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ERNDIM
Diagnostic proficiency testing
2003
Southern Europe

ANNUAL REPORT 2003

In 2003, 20 labs participated to the Proficiency Testing Scheme Southern Europe.
Organizing Center : Dr Christine Vianey-Saban, Service de Biochimie Pédiatrique, Hôpital Debrousse, Lyon, in collaboration with Pr Claude Bachmann, CHUV.

Geographical distribution of participants

Country	Number of participants
France	7
Italy	5
Spain	4
Portugal	2
Switzerland	1
Czech Republic	1
TOTAL	**20**

Logistic of the scheme

- 2 surveys 2003-1 : patient P1, P2 and P3
  2003-2 : patient P4, P5 and P6

- Origin of patients : 5 out the 6 urine samples have been kindly provided by participants
  - Patient P1 : Tyrosinemia type I - Dr Monique Fontaine, Service de Biochimie, Hôpital C. Huriez, Lille
  - Patient P2 : Short-chain 3-hydroxy-2-methylacyl-CoA dehydrogenase deficiency - Dr Antonia Ribes, Institut de Bioquimica Clinica, Barcelona
  - Patient P3 : Argininosuccinic aciduria - Hôpital Debrousse
  - Patient P4 : Isolated 3-methylcrotonyl-CoA carboxylase deficiency - Dr Begoña Merinero, CEDEM, Fac Ciencas, Madrid

- Patient P5 : Sialidosis - Dr Viktor Kozich, Institute of Inherited Metabolic Diseases, General faculty Hospital, Prague. This sample has been sent to all labs participating to the DPT scheme in Europe
- Patient P6 : MSUD - Dr Olivier Boulat, Laboratoire Central de Chimie Clinique, CHUV, Lausanne.

- Mailing : samples were sent by rapid mail (EMS Chronopost) at room temperature.

Timetable of the schemes

- February 17th : shipment of samples of Survey 1 by rapid mail and of the form by e-mail
- March 13nd : deadline for results submission (Survey 1)
- May 5th : report of Survey 1 by e-mail
- June 23rd : shipment of samples of Survey 2 by rapid mail and of the form by e-mail
- July 18th : deadline for results submission (Survey 2)
- August 5th : report of Survey 2 by e-mail
- November 7th meeting of participants in Madrid
- November 28th annual report by e-mail

Date of receipt of samples

	Survey 1	**Survey 2**
+ 24 hours	9	5
+ 48 hours	3	7
+ 72 hours	1	2
+ 7 days	1	1
+ 9 days		1
Not indicated	6	4

Date of reporting

Despite (or thanks to ?) the change for deadline in reporting results (changed from 6 weeks to 3 weeks), nearly all labs sent the form in time and the number of labs who did not send answer at all decreased, compared to last year.

	Survey 1	**Survey 2**
	(3 weeks)	(3 weeks)
Receipt of results :		
Before deadline	16 / 20	19 / 20
+ 1 day	1 / 20	
+ 1 day	1 / 20	
No answer	**2** / 20 (10 %)	**1** / 20 (5 %)

Scoring of results

The International Scientific Advisory Board of ERNDIM has decided to establish a scoring system. Thus 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.

No answer to one survey is scored as 0 for the 3 samples.

Meeting of participants

It took place in Madrid on September 7th from 12h00 to 14h00, during the V Congreso Nacional de Errores Congénitos del Metabolismo. We thank the Spanish groups who allowed the organization of this meeting in Madrid, since the number of scheme participants attending the Brisbane meeting was to low for organizing a satisfactory scheme meeting.

❖ Participants

Representatives from 9 labs were present :

Drs Isabelle Redonnet-Vernhet, Christine Vianey-Saban (France), Silvia Funghini, Elisabetta Pasquini, Cristiano Rizzo (Italy), Daisy Castiñeiras Ramos, Jose Angel Cocho, Cristobal Colon Mejeras, Begoña Merinero, Antonia Ribes, Encarnacio Riudor Taravilla, Pedro Ruiz-Sala (Spain), Laura Vilarinho (Portugal), Claude Bachmann (Switzerland)

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

- There are 4 centers in Europe for the DPT-scheme. 20 labs per center is a maximum. If there are more requests, a fifth center will be created.
- We will continue to use genuine patient samples, although it is difficult to get them.
- Certificate of participation : in 2002, certificates of participation have been sent to labs who sent results using the report form for at least one of the two surveys. It will be the same for 2003.
- A scoring system is introduced in 2003 for all centers. Information about the DPT-scoring will be collected in 2003 and 2004 in order to harmonize the results of this scoring.

