performed aminoacid analysis: 14 labs reported hyperaminoaciduria, whereas 4 labs concluded to a normal profile or to the increase of one aminoacid: problem of control values? Problem of pipecolic acid: most labs failed to detect it, although its concentration was approximately 70 mmol/mol creatinine (by LC-MS/MS and GC/MS). With “new” ninhydrines, there is a lack of sensitivity to detect pipecolic acid due to the removal of methylcellosolve from their composition.

Twenty labs performed organic acids. All of them reported an increase of 4-hydroxyphenyllactic acid: this is a frequent finding in disorders of peroxysomal biogenesis, although it is not specific. 3,6 epoxydicarboxylic acids, mainly C14 (but also C12, C13, and C14:1) were identified by 9 labs. This increase is probably due to the deficiency of epoxide hydrolase, a peroxysomal enzyme (*Pitt and Poulos, Clin Chim Acta 1993;223:23-9*). However these are not specific metabolites, since a slight

increase of 3,6 epoxydecanedioic (C10), and 3,6 epoxydodecanedioic acid (C12) can be observed in newborns. One participant informed us of an increase of these metabolites in bile occlusion and liver diseases. The spectra of TMS derivatives of 3,6 epoxydodecanedioic and 3,6 epoxytetradecanedioic acid are given below. Presence of 2-hydroxysebacic acid was reported by 6 labs. It is present in disorders of peroxysome biogenesis (possibly due to impaired oxidation of 2-hydroxy very-long-chain fatty acids) but it is sometimes slightly increased in premature newborns fed with medium chain triglycerides (*Muth et al, J Inher Metab Dis 2003; 26:583*).





- Among the 11 labs who performed oligosaccharides, 4 labs reported a profile consistent with alpha-mannosidosis. The 6 other labs reported an abnormal profile but not consistent with an oligosaccharidosis, and one lab reported a normal profile. During the meeting, one participant informed us that she performed an oligosaccharide profile on urine from another patient with a disorder of peroxysomal biogenesis and observed the same abnormal profile.

- Thirteen labs did look for mucopolysaccharides and 2 of them reported a profile that can evoke a mucopolysaccharidosis.

- Two labs did a bile acid analysis by tandem MS and found an increase of taurotetrahydroxycholestanoic acid (and taurodihydroxycholestanoic acid for one of them).

- Advice for further investigations was OK for those who reached a correct diagnostic suspicion, whereas other labs were proposing misleading follow-up measures.

- Scoring

    - Analytical: increase of 4-hydroxyphenyllactic and 3,6 epoxydicarboxylic acids or 2-hydroxysebacic acid, and/or pipecolic acid (score 2), wrong creatinine, increase of 4-hydroxyphenyllactic alone (score 1), organic acids not performed, elevation of all organic acids, oligosaccharide profile consistent with alpha-mannosidosis, mucopolysaccharide profile evocative of a mucopolysaccharidosis (score 0).

    - Interpretation: disorder of peroxysome biogenesis, Zellweger syndrome (score 2), alpha-mannosidosis, GM gangliosidosis, glycoproteinoses, mucopolysaccharidoses, sialic storage disorder = misleading diagnoses (score 0)

    - Recommendations: plasma VLCFA, pipecolic acid, phytanic acid, erythrocyte plasmalogens or investigation of peroxysomal disorder in fibroblasts (score 1), enzyme study(ies) of lysosomal storage disease(s) (score 0).

- **Patient P4 : multiple acyl-CoA dehydrogenase (MAD) deficiency due to Electron Transfer Flavoprotein (ETF) deficiency**

- This girl is the 9th child of consanguineous parents. The 1st and 6th child died at 2 days of life, without diagnosis. At 20 hours of life, she presented lethargy, hypotonia with peripheral hypertonicity and tremor. She had acidosis, hypoglycemia (0.7 mmol/L), hyperammonemia (139 µmol/L), and anemia with leucopenia. The urine sample was collected at two months of age, under treatment with L-carnitine, riboflavine, low fat and limited protein diet. Electron Transfer Flavoprotein activity was defective in fibroblasts (ETF = 0.15 nmol/min/mg prot - controls = 1.25 ± 0.32). Mutation analysis revealed that the patient is homozygote for the c.797C>T (p.Thr266Met) mutation of the ETFA gene: this is the most frequent disease-causing mutation of ETFA gene.

