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**ERNDIM**
**Diagnostic proficiency testing**
**2010**
**Southern Europe**
**Lyon Centre**

# ANNUAL REPORT 2010

In 2010, 21 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 | 5 |
| Spain | 4 |
| Portugal | 2 |
| Switzerland | 1 |
| **TOTAL** | **21** |

## Logistic of the scheme

- **2 surveys** 2010-1: patient P1, P2 and P3
  2010-2: patient P4, P5 and P6

- **Origin of patients**: 4 out of the 6 urine samples have been kindly provided by participants (they will get a 20% discount on the DPT fee for 2011)
  - Patient P1: Mevalonic aciduria – Dr E Riudor, Dr JA Arranz, Hospital Maternoinfantile Vall d'Hebron, Barcelona
  - Patient P2: Aminoacylase I deficiency – Dr O Sass, Labor für Klinische Biochemie und Stoffwechsel, Universitätklinikum Freiburg, Germany.
  - Patient P3: No metabolic disorder – Dr B Merinero, Universidad Autonoma, Madrid
  - Patient P4: Sialidosis type I – Centre de Biologie Est, Lyon - This sample has been sent to all labs participating to the DPT scheme in Europe
  - Patient P5: Glutaric aciduria type I – Dr M Fontaine, CHRU, Lille
  - Patient P6: Aspartylglucosaminuria – Centre de Biologie Est, Lyon

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

## Timetable of the schemes

- 10 May 2010: shipment of samples of Survey 1 and Survey 2 by DHL and of the forms by e-mail
- 4 June 2010: deadline for result submission (Survey 1)
- 21 June 2010: report of Survey 1 by e-mail
- 23 June 2010: analysis of samples of the second survey
- 14 July 2010: deadline for result submission (Survey 2)
- 9 August 2010: report of Survey 2 by e-mail
- 31 August 2010: meeting in Istanbul before the SSIEM meeting
- 31 January 2011: annual report with scoring sent by e-mail

## Date of receipt of samples

| | **Survey 1 + 2** |
|---|---|
| + 24 hours | 18 |
| + 48 hours | 2 |
| + 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: | 21 labs | 21 labs |
| Before deadline | 18 | 20 |
| + 3 days | 1 | |
| + 1 week | 1 | 1 |
| + 2 weeks | 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 criteria 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 Istanbul on 31 August 2010 from 9.00 to 10.30, before the SSIEM Meeting.

### ❖ Participants

Representatives from 12 labs were present.

### ❖ Information from the Executive Board and the Scientific Advisory Board

- **Scoring and certificate of participation**: from 2010, scoring is done by 2 scheme organizers. For the Lyon Centre, the results have also been scored by Dr Viktor Kozich from Prague Centre. Certificate of participation for 2010 will be issued for participation and it will be additionally notified whether the participant has received a warning letter. This warning letter is sent out if the performance is less than 60% (score < 18 / 30).
- Two warning letters will be sent in 2010.
- 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 website reporting system will be mandatory in 2011.
- **Training**: SSIEM Academy training courses.
    - A 2 days course has been organized on Monday and Tuesday 4 & 5 October 2010 in Manchester on disorders of mucopolysaccharides / oligosaccharides, purines / pyrimidines and peroxisomes. Thirty nine biochemists attended this meeting. The lectures are available on the website.
    - The next course will take place on Wednesday and Thursday 23 & 24 November 2011 in Amsterdam: 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 "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.

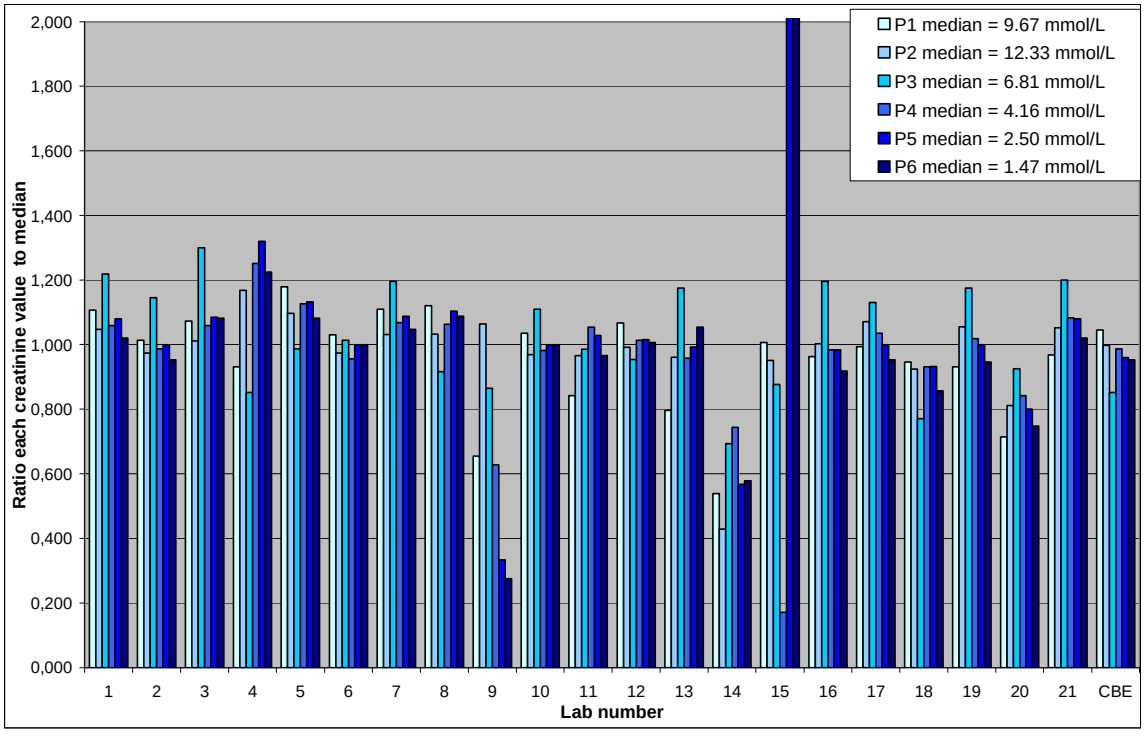
  Please send us an e-mail on the day you send the samples.

- **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

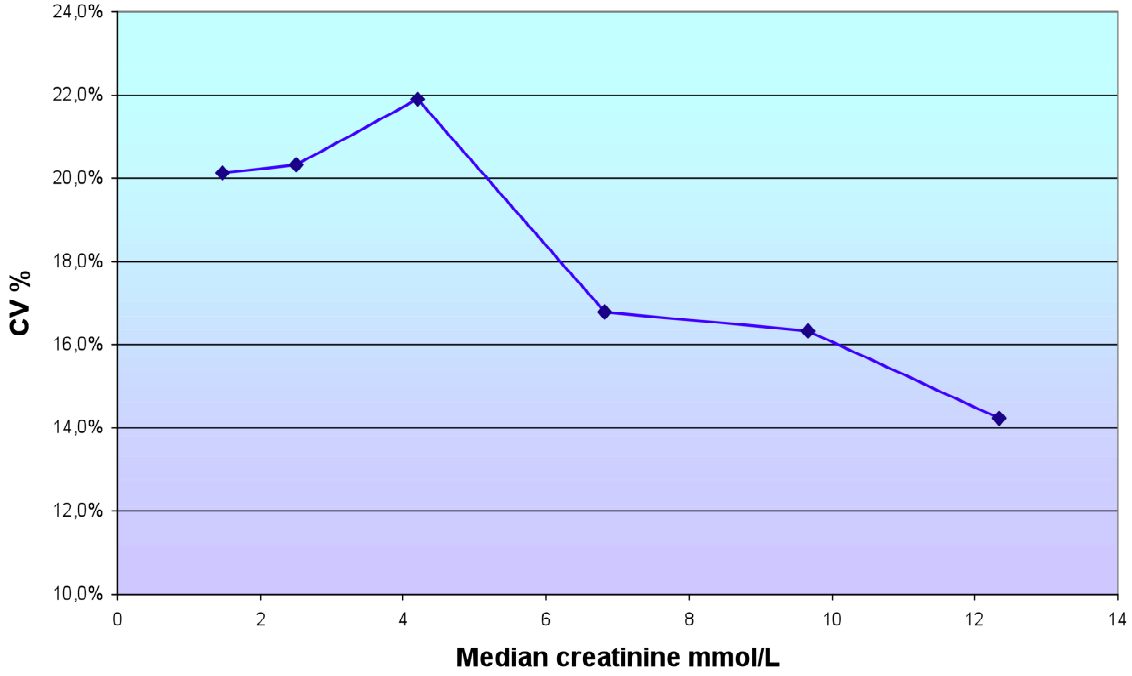
- **Creatinine measurement**

Creatinine determination was correct for most labs, except for lab 15 who had completely wrong values for survey 2 (?); he had the same problem last year. Lab 9, 14, and, to a lesser extent, lab 20 have systematically low values.




The CV is > 14% for all samples (14 – 22 %) and this is higher than the interlab CV 2009 for Special

Assay in urine (6.5 %, n = 108) and for Quantitative Organic Acids (5.8 %, n = 68). The CVs are higher for the samples with the lowest creatinine value.



- **Patient P1 – Mevalonic aciduria (mevalonate kinase deficiency, hyper IgD syndrome)**

This 20 year-old boy began to have symptoms in the neonatal period with systemic inflammation: high fever, adenopathies, serositis, intestinal inflammation, persistent arthritis, conjunctivitis, cataract and acne. He was diagnosed at the same time than his 9 year-old brother who presented a critical clinical picture. At diagnosis, the mevalonate ranged from 2.1 – 9.8 mmol/mol creatinine, but now it is much higher. IgD level was 234 UI/L (controls <90) at the time of collection of the urine sample. Genetic study of *MVK* gene revealed that he is compound heterozygote for 2 sequence alterations: p.His20Asn / p.Arg215Gln. Several therapeutic trials did not lead to substantial modification of his clinical state. Recently, the use of anakinra (Kineret©), an antagonist of IL-1 receptor, permitted a better control.