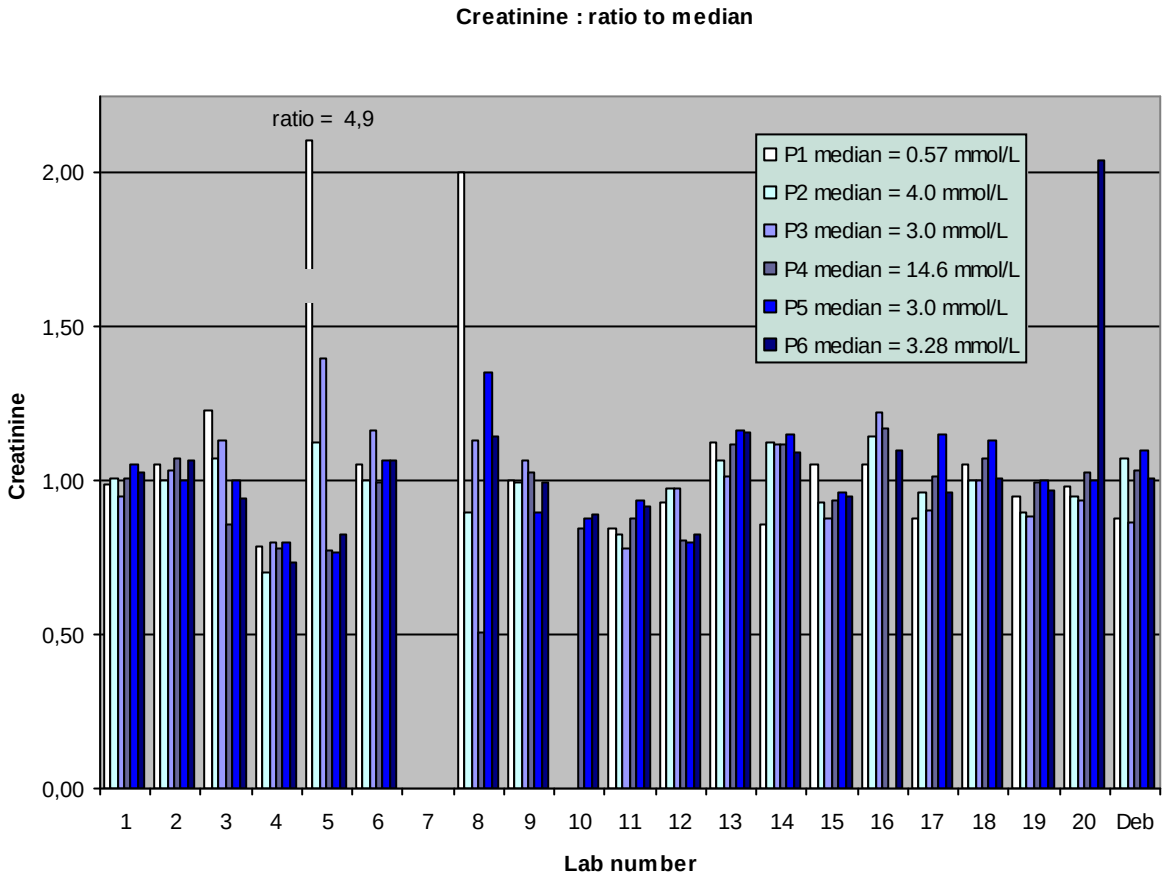
- We remind you that every year, each participant must provide to the scheme organizer at least 300 ml of urine from a patient affected with an established inborn error of metabolism or a “normal” urine, together with a short clinical report. Each urine sample must be collected from a single patient (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.

  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 44 tubes), add stoppers and freeze. 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**

The reliability of creatinine measurements is similar to last year. Lab 4 has still a systematic error but informed us that they have now solved their problem. Lab 5, 8 have problems of imprecision, especially for low values. Lab 20 got a high value for sample 6 : error of dilution ?