- All labs gave a correct diagnosis.

- Aminoacid analysis was performed by 18 labs. Eleven of them reported an increase of glycine, 4 an increase of tyrosine, and 2 an increase of sarcosine. By LC-MS/MS, we identified an important increase of dimethylglycine (407 mmol/mol creat - controls <30) and of sarcosine (42 mmol/mol creat - controls <5), consistent with MAD deficiency (dimethylglycine dehydrogenase and sarcosine dehydrogenase are ETF dependent enzymes).

- All labs identified an increase of ethylmalonic and glutaric acids acid (with a great variability in quantitative results, although these 2 organic acids are included in the ERNDIM QAP “quantitative organic acids”). Most labs also reported an increase of 2-hydroxyglutaric acid, isobutyrylglycine, and isovalerylglycine. Half of them noted an increase of hexanoylglycine, butyrylglycine, and 2-methylbutyrylglycine. Excretion of dicarboxylic acids was normal since the patient was already treated.

- Interpretation and recommendations were satisfactory.

- Scoring

    - Analytical : increase excretion of ethylmalonic, glutaric, 2-hydroxyglutaric acids and acylglycines (score 2), acylglycines not detected, wrong creatinine (score 1)

    - Interpretation : multiple acyl-CoA dehydrogenase deficiency (score 2)

    - Recommendations : plasma/blood acylcarnitines, enzyme assay in fibroblasts or mutation analysis (score 1), no advice for further investigations (score 0)

- **Patient P5 : alpha-mannosidosis due to alpha-mannosidase deficiency**

- This urine sample was collected from a 34 year old patient. She had surgery at 3 years of age for craniostenosis. She has mental retardation, strabismus, deafness and dysmorphia ascribed to Crouzon syndrome. Insulino-dependent diabetes began at the age of 22 years. She was hospitalized in rheumatology because of foot pain: X-ray revealed important abnormalities. She also had neutropenia, and thrombopenia. The myelogram revealed vacuolized macrophages evocating storage cells. Alpha-mannosidase deficiency was confirmed in leukocytes.

- A big problem occurred with this urine: it has been stored frozen in aliquots of 8 ml in plastic tubes for one year. Some urine samples had a characteristic profile of alpha-mannosidosis, whereas, in other samples, a normal oligosaccharide profile was observed. It is not clear whether this is due to a problem of storage (but all of them have been stored in the same conditions), or whether this is due to a problem of homogenization of the urine sample during aliquoting? We have no answer. We already experienced this problem in alpha-mannosidosis (with a control sample sent by the QC scheme of Manchester “Qualitative and quantitative MPS diagnosis”). Moreover, this patient had diabetes and this complicated the interpretation of results. The important conclusion concerning this sample is that the same pre-analytical problem can happen with urine samples sent for diagnosis as well; the results have to be interpreted together with the clinical information and if there are unexpected findings a control sample must be required.

- The diagnosis given by the participants depended of the quality of the urine sample received. Eight labs concluded that alpha-mannosidosis was the most probable diagnosis, and 4 that a lysosomal disorder cannot be excluded. Because of the important glucosuria, 2 labs gave diabetes as sole diagnosis. Four labs concluded to a mucopolysaccharidosis or to Gaucher type I, and 4 could not reach a diagnosis.

- Preinvestigations: 19 labs searched for glucose in urine, and all, except one, found a positive test (when quantified, glucose ranged from 55 to 500 mmol/mol of creatinine).

- Sixteen labs performed amino acids and did not find any significant abnormality.

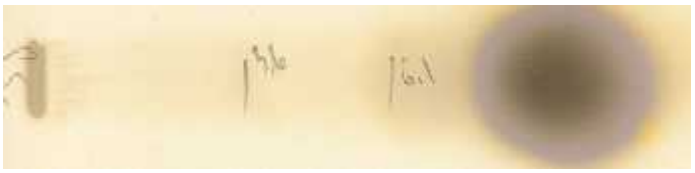
- Seventeen labs performed organic acids and all, except one, reported ketosis. One lab misidentified glucose as fucose.