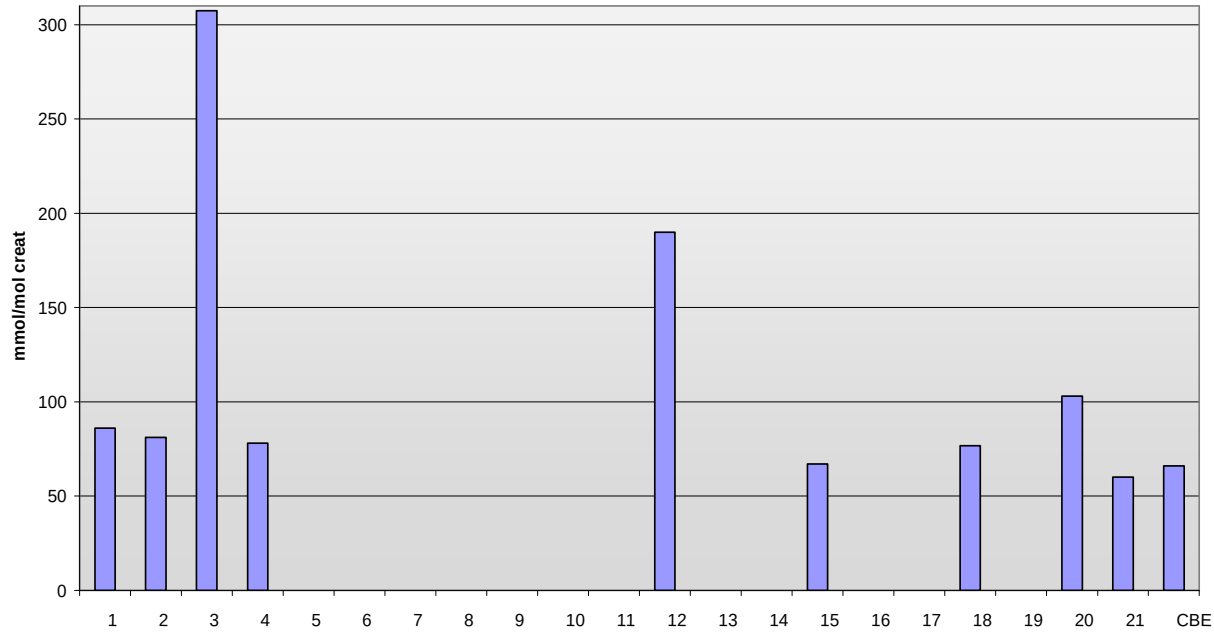
### Diagnosis

All labs reached the good diagnosis of mevalonate kinase deficiency; however, one did not find abnormality, but suggested hyper IgD syndrome because of the clinical picture and treatment.

**Aminoacid** analysis was performed by all labs, and was found to be normal.

All labs also performed **organic acid** analysis: 18 reported an increase of mevalonolactone, and 7 an increase of mevalonic acid. Drug metabolites were also reported: ibuprofen (6 labs), paracetamol (4 labs), « drugs » (1 lab), and caffein (1 lab). The urine sample from the lab who did not detect mevalonic acid and mevalonolactone was re-analyzed by the scheme organizers and found positive.

**Mevalonolactone median = 81 mmol/mol creat**




Accurate quantification of mevalonolactone requires stable isotope dilution. DL-mevalonolactone-$d_3$ is available as internal standard from CDN Isotopes [ref D-3050]. Mevalonic acid is now included in the ERNDIM Quantitative Organic Acid scheme

**Advice for further investigations** was OK for all labs.

**Scoring**

- Analytical: increase of mevalonolactone and / or mevalonic acid (score 2)
- Interpretation of results: Mevalonate kinase deficiency (score 2), MK deficiency according to clinical data (score 1).
- Recommendations: mevalonate kinase activity (lymphocytes or fibroblasts) and/or mutation analysis *MVK* gene and/or Ig D in plasma (score 1).

- **Patient P2 – aminoacylase I deficiency**

This urine sample has been provided by Dr Oliver Sass (Freiburg). This 11 year-old boy is the eldest child of consanguineous Turkish parents residing in Germany. Two younger brothers are unaffected. He started walking at age 1 year. At age 3 years, the parents first observed muscle weakness that prompted a thorough investigation at age 11 years. At examination, he had a low-normal muscle tone and a shambling gait. There was no abnormality in plasma amino acids. Urinary organic acid profile (methyl esters) revealed abnormal peaks of *N*-acetylalanine, *N*-acetylvaline, *N*-acetylisoleucine, *N*-acetylglycine, *N*-acetylleucine, *N*-acetylmethionine, *N*-acetylglutamic acid. Mutation analysis *ACY1* gene revealed he is homozygous for a 2-bp insertion (c.1105-1106insAC) predicting a frame shift beginning with amino acid residue 369. Notably, the mutation does not lead to premature termination of translation but predicts a C-terminal mutated protein (p.369PfsX46) that is longer than the wild-type protein (408 amino acid residues) and affects the C-terminal peptidase domain. Segregation analysis confirmed heterozygous carrier status in both parents. ACY1 activity in EBV-transformed lymphoblasts was 0.08 nmol/min/mg prot (control values: mean 1.24; SD 0.53 nmol). Ultrasound, electromyography, and NMR tomography of the musculature from the lower extremities showed unremarkable results. Muscle biopsy did not show significant myopathological abnormalities. The boy's cognitive development appears normal. At present, he attends high school. This patient has been published: Sass et al. J Hum Genet. 2006; 78: 401–40.

**Diagnosis**

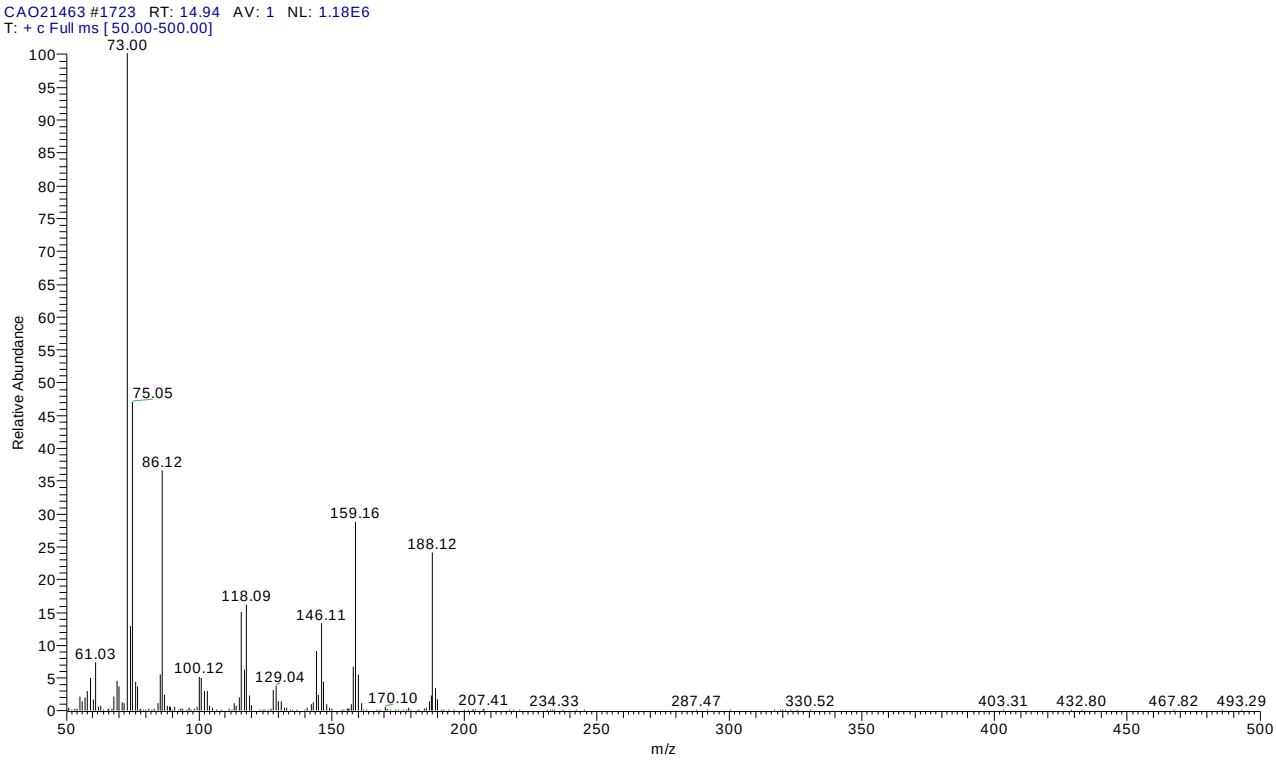
Twelve labs concluded to aminoacylase I deficiency, whereas 9 labs gave no or a wrong diagnosis.

**Aminoacid** analysis (20 labs) revealed slight abnormalities probably due to a poor storage of the urine sample. All labs performed **organic acids**, but only 12 of them identified the abnormal presence of N-acetylaminoacids.