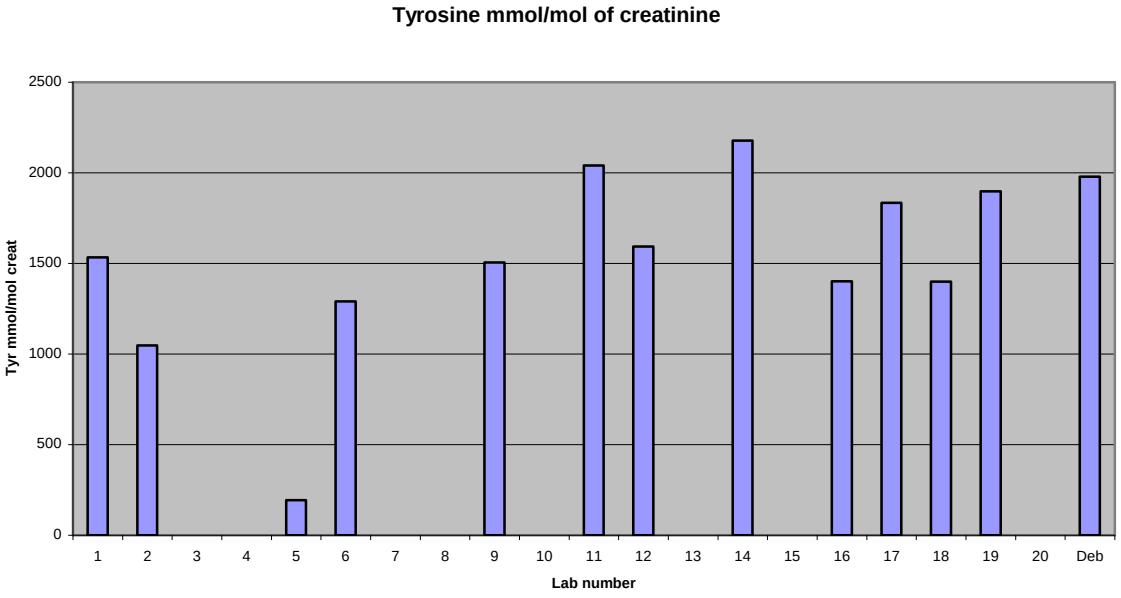


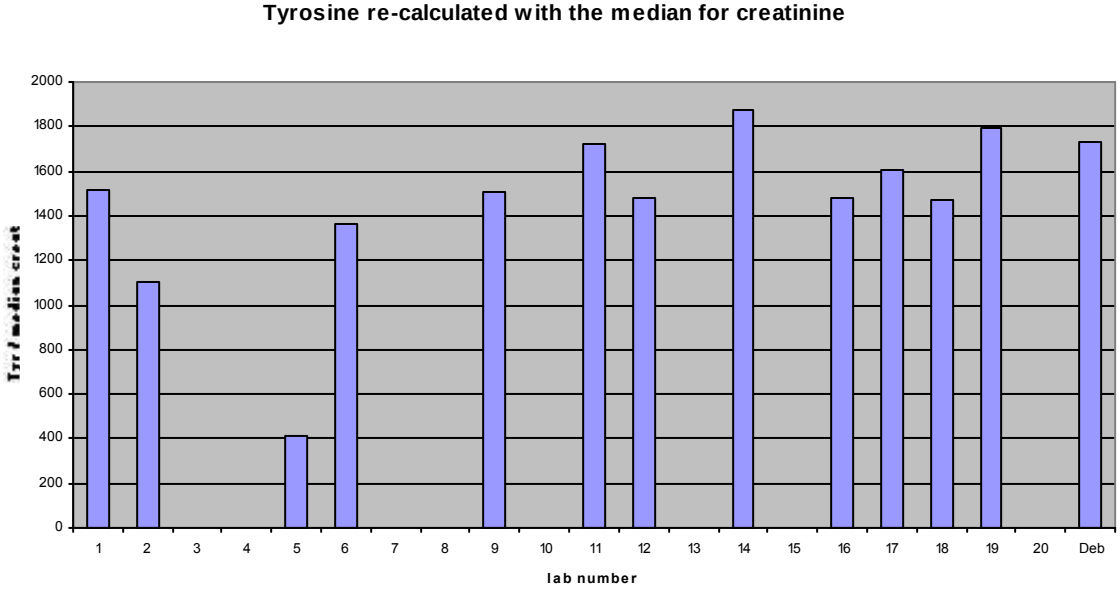
- **Patient P1 – tyrosinemia type I : fumarylacetoacetase deficiency**