- Only 19 labs performed oligosaccharides. Eleven reported an abnormal profile but only 5 of them concluded to alpha-mannosidosis. Four labs reported a normal profile. Among these 19 labs, 10 reported an abnormal band above lactose, compatible with glucose.

The figure above illustrates that some urine samples had a normal oligosaccharide profile (1st and 2nd sample), whereas the 3rd urine sample had a characteristic profile of alpha-mannosidosis.

P5 1st urine sample

P5 2nd urine sample, stored15 days at room temperature



P5 3rd urine sample

α-mannosidosis

- Mucopolysaccharides: 7 labs reported a normal profile, one lab reported a profile consistent with mucopolysaccharidosis type III and one lab reported an increase of MPS.
- Advice for further investigations depend of diagnosis.
- Scoring
    - Analytical: oligosaccharide pattern characteristic for alpha-mannosidosis, abnormal profile of oligosaccharides and glucosuria (score 2), normal profile of oligosaccharides, oligosaccharides not performed and normal mucopolysaccharide profile, glucosuria (score 1), mucopolysaccharide profile consistent with MPS type 2 or 3 (score 0)
    - Interpretation: alpha-mannosidosis, lysosomal storage disease cannot be excluded (score 2), no diagnosis, possible mucopolysaccharidosis, lysosomal storage disease not diagnosed by the methods used (score 1), mucopolysaccharidosis (score 0)
    - Recommendations: alpha-mannosidase activity, or oligosaccharides profile must be repeated on a new urine sample or by a specialized lab, or enzymes of oligosaccharidoses, identification of storage cell materiel (score 1), plasma studies, ascertain Crouzon syndrome, enzyme assay MPS type 2 or 3 (score 0)

- **Patient P6 : 4-hydroxybutyric aciduria – succinic semialdehyde dehydrogenase (SSADH) deficiency**

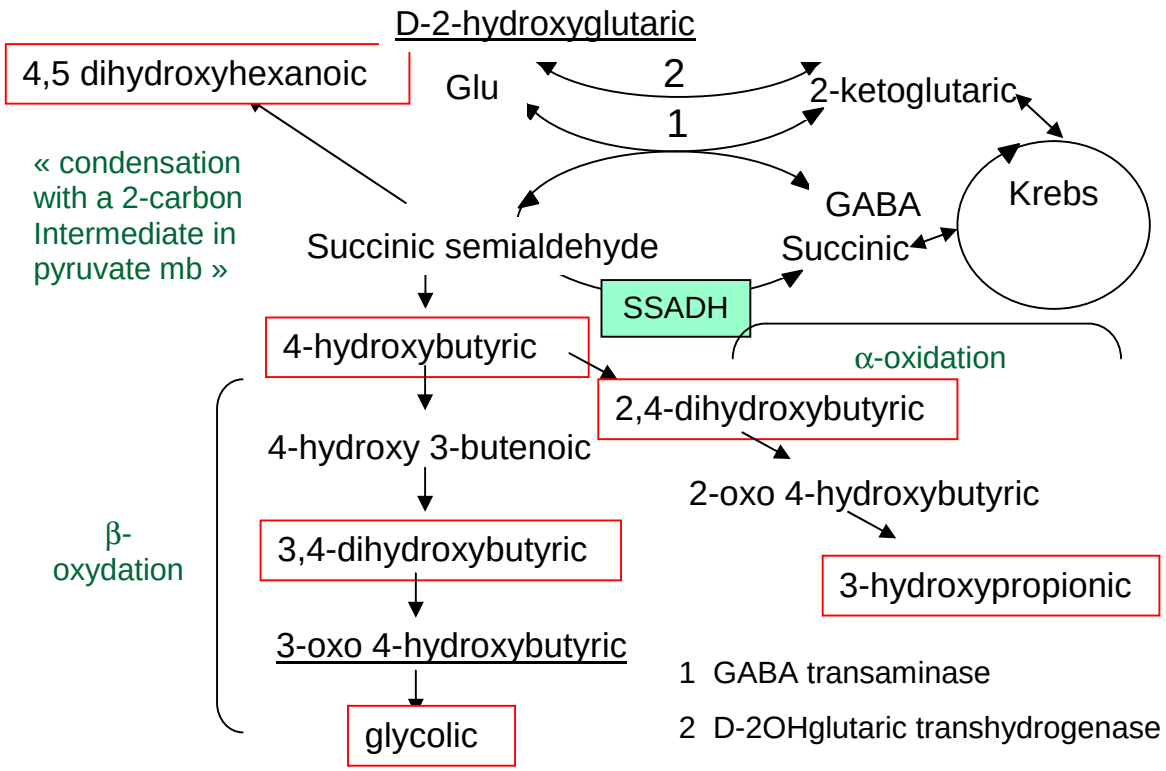
- This 7 year-old boy present a severe psychomotor retardation, with hypotonia during the first months of life, plagiocephaly, and strabismus. There is no regression. MRI and caryotype are normal, and investigation for fragile-X is negative. His younger brother who has the same clinical presentation is also affected. Diagnosis of SSADH deficiency has been confirmed by mutation analysis of *ALDH5A1* gene.