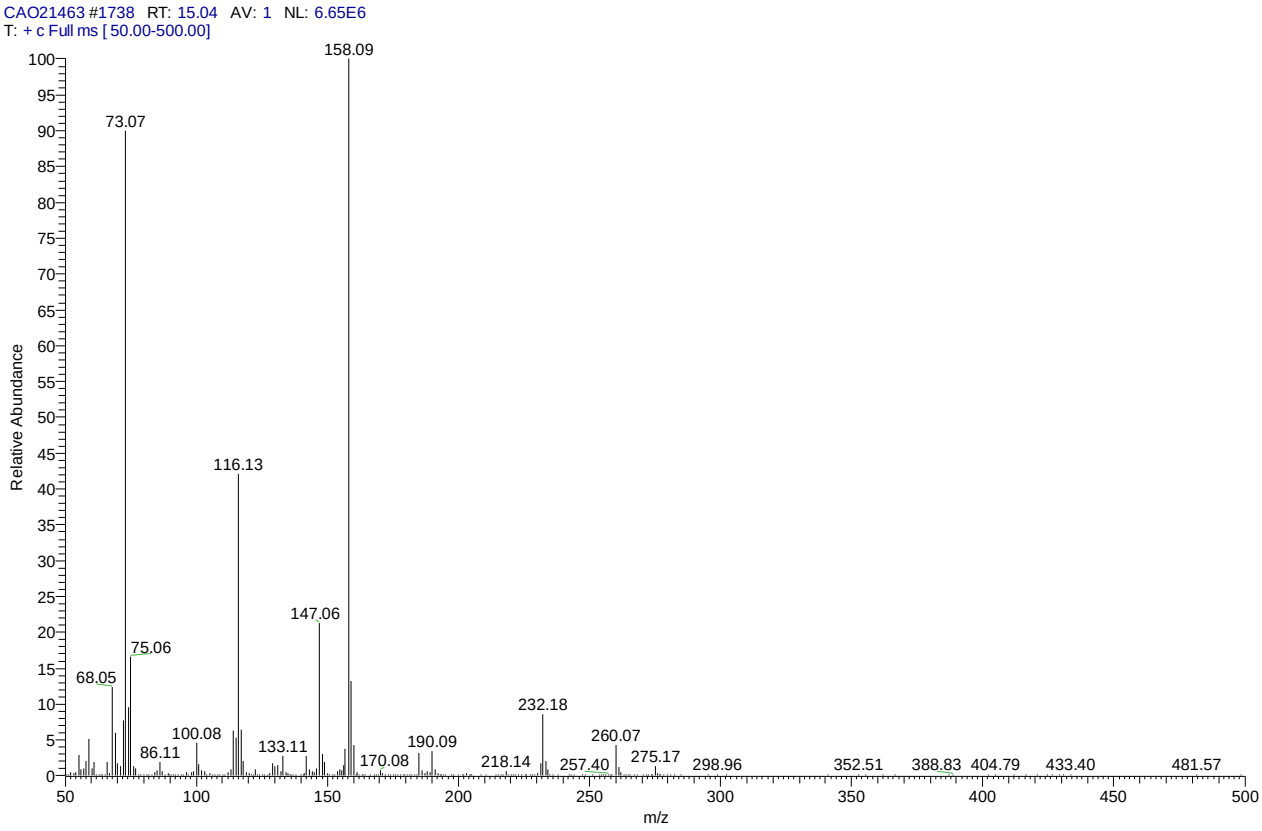
| N-acetylaminoacid | Number of labs |
|---|---|
| N-acetylglutamic acid | 12 |
| N-acetylglycine | 9 |
| N-acetylalanine | 9 |
| N-acetylleucine | 9 |
| N-acetylvaline | 8 |
| N-acetylaspartic acid | 6 |
| N-acetylisoleucine | 5 |
| N-acetylmethionine | 5 |
| N-acetylserine | 4 |
| N-acetylthreonine | 2 |
| N-acetylglutamine | 1 |
| N-acetylalloisoleucine | 1 |

**Spectra of N-acetyl aminoacids (in order of elution)**
(thanks to Dr JF Benoist, Dr C Caruba and Dr C Ottolenghi for their help)

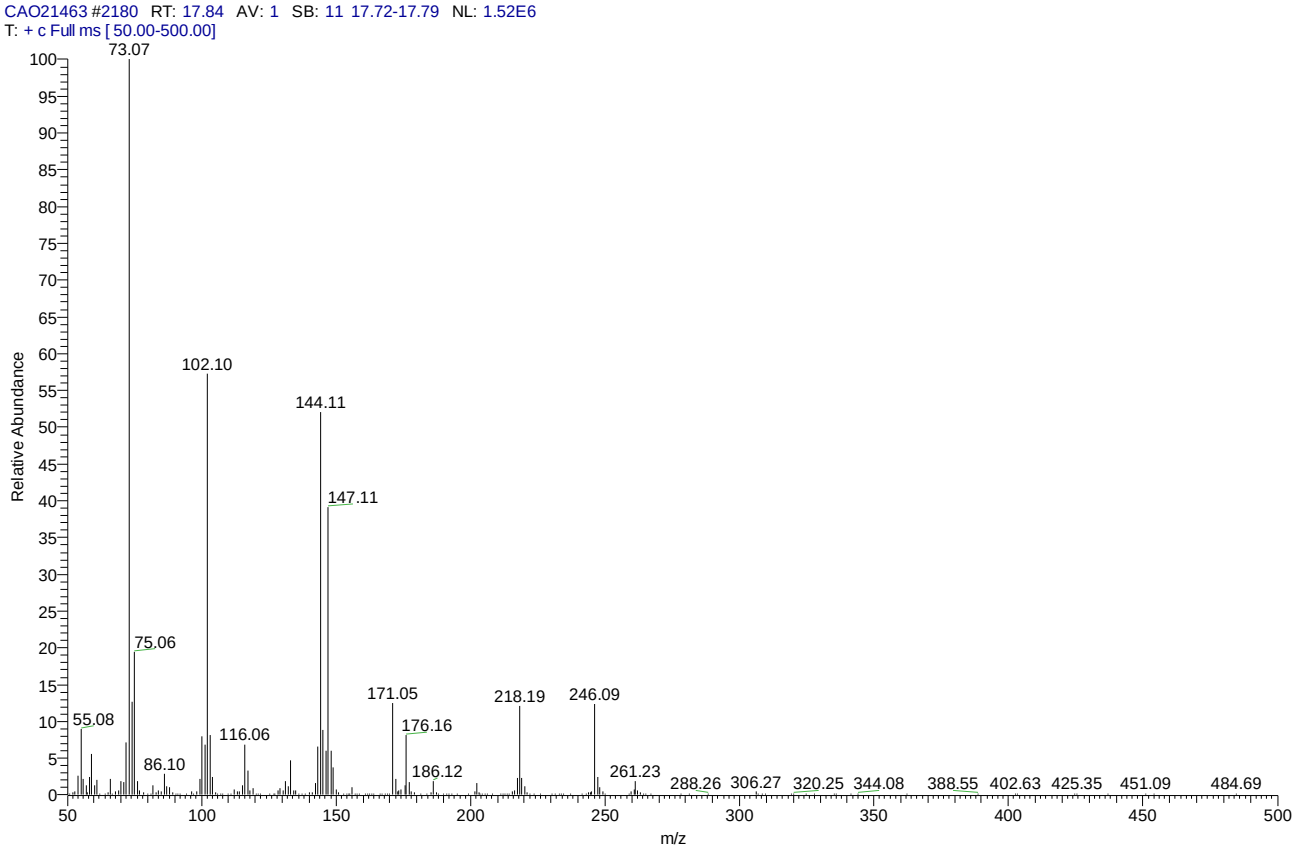
- **N-acetylalanine TMS1 :** eluted between urea and phosphoric acid



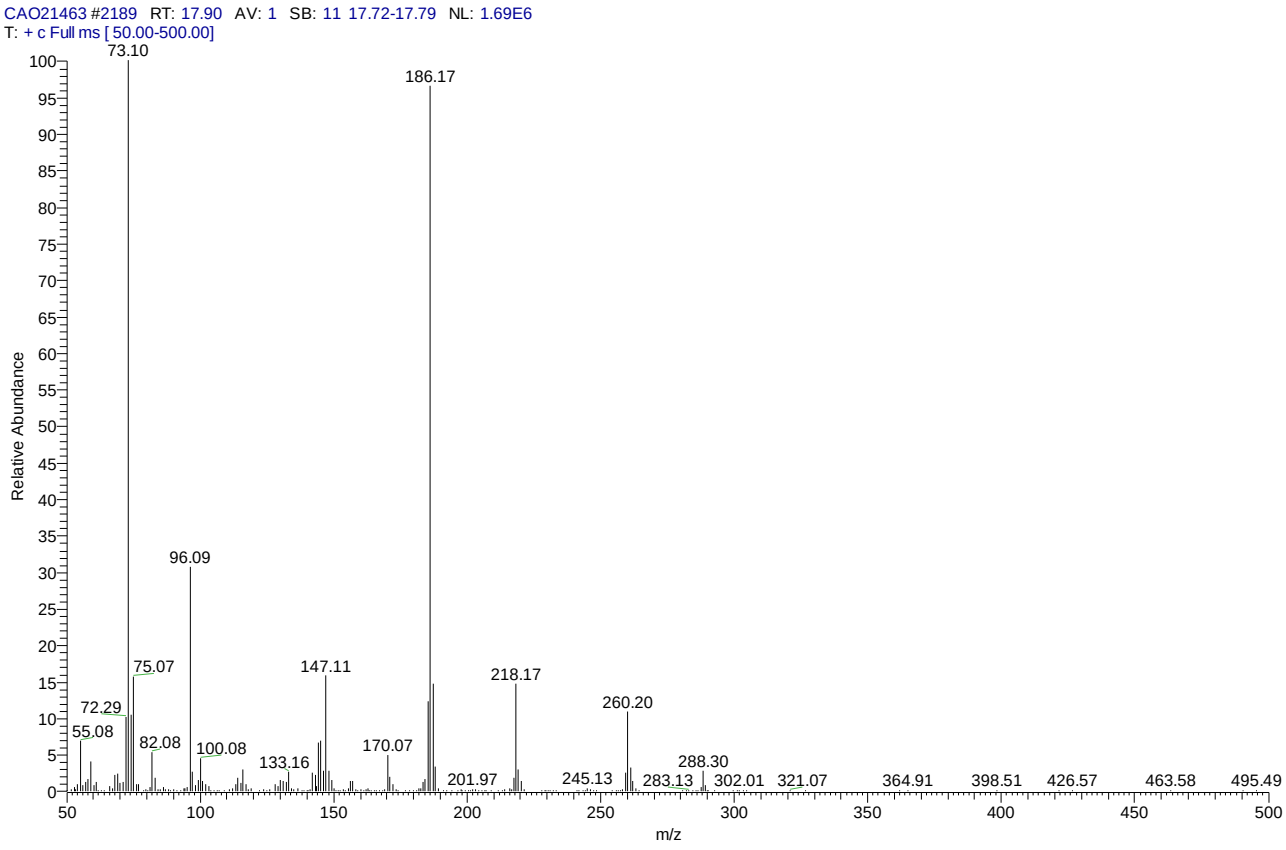

- **N-acetylalanine TMS 2 :** eluted between N-acetylalanine TMS 1 and phosphoric acid