<u>18 reports</u>

- The urine sample was collected from a 4 month-old boy with confirmed fumarylacetoacetase deficiency. He had failure to thrive, liver failure. Biological investigations showed coagulopathy, metabolic acidosis and hyponatremia.

- 16 labs performed amino acid analysis : all except two reported an important increase of tyrosine in a generalized hyperaminoaciduria (all except one). Three labs reported an increase of delta-aminolevulinic acid which is of diagnostic value. Quantification of tyrosine, when performed, was correct (median = 1520 mmol/mol creatinine, CV = 33%). We re-calculated tyrosine excretion using the median creatinine value obtained by all labs (see figure below) : the CV improved to 25%, emphasizing again the need of an accurate measurement of creatinine.





- All labs reported the presence of succinylacetone. There was a very high variability in quantitative results from 12 to 4406 mmol/mol of creatinine. A problem of storage can only partly explain these results, since we measured again succinylacetone after 15 days at room temperature : the value decreased from 700 to 380 mmol/mol of creatinine. It is most likely that the use of a stable isotope as internal standard would improve the measurement of succinylacetone but is not available so far. Another easy alternative for an accurate follow up of patients is the use of the inhibition colorimetric method (Grenier et al, Clin Chim Acta 1982;123:93-99).

- Interpretation of results and recommendations were good.

- Scoring

    - Analytical : identification of succinylacetone and increase of Tyr (score 2), wrong creatinine, amino acids not performed (score 1)

    - Interpretation : tyrosinemia type I (score 2)

    - Recommendations : plasma AA, fumarylacetoacetase activity or mutation analysis (score 1)

- **Patient P2 : 3-hydroxy-2-methylbutyryl-CoA dehydrogenase deficiency also called short-chain 3-hydroxy-2-methylacyl-CoA dehydrogenase (SC-HMAD) deficiency**

18 reports

- This 3 year-old girl presented psychomotor retardation since the first months of life and had gait disturbance. Her brother died in the neonatal period with lactic acidosis. The enzyme deficiency has been confirmed.

- SC-HMAD deficiency has been described for the first time in 2000 (Zschocke et al, Pediatr Res 2000;48:852-5). Approximately 10 patients have been reported : it is a neurodegenerative disease, a clinical presentation completely different from MAT. The gene has been located on X chromosome but the functional role of the enzyme is not yet elucidated (Ofman et al, Am J Hum Genet 2003;72:1300-7).

- All labs reported an increase of 3-hydroxy-2-methylbutyric acid and tiglyglycine but only 7 labs noticed that methylacetoacetic acid was not elevated. Tiglylglycine quantification when performed was correct (median = 16.5 mmol/mol creat - P95 reference value = 4.9), except for one lab due to a wrong creatinine value.

- Interpretation and/or recommendations were correct for those who noticed that methylacetoacetate excretion was normal. 7 labs concluded to MAT deficiency.

- Scoring

    - Analytical : increase of 3-hydroxy-2-methylbutyric acid and of tiglyglycine, no increase of methylacetoacetic acid, no ketone bodies (score 2), increase of 2-methyl-3-hydroxybutyric acid and of tiglyglycine, no mention of methylacetoacetic acid (score 1)

    - Interpretation : SC-HMAD deficiency (score 2), mitochondrial acetoacetyl-CoA thiolase MAT deficiency (score 1)

    - Recommendations : SC-HMAD activity or Ile load (score 1), MAT activity only (score 0)

- **Patient P3 : argininosuccinic aciduria due to argininosuccinate lyase deficiency**

18 reports

- This boy, born from consanguineous parents, has been diagnosed after 2 episodes of "encephalitis". Ammonemia has been measured during the second episode. The urine sample had been collected when he was under treatment.