- All labs, except one, concluded to 4-hydroxybutyric aciduria, and one lab concluded to mucolipidosis type 4 (although the presence of 4-hydroxybutyric was reported in organic acids !).

- The 18 labs who performed amino acid analysis reported no specific abnormality.

- Organic acids: all labs demonstrated an increase of 4-hydroxybutyric acid excretion, with a high variability of quantitative results (CV = 271%), possibly due to a problem of storage of the sample. Erythro- and threo-4,5-dihydroxyhexanoic acids and/or lactones were reported by 13 labs, an increase of glycolic acid by 8 labs, of 3,4-dihydroxybutyric by 7, and of 3-hydroxypropionic and 2,4-dihydroxybutyric acids by 3 labs.

  Beside 4-hydroxybutyric acid, several organic acids have been reported to be increased in 4-hydroxybutyric aciduria. Cornelis Jakobs and Mike Gibson were asked about the specificity of theses compounds with respect to 4-hydroxybutyric aciduria: only 4,5-dihydroxyhexanoic acids (erythro-, threo- and the lactones which are dependent upon the pH of extraction) are specific of SSADH deficiency, but others can be elevated. In case of exogenous intake (Gamma-OH®), only 4-hydroxybutyric is elevated. The way to confirm diagnosis is to measure SSADH activity in lymphocytes or lymphoblasts and/or to perform mutation analysis of *ALDH5A1* gene.



- Advice for further investigations was OK. Only one lab adviced to investigate the brother !
- Scoring
    - Analytical: increase of 4-hydroxybutyric acid excretion (score 2), wrong creatinine (score 1)
    - Interpretation: 4-hydroxubutyric aciduria or succinic semialdehyde dehydrogenase deficiency (score 2), mucolipidosis type 4 (score 0)
    - Recommendations: 4-hydroxubutyric in plasma or CSF, SSADH activity, mutation analysis *ALDH5A1* gene (score 1), investigation of mucolipidoses, no advice for further investigations (score 0)

Scores of participants

- **Survey 2005-1**

Lab n°	Patient P1 Isovaleric acidemia				Patient P2 Tyrosinemia type II				Patient P3 Peroxysome biogenesis defect			
	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**	1	2	1	**4**
3	2	2	1	**5**	2	2	1	**5**	1	0	0	**1**
4	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
5	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
6	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
7	1	2	1	**4**	2	2	1	**5**	1	0	0	**1**
8	2	2	1	**5**	2	2	0	**4**	1	2	1	**4**
9	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
10	1	2	1	**4**	2	2	1	**5**	1	0	0	**1**
11	1	2	1	**4**	2	2	1	**5**	1	0	0	**1**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
14	2	2	1	**5**	2	2	1	**5**	0	0	0	**0**
15	1	2	1	**4**	1	2	1	**4**	1	2	1	**4**
16	2	2	1	**5**	1	2	1	**4**	2	2	1	**5**
17	2	2	1	**5**	1	2	1	**4**	2	2	1	**5**
18	1	2	1	**4**	2	2	1	**5**	1	0	0	**1**
19	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
20	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
21	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
22	1	2	1	**4**	2	2	1	**5**	0	0	0	**0**