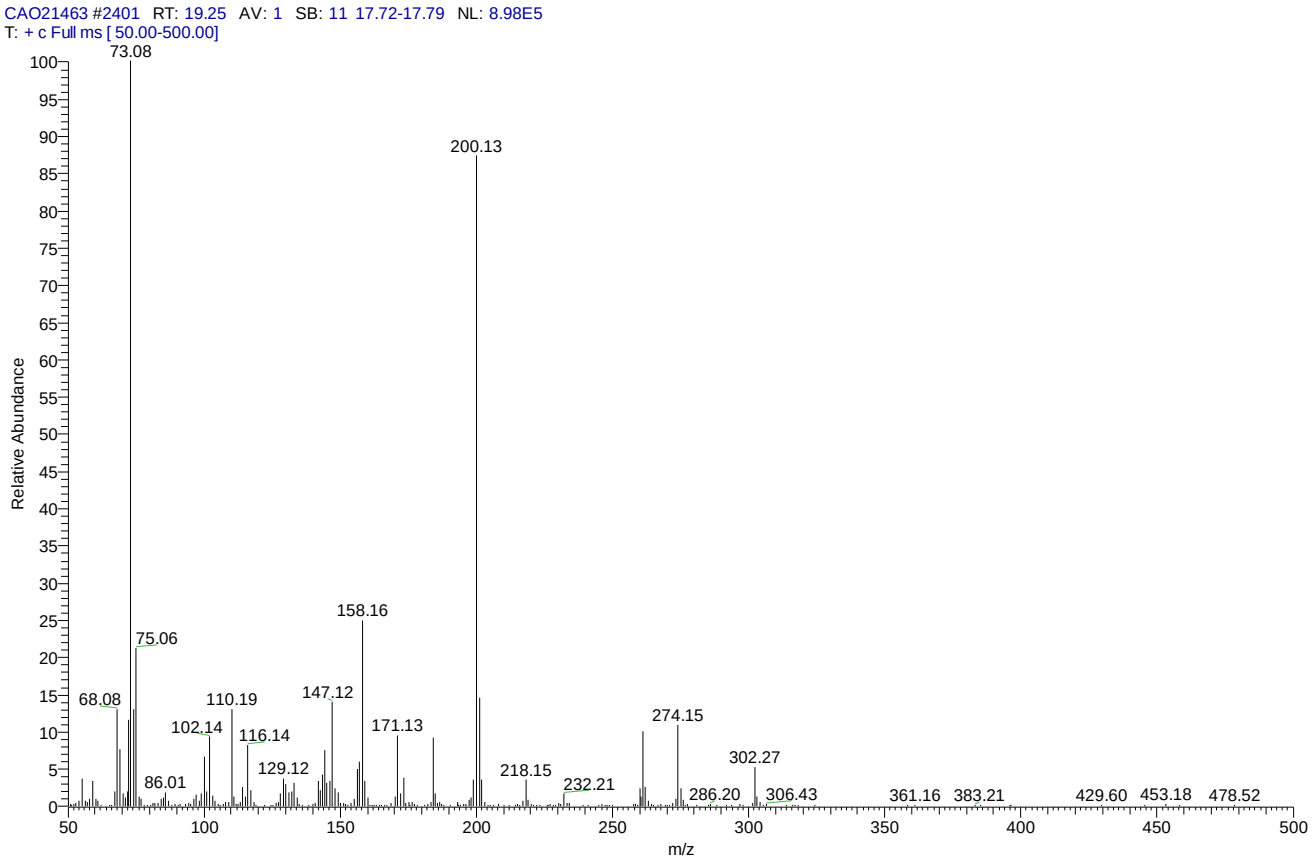
- **N-acetylglycine TMS 2 :** eluted between 2,3-dihydroxybutyric and glutaric acids



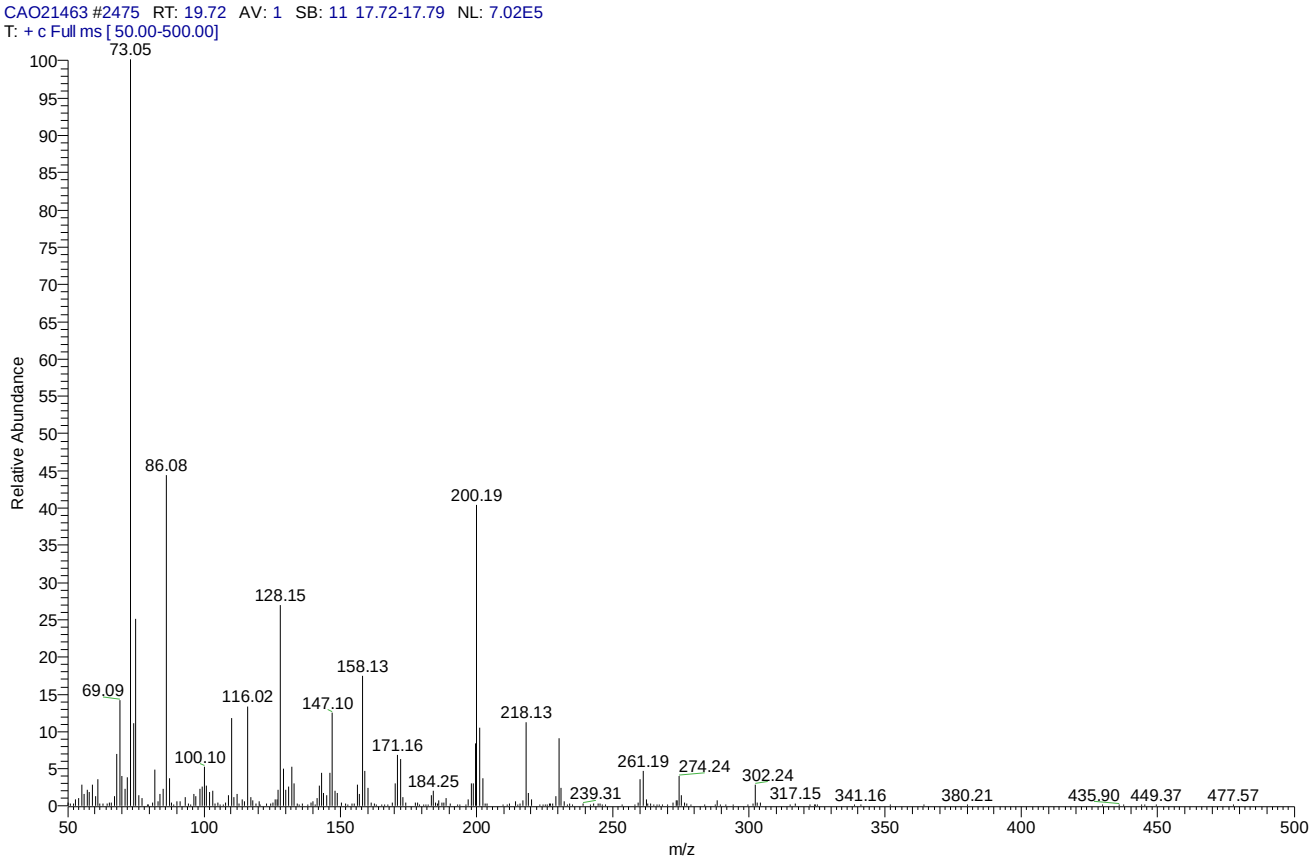

- **N-acetylvaline TMS 2 :** eluted between N-acetylglycine TMS 2 and glutaric acid



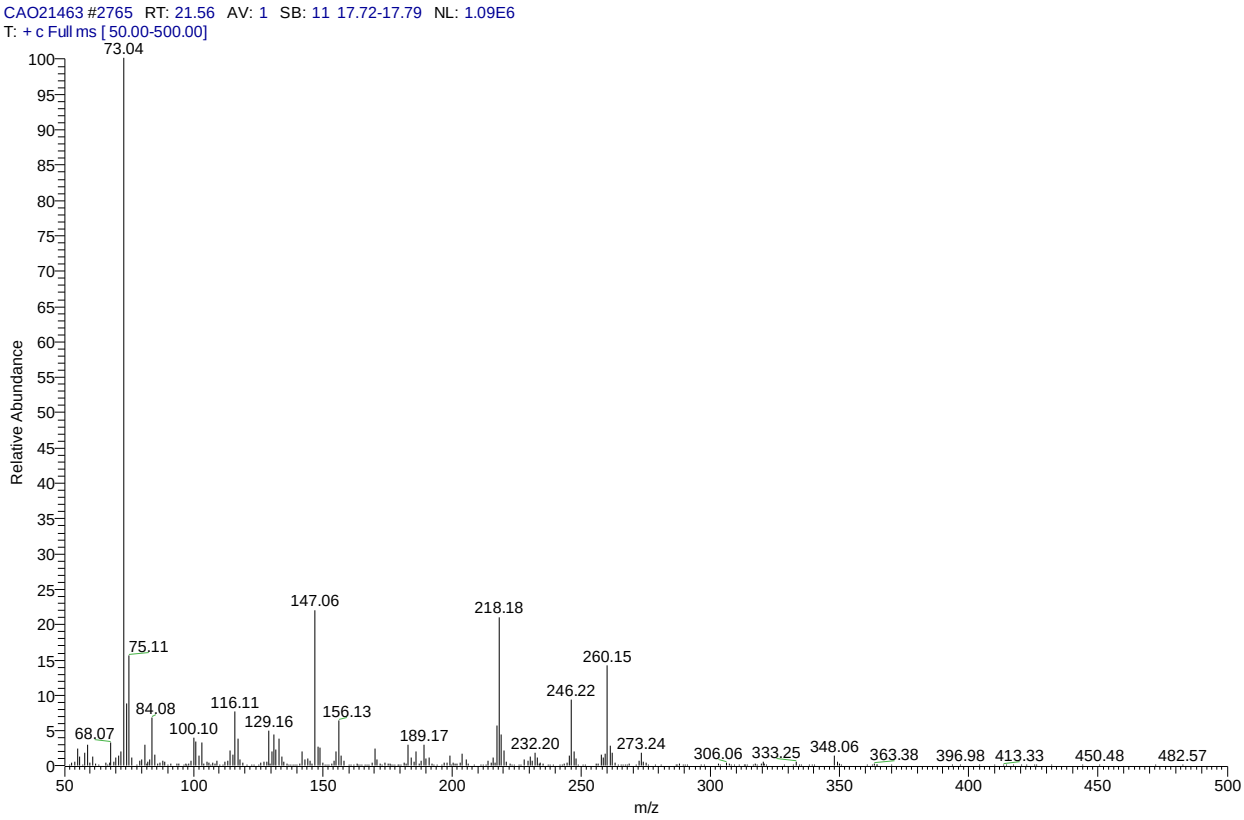
- **N-acetylleucine TMS 2 :** eluted after glutaric acid