- All labs performed amino acid analysis and all except one identified argininosuccinic acid (ASA) and anhydrides. Results of quantification were highly variable. Quantification of ASA and anhydrides is difficult to achieve since absorbance is different for ASA and its anhydrides and the distribution among the 3 species depends of storage and pH of the sample. To measure accurately ASA for the follow-up of patients, it is recommended to transform ASA into anhydride 1 by heating the urine (or plasma after deproteinization) samples during 45 min at 100°C, pH 2.2.

- 17 labs performed organic acids and 2 of them reported a decreased citrate level (which is known to be low in ASA-uria). Orotic acid (8 labs), orotidine (1 lab) and uracile (1 lab) excretion was normal since the patient was already treated.

- Interpretation and/ or recommendations were satisfactory

- Scoring

    - Analytical : increase of argininosuccinic acid and anhydrides (score 2)

    - Interpretation : argininosuccinic aciduria (score 2)

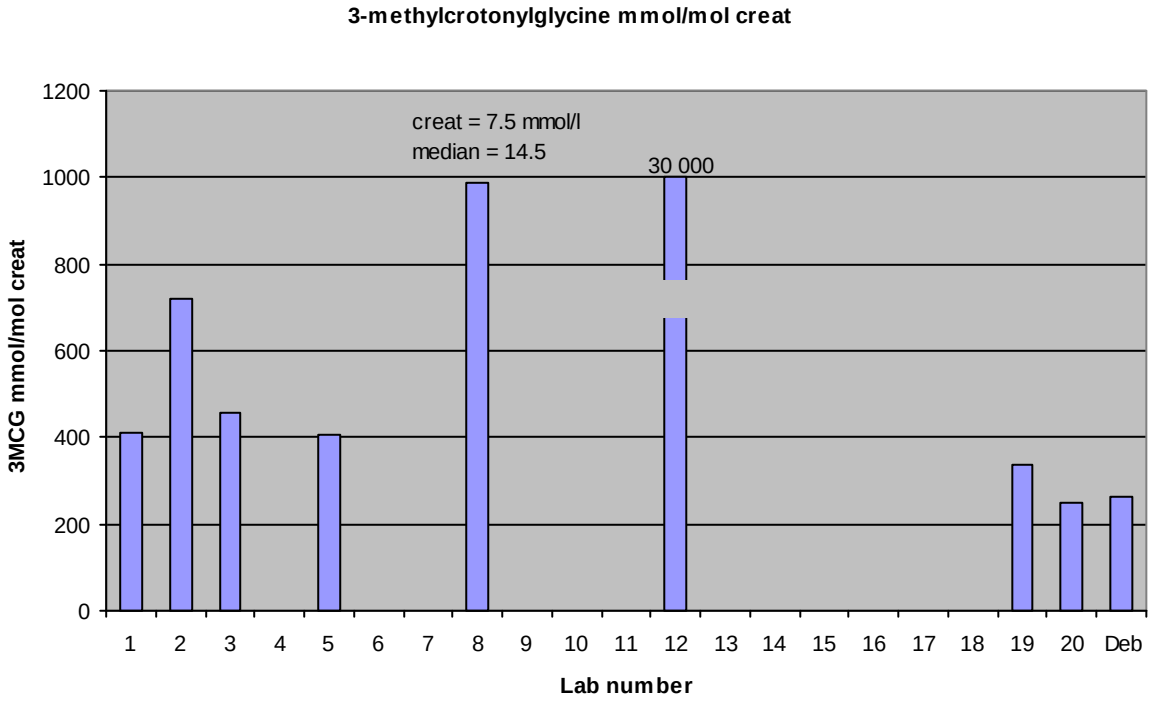
    - Recommendations : plasma AA (Arg) and argininosuccinate lyase activity or mutations (score 1)

- **Patient P4 : isolated 3-methylcrotonyl-CoA carboxylase deficiency**

19 reports

- This 11 year-old boy had a 2 years history of muscular weakness and recurrent episodes of abdominal pain and nervous depression. The urine sample was collected when he was 15 year-old, under carnitine treatment. MCC deficiency was confirmed in lymphocytes and fibroblasts. Mutation analysis allowed the identification of one mutation on MCCA gene, the gene coding for alpha-subunit of MCC. The other mutation is under investigation.