❖ **Survey 2005-2**

Lab n°	Patient P4 MAD deficiency				Patient P5 ~-mannosidosis				Patient P6 4-hydroxybutyric aciduria			
	A	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**
1	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
2	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
3	2	2	0	**4**	2	1	0	**3**	2	2	0	**4**
4	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
5	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
6	2	2	1	**5**	2	1	1	**4**	1	2	1	**4**
7	2	2	1	**5**	1	1	0	**2**	2	2	1	**5**
8	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**
9	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
10	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
11	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	1	0	0	**1**	2	2	1	**5**
14	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
15	2	2	1	**5**	0	1	0	**1**	1	2	1	**4**
16	2	2	1	**5**	2	2	0	**4**	2	2	1	**5**
17	2	2	1	**5**	1	2	0	**3**	2	2	1	**5**
18	2	2	1	**5**	1	1	0	**2**	2	2	1	**5**
19	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
20	2	2	1	**5**	1	2	0	**3**	2	2	1	**5**
21	2	2	1	**5**	2	2	1	**5**	1	2	1	**4**
22	1	2	1	**4**	1	1	1	**3**	2	0	0	**2**

❖ **Total scores**

Lab number	Survey 2005-1	Survey 2005-2	Cumulative score	Cumulative score (%)
1	15	13	**28**	**93 %**
2	14	15	**29**	**97 %**
3	11	11	**22**	**73 %**
4	14	15	**29**	**97 %**
5	14	15	**29**	**97 %**
6	15	13	**28**	**93 %**
7	10	12	**22**	**73 %**
8	13	14	**27**	**90 %**
9	14	15	**29**	**97 %**
10	10	13	**23**	**77 %**
11	10	13	**23**	**77 %**
12	15	15	**30**	**100 %**
13	14	11	**25**	**83 %**
14	10	13	**23**	**77 %**
15	12	10	**22**	**73 %**
16	14	14	**28**	**93 %**
17	14	13	**27**	**90 %**
18	10	12	**22**	**73 %**
19	14	15	**29**	**97 %**
20	14	13	**27**	**90 %**
21	14	14	**28**	**93 %**
22	9	8	**17**	**57 %**

There is no “poor performer”. Seventeen labs are “good performers”.

❖ **Summary of scores**

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Recommen-dations (%)	Total (%)
Patient P1	Isovaleric ac.	75	100	100	**90**
Patient P2	Tyrosinemia II	93	100	95	**96**
Patient P3	Peroxysome	68	68	68	**68**
Patient P4	MAD def.	95	100	95	**97**
Patient P5	α-mannosidosis	73	73	64	**71**
Patient P6	4-hydroxybutyric ac.	91	95	91	**93**

DPT-scheme in 2006

Same “rules” as in 2005 :

- Two surveys of 3 urines, including “normal” patients
- Results have to be sent within 3 weeks
- Scoring will be analyzed for all centres

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. The cluster lab will also receive the results.

Please send quantitative data for amino acids and, when possible, for organic acids.

Meeting in 2006

There is no SSIEM Symposium next year, because of the international IEM meeting in Japan. An ERNDIM meeting will be organized in Prague in October 2006. Further information concerning this meeting will be available soon.

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

URINE SAMPLES ALREADY SENT

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

• **2002 : 1**	**P1**	**LCHAD deficiency**	
	P2	**Sulphite oxidase deficiency**	
• **2002 : 2**	**P3**	**Biotinidase deficiency**	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	**Tyrosinemai type II**	
	P3	**Disorder of peroxysome biogenesis**	
• **2005:2**	**P4**	**Multiple acyl-CoA dehydrogenase deficiency**	
	P5	**~-mannosidosis**	
	P6	**4-hydroxybutyric aciduria**	