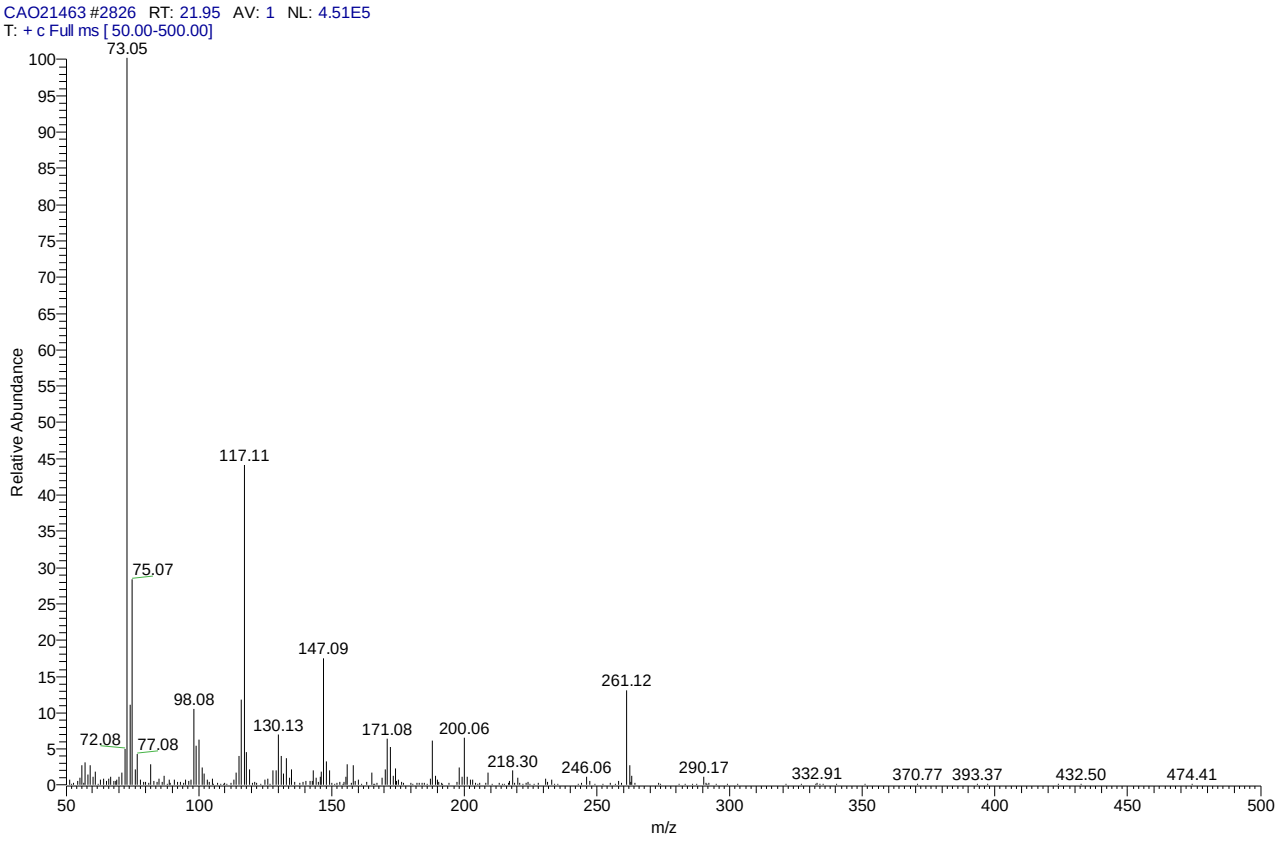

- **N-acetylisoleucine TMS 1 and TMS 2 :** coeluted before malic acid



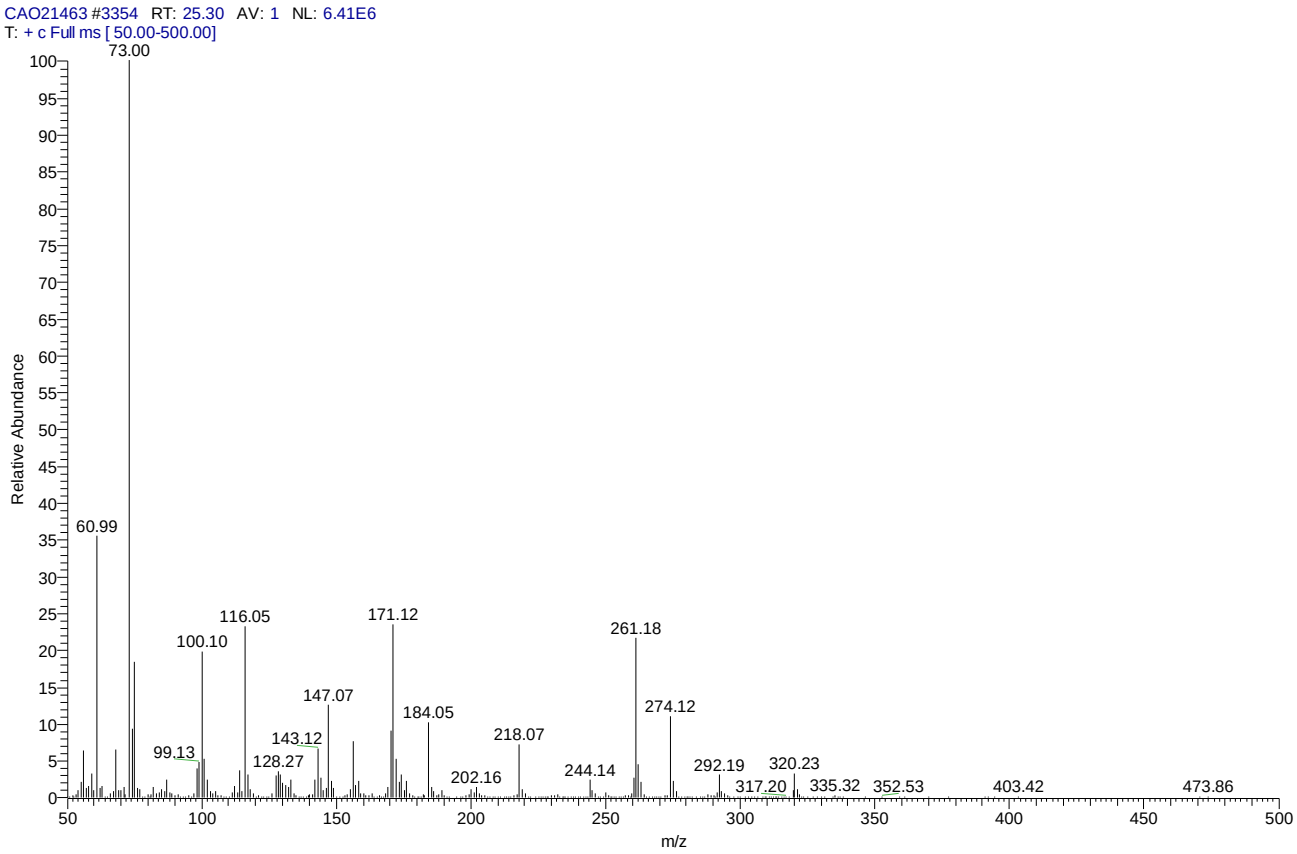
- **N-acetylserine TMS 3 :** eluted between adipic acid and 4-phenylbutyric acid (IS)