- All labs identified an increase of 3-methylcrotonylglycine and of 3-hydroxyisovaleric acid. Half of labs reported the absence of propionate metabolites. Quantification of 3MCG, when performed was acceptable (median = 434 mmol/mol creatinine), except for lab 8 (due to a wrong creatinine) and lab 12 (problem of calculation ?). The interlaboratory variance was too important for 3OHIVA and this is probably due to the absence of an available standard.



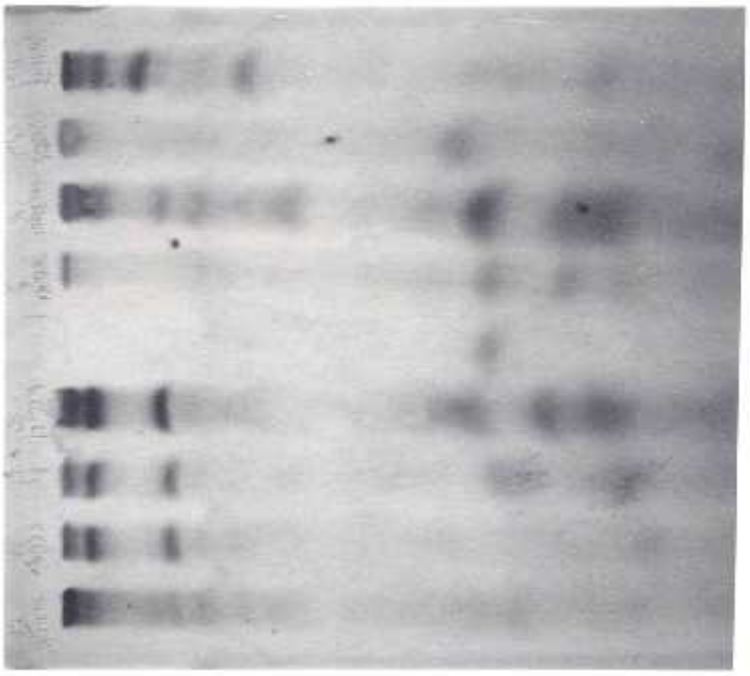
- Interpretation and recommendations were satisfying.
- Scoring
    - Analytical : increase of 3-methylcrotonyglycine and of 3-hydroxyisovaleric acid (score 2)
    - Interpretation : isolated 3-methylcrotonyl-CoA carboxylase deficiency (score 2)
    - Recommendations : 3-methylcrotonyl-CoA carboxylase activity or mutations (score 1)

- **Patient P5 : sialidosis (also called mucolipidosis type I) due to ~- neuraminidase (also called sialidase) deficiency**

<u>19 reports</u>

- This urine sample from this 15 year-old boy has been sent to all labs in Europe participating to the DPT-scheme. Diagnosis was confirmed by measurement of α-neuraminidase activity in fresh cultured skin fibroblasts (3% of control value) while β-galactosidase activity was normal.
- Only 13 labs performed oligosaccharides. All except one reported an abnormal profile but only 7 of them concluded to the presence of sialyloligosaccharides. Three labs gave a wrong interpretation (mannosidosis or GM1 gangliosidosis). One lab reported a normal profile. The next two pictures illustrate the TLC profile we obtained in urine from patient P5 compared to other oligosaccharidoses. The method used is Humbel and Collart, Clin Chim Acta 1975; 60:143-145. Urine samples (corresponding to 0.13 µmol creatinine) are applied to Silicagel plates using an automatic sampler. The solvent used is n-butanol / acetic acid 96% / water (2:1:1 v:v:v) and migration lasts overnight. Plates are soaked in orcinol 0.2% in H_2SO_4 6 % (w:v) and warmed at 150°C during 10 min. When there are

problem of identification, plates are migrated twice before adding the dye reagent, as illustrated on the pictures. Viktor Kozich and Evza Pospisilova, the scheme organizers of DPT Central Europe and who sent this urine sample, recommend to also use resorcinol detection which is specific for sialyloligosaccharides.



GM1 gangliosidosis

Control

Fucosidosis

Control

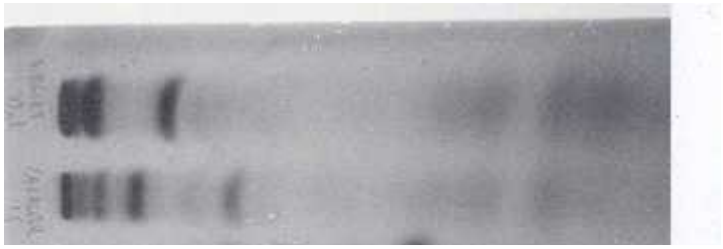
Lactose

Sialidosis

P5

P5 +15days

Mucolipidosis type II



Sialidosis

GM1 gangliosidosis

Fucosidosis

Lactose

Mucolipidosis type II

Galactosemia

α-mannosidosis

- Fifteen labs performed mucopolysaccharides. Among them, one lab, who did not perform oligosaccharides, reported an abnormal band of keratan sulfate, without an increase of GAG quantification and concluded to Morquio disease.

- Interpretation and recommendations : only 4 labs concluded to sialidosis or galactosialidosis (same oligosaccharide profile) and 3 labs were too restrictive concluding to sialidosis. Four labs could not reach a diagnosis whereas 5 gave a misleading diagnosis.

- Scoring

    - Analytical : oligosaccharide pattern characteristic for sialidosis or galactosialidosis (score 2), abnormal profile (score 1)

    - Interpretation : sialidosis or galactosialidosis (score 2), sialidosis (score 1)

    - Recommendations : α-neuraminidase and β-galactosidase activity, or oligosaccharides must be performed by a specialized lab (score 1)

- **Patient P6 : Maple Syrup Urine Disease (MSUD) due to branched-chain 2-ketoacid dehydrogenase (BCKD) deficiency**

<u>19 reports</u>

- This 35 year-old “classical” MSUD patient was diagnosed at 10 days of age by plasma amino acid analysis because of lethargy since the first days of life and a characteristic odor. She works as nurse assistant and has an IQ of about 105. She is not thiamine responsive. This urine sample has been collected between February and May 2003, when she had elevated values for plasma branched-chain amino acids (BCAA). The following table highlights that BCAA are highly reabsorbed by kidney tubule (FTR = % fractional tubular reabsorption with respect to creatinine).

	Plasma 10/02/03 µmol/L	**Urine sent mmol/mol creat**	**Plasma 19/05/03 µmol/L**	**Urine reference value**	**FTR %**
Val	423	14	428	<8	99.74
Leu	1000	46	923	<7	99.63
alle	200	6	193	ND	99.63
Ile	342	11	332	<8	99.76

- 17 labs measured amino acids and all except 2, reported a slight increase of BCAA. For one of the 2 labs who reported a normal profile, the quantification was correct, it was a problem of reference value.

- 19 labs performed organic acid profile. All labs except 3 reported an increase of branched-chain 2-ketoacids with a wide range of results when quantified. It is possible that this can be due to a problem of storage because the sample has been sent during the summer heatwave. Dr Bachmann's labs compared the results obtained on the urine sample before they sent it to our lab and the results they obtained when they received it as a participant. 2-oxoisocaproate and 2-oxo-3-methylvalerate concentrations dramatically decreased (see below) but were still elevated. Increase of branched-chain 2-hydroxyacids was reported by all labs.

	Urine orig	Urine sent	Reference value
2-hydroxyisovalerate	**433**	**312**	**<2**
2-oxoisovalerate	**204**	**210**	**<1**
2-hydroxyisocaproate	**9**	**5**	**<3.5**
2-oxoisocaproate	**1485**	**357**	**<2**
2-hydroxy-3-methylvalerate	**45**	**28**	**<2**
2-oxo-3-methylvalerate	**4268**	**861**	**<5**

- DNPH reaction was found positive by 5 labs. Only 2 labs reported the characteristic odor of urine.

- Interpretation was correct except that some labs concluded to possibly intermediate or intermittent phenotype; it is not clear if this is caused by lack of awareness of the reference limits in adults or by not recognizing that the very efficient tubular reabsorption leads to a "mild" pattern. Recommendations were good for everybody.