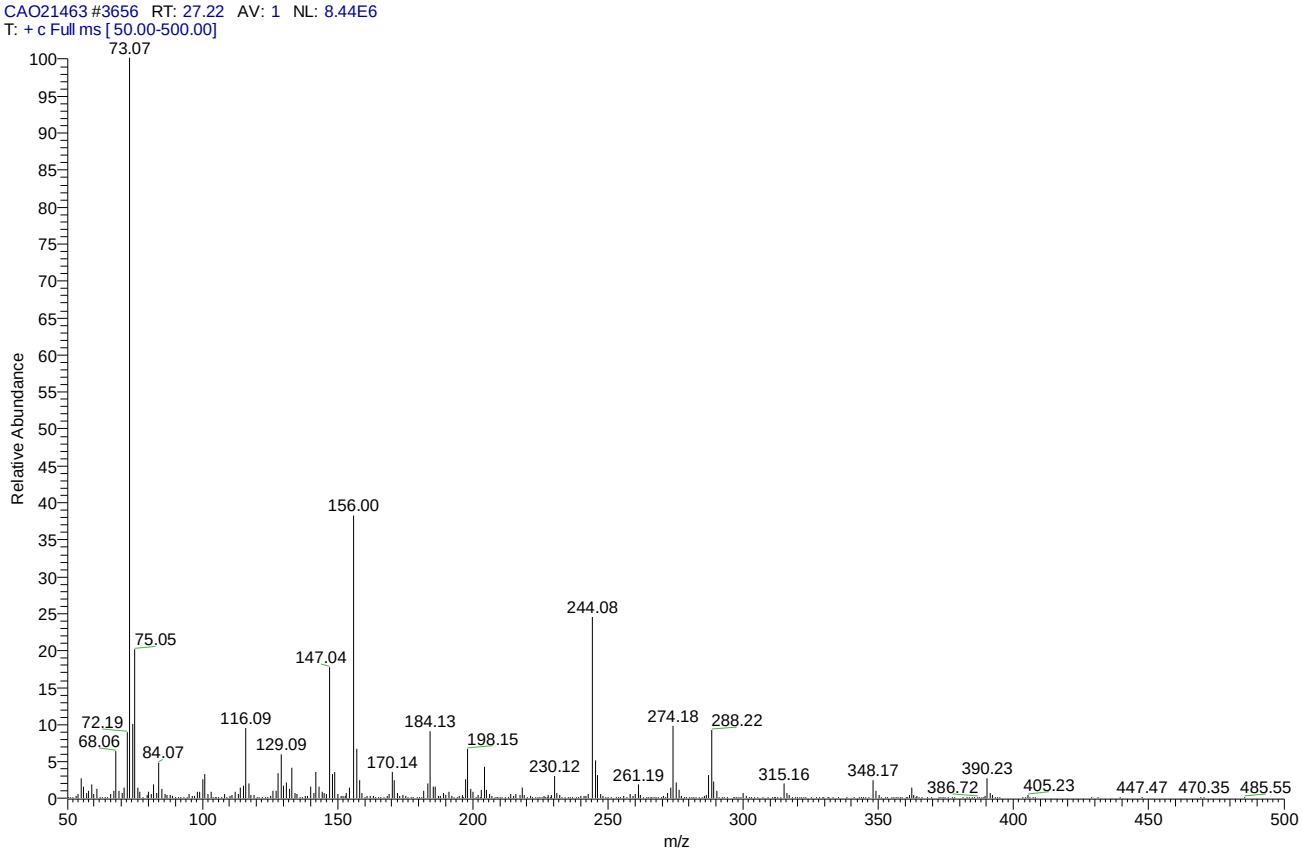

- **N-acetylthreonine TMS 2 :** eluted after 4-phenylbutyric acid (internal standard)



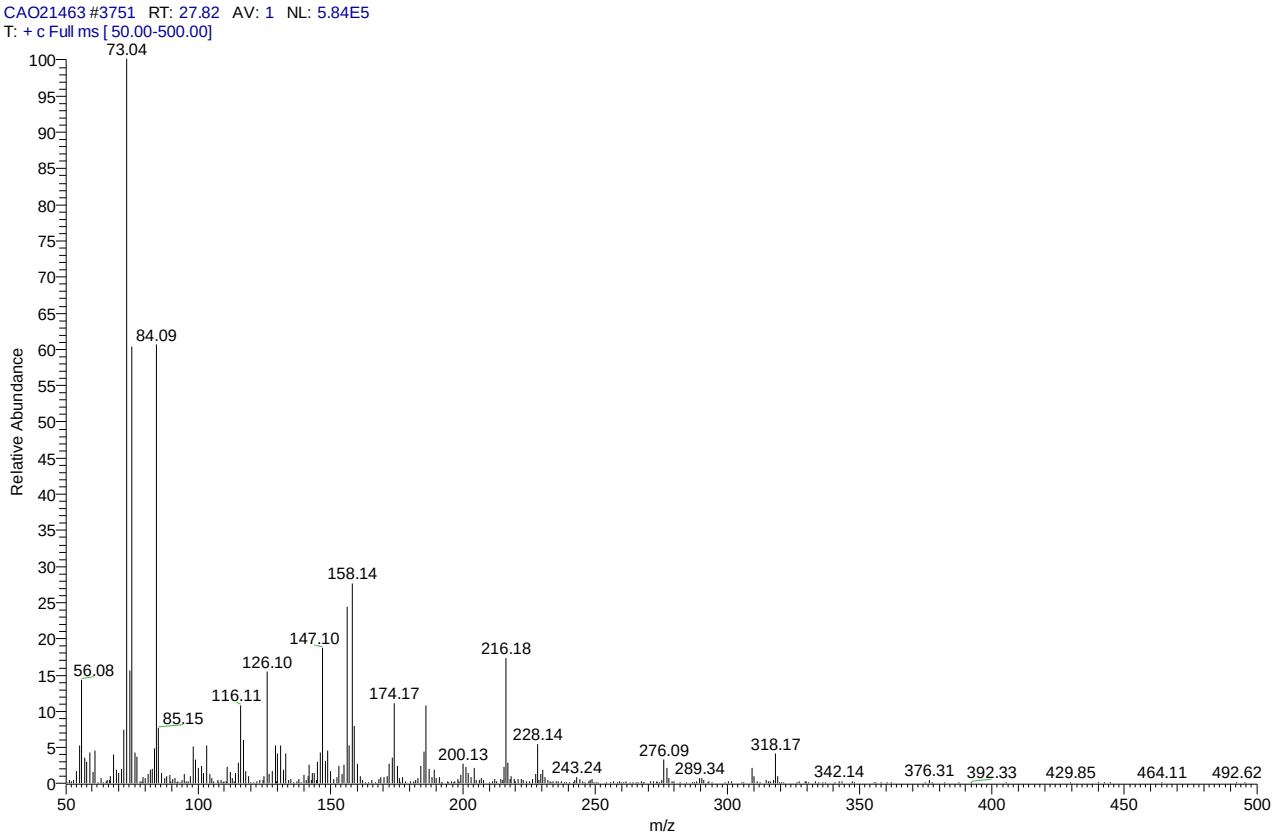
- **N-acetylmethionine TMS 2 :** eluted between 4-hydroxyphenylacetic and suberic acid




- **N-acetylglutamate TMS 3 :** eluted after cis-aconitic acid (co-eluted with homovanillic acid)



- **N-acetylglutamate TMS 2 :** eluted between N-acetylglutamate TMS 3 and azelaic acid




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

**Scoring**

- Analytical: Identification of at least 3 *N*-acetylamino acids (score 2), unknown peaks, some look amino acids (score 1)
- Interpretation of results: aminoacylase 1 deficiency (score 2).
- Recommendations: Mutation analysis *ACY1* gene and/or aminoacylase 1 activity and/or repeat organic acids on a new urine sample (score 1).

- **Patient P3 – No metabolic disorder**

This boy, aged 9 years, is born from non consanguineous parents; an elder sister presented with anorexia nervosa. He was first investigated at 2 years of age due to psychomotor delay, more pronounced in the speech area. He presented failure to thrive since the first year of age. At that time, normal results were obtained for brain MRI, caryotype (X-fragile), routine biochemical analyses, ophtalmological assessment, auditive evoked potentials. EEG revealed unspecific irregular cerebral bioelectric activity. He was reinvestigated at 6 years of age: restless, repetitive but not aggressive behaviour, and psychomotor delay evocating Asperger syndrome. EEG was normal at that time. He attended a school for handicapped children. He mental ability was that of a 3-year child. He has been lost for follow-up for the last 2 years.

**Diagnosis**

Sixteen labs reported no relevant abnormalities with the performed investigations and 2 labs did not conclude. Conversely, 3 labs gave a wrong diagnosis.

All labs reported a normal **amino acid** profile. All labs except one also reported a normal **organic acid** profile.

**Mucopolysaccharide** analysis was performed by 17 labs: all reported normal results. Among the 19 labs who performed **oligosaccharides**, 2 of them (same cluster) suggested Schindler disease type III. In the scheme organizers' experience, the profile for Schindler disease is different.

One lab over the 3 labs who performed **purines and pyrimidines**, reported an increase of SAICAR. Other investigations (creatine & guanidinoacetate, Bratton & Marchall, TLC sialic acid, homocystein, acylcarnitines, orotic acid) were all normal.

**Advice for further investigations:** only 13 labs but various!

**Scoring**

- Analytical performance: all investigations (amino acids, organic acids, oligosaccharides, mucopolysaccharides): no significant abnormality (score 2), all except one investigations: no significant abnormality (score 1).
- Interpretation of results: no significant abnormality with the performed investigations (score 2), no conclusion (score 1)
- Recommendations: other assays or repeat assays or probably not an IEM (score 1)

- **Patient P4 – sialidosis type I (common sample)**

This urine sample is from a 43 year old woman who presented progressive walking difficulties, osteoporosis, cholestasis and myoclonic epilepsy. She also had a loss of visual acuity. Opthalmoscopy revealed a cherry red spot. This patient is affected with sialidosis type I: α-D-neuraminidase deficiency, also called sialidase deficiency or cherry-red spot-myoclonus syndrome or mucolipidosis type I. Detailed clinical and biochemical data of this patient have been presented in the ERNDIM session at SSIEM meeting (see presentation attached).

**Diagnosis**

Seventeen labs gave the write diagnosis of sialidosis or galactosialidosis (best score among all centres!), 2 labs concluded to a most probable sphingolipidosis, 1 lab to an oligosaccharidosis, probably GM1 gangliosidosis, and 1 lab to GM2 gangliosidosis.

Fourteen out of the 16 labs who performed **aminoacid** analysis reported an increase of glycine secondary to valproate therapy. And 13 labs out of the 15 who performed **organic acids** identified metabolites of valproate. Only 8 of them identified levotiracetam, although the spectrum of its metabolites has been distributed in the annual report 2009.

All labs except one performed **oligosaccharide** analysis: 14 identified a profile consistent with sialidosis or galactosialidosis (same profile), 3 reported sialyloligosaccharides. The 3 remaining labs reported an abnormal profile but the conclusion was wrong: sphingolipidosis, probably GM1 gangliosidosis and GM2 gangliosidosis. The 3 labs who performed TLC for sialic acid identified sialyloligosaccharides. One lab reported increased quantification of conjugated sialic acid whereas free sialic acid was normal.

**Advice for further investigations** was appropriate for those who reached a correct diagnosis.

**Scoring**

- Analytical: oligosaccharide profile suggestive of sialidosis or galactosialidosis, and/or increase of silalyloligosaccharides, and/or increase of conjugated sialic acid (score 2), abnormal oligosaccharide profile (score 1)
- Interpretation of results: sialidosis or galactosialidosis (score 2), oligosaccharidosis, or diagnosis of sialidosis based on clinical data, or "sialidosis or GM1gangliosidosis" (score 1), GM1 or GM2 gangliosidosis, or sphingolipidosis (score 0)
- Recommendations: α-neuraminidase activity (leukocytes, or fibroblasts) and / or mutation analysis *NEU1* gene (score 1)

- **Patient P5 – glutaric aciduria type I (glutaryl-CoA dehydrogenase deficiency)**

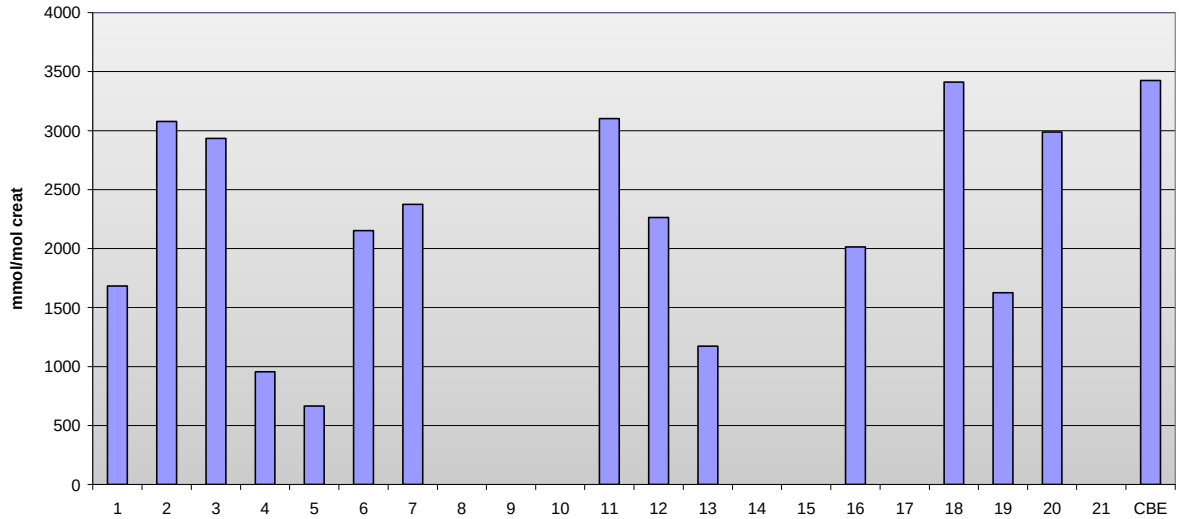
This 3 year-old girl had a normal psychomotor development. She suddenly felt tired in the morning. Then she vomited twice during the day, without diarrhoea, nor fever. The day after, she was found comatose in her bed. At admission in the intensive care unit, she had hypoglycaemia (0.44 mmol/L) without significant ketonuria (+). Skull size was +2SD. Cerebral MRI was suggestive of glutaric aciduria type I. The urine sample has been collected before treatment. She recovered under glucose infusion, riboflavine and carnitine supplementation, lysine and tryptophane restricted diet. Mutation analysis of *GCDH* gene is under investigation.

**Diagnosis**

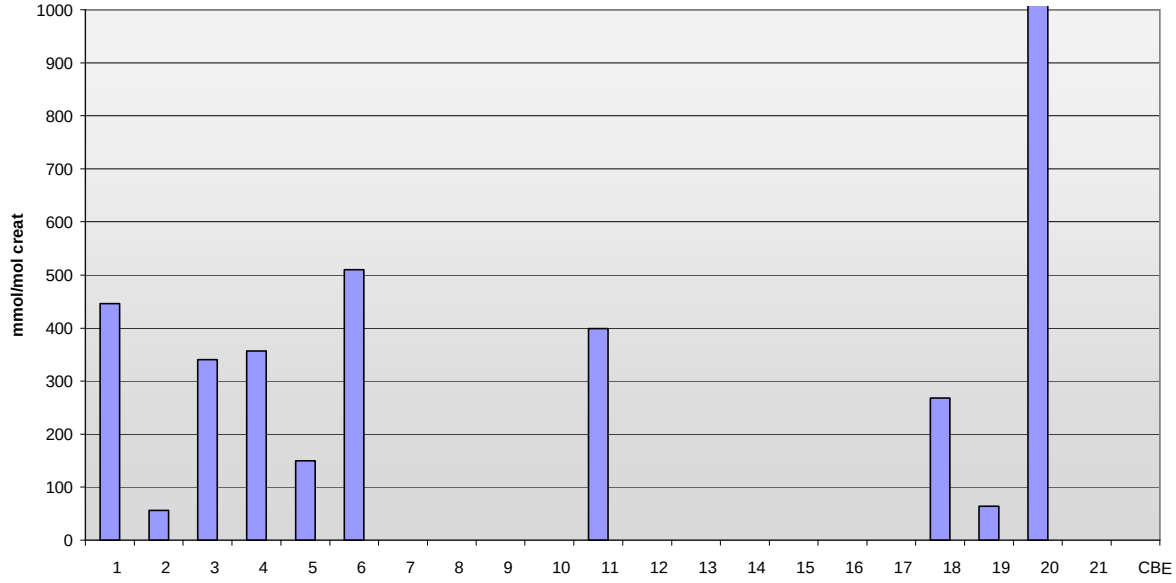
All labs concluded to glutaric aciduria type I.

All labs performed **organic acids** and reported an increase of glutaric acid (range: 665 – 3411 mmol/mol creat) and 3-hydroxyglutaric (range: 56 – 1075 mmol/mol creat) but with a high CV for both of them (see below), although glutaric acid belongs to the Quantitative Organic Acid scheme of ERNDIM. Standard for 3-hydroxyglutaric acid is available from Dr Herman Ten Brink in Amsterdam: HJ.tenBrink@vumc.nl. and its stable isotope (*3-hydroxy –1, 5- pentanedioic – 2,2,3,4,4 –$d_5$ acid* - C5H3D5O5) is available from CDN isotopes (reference D-639), stockist Cil Cluzeau Info Labo. E-mail: cil@cluzeau.com.

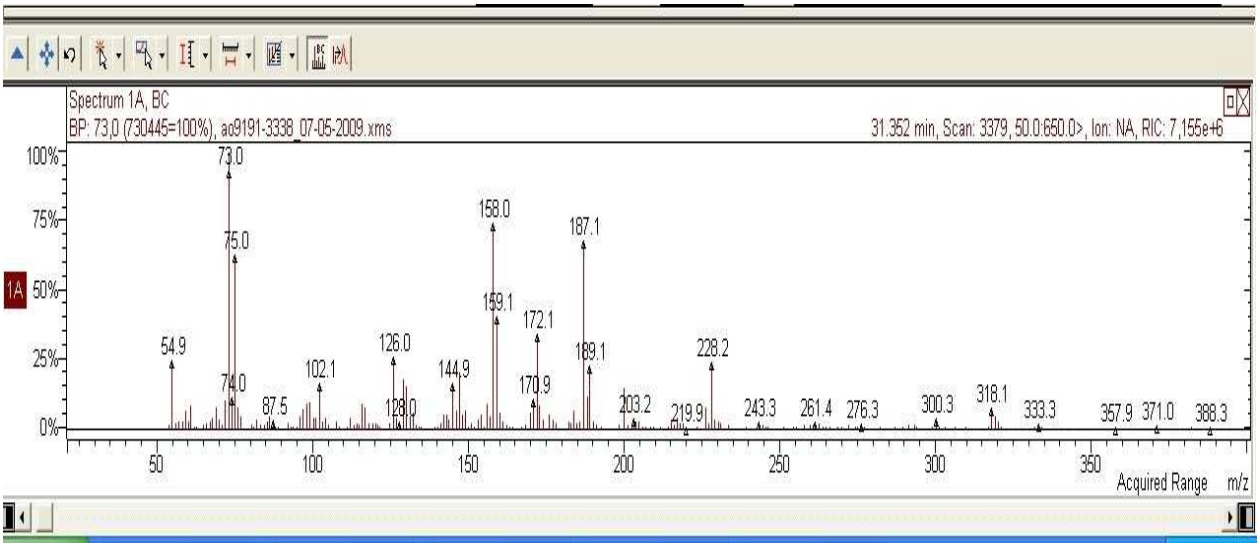
**Glutaric acid - median = 2 261mmol/mol creat - CV = 40%**



**3-hydroxyglutaric acid (median = 349 mmol/mol creat - CV = 84%)**




Only 1 lab reported the presence of glutarylglycine whose spectrum was circulated in the 2009 Annual Report.




Three labs reported an increase of glutarylcarnitine (C5DC): 43; 52.4; 96.9 mmol/mol creatinine - control < 2.0 (scheme organizers: 73 mmol/mol creatinine).

**Advice for further investigations** 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)
- Recommendations: mutation analysis *GCDH* gene and/or glutaryl-CoA dehydrogenase activity and/or blood acylcarnitine profile (score 1)

## Patient P6 – Aspartylglucosaminuria (aspartylglucosaminidase deficiency)

The patient, a boy, is the first child of non consanguineous parents. His mother had 5 miscarriages before this pregnancy. She remained in bed during the duration of this pregnancy. At birth, the patient presented with inguinal hernia and metatarsus varus. He had recurrent viral infections and diarrhea. A speech delay was noted at 2 years 1/2. Clinical examination at 4 years of age revealed slightly dysmorphic features, moderate pyramidal syndrome of lower limbs, slight leukodystrophy, hyperactivity, leading to a metabolic workup. Plasma amino acids (LC-MS/MS) allowed to detect aspartylglucosamine (32 µmol/L – controls: undetectable). Urine oligosaccharides and amino acids confirmed the diagnosis (aspartylglucosamine = 461 mmol/mol creat – controls: undetectable).
Aspartylglucosaminidase activity in leukocytes was 3.2 µkat/kg prot (simultaneous control = 67.9).
At 4 years of age, he underwent bone marrow transplantation (BMT). He subsequently presented several febrile episodes, epidermolysis, hypotension and digestive problems. He had a colonoscopy in April 2007 but a septic shock occurred leading to death 3 months after the BMT.

The urine sample has been collected at the time of the diagnosis.