- Scoring

    - Analytical : increase of branched-chain 2-keto- and 2-hydroxyacids and of branched-chain aminoacids (score 2) increase of branched-chain 2-hydroxyacids, normal branched-chain aminoacids (score 1)

    - Interpretation : MSUD (score 2)

    - Recommendations : aminoacids or BCKD activity or mutation analysis (score 1)

Scores of participants

- **Survey 2003-1**

Lab n°	Patient P1 Tyrosinemia type I				Patient P2 SC-HMAD deficiency				Patient P3 Argininosuccinic ac.			
	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**	1	1	0	**2**	2	2	1	**5**
4	2	2	1	**5**	1	1	0	**2**	2	2	1	**5**
5	1	2	1	**4**	2	2	1	**5**	0	0	0	**0**
6	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
7	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
8	1	2	0	**3**	1	1	0	**2**	0	0	0	**0**
9	2	2	1	**5**	1	1	0	**2**	2	2	1	**5**
10	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
11	2	2	1	**5**	2	1	0	**3**	2	2	1	**5**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
13	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
14	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
15	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
16	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
17	2	2	1	**5**	2	1	0	**3**	2	2	1	**5**
18	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
19	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
20	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**

❖ **Survey 2003-2**

Lab n°	Patient P4 3-methylcrotonyl-CoA deficiency				Patient P5 Sialidosis (mucolipidosis type I)				Patient P6 MSUD			
	A	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**
1	2	2	1	**5**	0	0	1	**1**	2	2	1	**5**
2	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
3	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
4	2	2	1	**5**	0	0	1	**1**	2	2	1	**5**
5	2	2	1	**5**	2	1	1	**4**	2	2	1	**5**
6	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
7	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
8	2	2	1	**5**	2	1	1	**4**	1	2	1	**4**
9	2	2	1	**5**	0	0	0	**0**	1	2	1	**4**
10	2	2	1	**5**	2	1	1	**4**	2	2	1	**5**
11	2	2	1	**5**	1	0	0	**1**	2	2	1	**5**
12	2	2	1	**5**	1	0	0	**1**	2	2	1	**5**
13	2	2	1	**5**	1	1	1	**3**	1	2	1	**4**
14	2	2	1	**5**	0	0	0	**0**	1	2	1	**4**
15	2	2	0	**4**	0	0	0	**0**	2	2	1	**5**
16	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**
17	2	2	1	**5**	2	1	1	**4**	1	2	1	**4**
18	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**
19	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**
20	2	2	1	**5**	1	0	0	**1**	2	2	1	**5**

❖ **Total scores**

Lab number	Survey 2003-1	Survey 2003-2	Cumulative score	Cumulative score (%)
1	15	11	**26**	87 %
2	15	15	**30**	100 %
3	12	10	**22**	73 %
4	12	11	**23**	77 %
5	9	14	**23**	77 %
6	15	15	**30**	100 %
7	0	0	**0**	0 %
8	5	13	**18**	60 %
9	12	9	**21**	70 %
10	0	14	**14**	47 %
11	13	11	**24**	80 %
12	15	11	**26**	87 %
13	13	12	**25**	87 %
14	13	9	**22**	73 %
15	15	9	**24**	80 %
16	13	10	**23**	77 %
17	13	13	**26**	87 %
18	15	15	**30**	100 %
19	13	13	**26**	87 %
20	15	11	**26**	87 %

❖ Summary of scores

We excluded from this table, the labs who did not send results. The percentages given are the scores obtained from labs who sent a report.

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Recommen-dations (%)	Total (%)
Patient P1	Tyr. Type I	94	100	94	**97**
Patient P2	SC-HMAD	81	75	67	**74**
Patient P3	Argininosuc.	89	89	83	**88**
Patient P4	MCC	100	100	95	**99**
Patient P5	Sialidosis	50	32	58	**44**
Patient P6	MSUD	87	100	100	**95**

DPT-scheme in 2004

Same “rules” as in 2003 :

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

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 2004

The next meeting for the DPT-scheme Southern Europe will take place before the 41st Symposium of SSIEM in Amsterdam, on Tuesday 31st August in the morning.

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ANNEX 1

PROFICIENCY TESTING – SOUTHERN EUROPE
URINE SAMPLES ALREADY SENT

• **1998 : 1**	**A**	**OCT**	
	B	**Propionic**	
• **1999 : 1**	**C**	**MPS I ou 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**	