### Diagnosis

Only 7 labs concluded to aspartylgucosaminuria and this is worse than in 2007 (see below). This is the same urine sample than in 2007 which has been stored at -20°C. A possible problem of storage can explain the worsening of results.

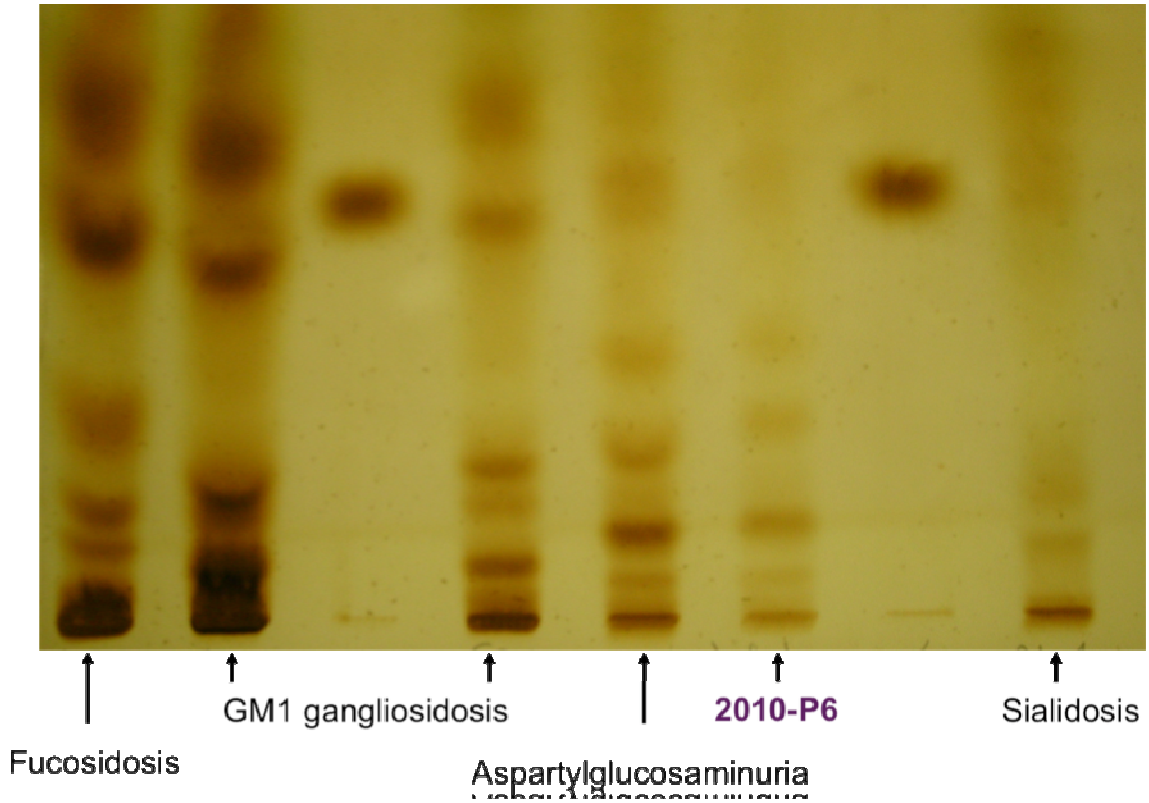
| Diagnosis | 2010 | 2007 |
|---|---|---|
| Aspartylglucosaminuria | 7 | 9 |
| Oligosaccharidosis | 2 | 3 |
| Wrong oligosaccharidosis | 2 | 3 |
| Wrong or no diagnosis | 11 | 7 |

All labs except one performed **aminoacid analysis**. Among them, 6 labs identified aspartylglucosamine (they were 9 in 2007); one reported an increase of asparagine suggestive of aspartylglucosaminuria and one an unidentified substance with retention time close to urea.

In 2007, we got the following information from participants:
- Jeol analyser, aspartylglucosamine is eluted with urea, but abnormal ratio 570 nm / 440 nm
    - Urea : 570 / 440 nm ratio = 12
    - Patient: 570 / 440 nm ratio = 9
    - Patient after hydrolysis of urine : 570 / 440 nm ratio = 12
- Hitachi analyser, it is eluted before or with urea (abnormal 570 / 440 nm ratio in the last case)
- Biochrom analyser, it is separated from urea
- LC-MS/MS (scheme organizer), it is easy to detect

Standard for aspartylglucosamine [2-acetamido-1-N(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine or 2-acetamido-1-β-(L-aspartamido)-1,2-dideoxy-D-glucose or β-D-GlcNac] is available from Sigma (Ref A6681) http://www.sigmaaldrich.com.

Nineteen labs performed oligosaccharide analysis: 7 labs identified a profile consistent with aspartylglucosaminuria (9 in 2007), 4 labs reported an abnormal profile but did not give conclusion, and 2 concluded to a wrong oligosaccharidosis. Surprisingly, 6 labs reported no abnormality. It must be emphasized that it was a difficult sample since the profile was relatively weak as illustrated below.




The 18 labs who performed mucopolysaccharides did not report any abnormality.

**Advice for further investigation** was OK for those who gave a correct diagnosis, but misleading for those who concluded to a wrong oligosaccharidosis.

**Scoring**

- Analytical: identification of aspartylglucosamine (score 2), abnormal oligosaccharide profile (score 1)
- Interpretation: Aspartylglucosaminuria (score 2), oligosaccharidosis (score 1), wrong lysosomal storage disease (score 0)
- Recommendations: aspartylglucosaminidase activity and/or mutation analysis *AGU* gene and/or refer urine sample to a specialized lab (score 1)

**Scores of participants**

- **Survey 2010-1**

| Lab n° | Patient P1 Mevalonic aciduria | | | | Patient P2 Aminoacylase I deficiency | | | | Patient P3 No metabolic disease | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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 | 1 | 1 | 3 |
| 3 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 4 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 1 |
| 6 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 3 |
| 7 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 4 |
| 8 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 5 |
| 9 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 5 |
| 10 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 1 | 2 | 1 | 4 |
| 11 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 12 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 13 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| 14 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 15 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 1 | 1 | 2 | 1 | 4 |
| 16 | 0 | 1 | 1 | 2 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 17 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 18 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 1 | 2 | 2 | 1 | 5 |
| 19 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 1 |
| 20 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 5 |
| 21 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 | 2 | 2 | 1 | 5 |

- **Survey 2010-2**

| Lab n° | Patient P4 Sialidosis type I (common sample) | | | | Patient P5 Glutaric aciduria type I | | | | Patient P6 Aspartylglucosaminuria | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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 | 0 | 1 | 2 |
| 3 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 4 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 1 | 1 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 |
| 7 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 1 |
| 8 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 1 | 1 | 1 | 3 |
| 9 | 1 | 0 | 0 | 1 | 2 | 2 | 1 | 5 | 0 | 0 | 1 | 1 |
| 10 | 1 | 0 | 0 | 1 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 1 |
| 11 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 12 | 2 | 1 | 1 | 4 | 2 | 2 | 1 | 5 | 1 | 1 | 1 | 3 |
| 13 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 14 | 1 | 0 | 1 | 2 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 |
| 15 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 |
| 16 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 |
| 17 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 18 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 0 | 0 | 1 | 1 |
| 19 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 |
| 20 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 1 |
| 21 | 2 | 2 | 1 | 5 | 2 | 2 | 1 | 5 | 0 | 0 | 0 | 0 |

❖ **Total scores**

| Lab number | Survey 2010-1 | Survey 2010-2 | Cumulative score | Cumulative score ( % ) |
|---|---|---|---|---|
| 1 | 15 | 15 | 30 | 100% |
| 2 | 13 | 12 | 25 | 83% |
| 3 | 15 | 15 | 30 | 100% |
| 4 | 15 | 15 | 30 | 100% |
| 5 | 11 | 10 | 21 | 70% |
| 6 | 8 | 6 | 14 | 47% |
| 7 | 9 | 11 | 20 | 67% |
| 8 | 10 | 13 | 23 | 77% |
| 9 | 10 | 7 | 17 | 57% |
| 10 | 14 | 7 | 21 | 70% |
| 11 | 15 | 15 | 30 | 100% |
| 12 | 15 | 12 | 27 | 90% |
| 13 | 6 | 15 | 21 | 70% |
| 14 | 15 | 7 | 22 | 73% |
| 15 | 10 | 10 | 20 | 67% |
| 16 | 12 | 10 | 22 | 73% |
| 17 | 15 | 15 | 30 | 100% |
| 18 | 11 | 11 | 22 | 73% |
| 19 | 11 | 15 | 26 | 87% |
| 20 | 10 | 11 | 21 | 70% |
| 21 | 10 | 10 | 20 | 67% |

**Performance**

| | Number of labs | % total labs |
|---|---|---|
| Excellent performers (100 % of good responses) | 5 | 23 % |
| Poor performers (< 60 % good responses) | 2 | 9 % |

❖ **Summary of scores**

| Sample | Diagnosis | Analytical (%) | Interpretation (%) | Recommen-dations (%) | Total (%) |
|---|---|---|---|---|---|
| **Patient P1** | Mevalonic aciduria | **95%** | **98%** | **100%** | **97%** |
| **Patient P2** | Aminoacylase I | **62%** | **57%** | **57%** | **59%** |
| **Patient P3** | Non metabolic | **86%** | **79%** | **81%** | **82%** |
| **Patient P4** | Sialidosis type I | **88%** | **79%** | **90%** | **85%** |
| **Patient P5** | Glutaric ac. type I | **100%** | **100%** | **100%** | **100%** |
| **Patient P6** | Aspartylglucosam. | **48%** | **38%** | **52%** | **45%** |

**DPT-scheme in 2011**

- 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) will be used. It will be mandatory to send results through the website before the deadline.
- **Scoring of results**: *"Poor performance"* is not acceptable terminology; it should be *"lack of attainment of adequate performance".* Like in 2010, scoring will be performed by two different scheme organizers. For the Lyon centre, this will again be done by Viktor Kozich from Prague. At the Scientific Advisory Board meeting in December in London, there was again discussion as to how satisfactory performance should be defined. ERNDIM tests ability of current practice to identify abnormalities in rare disorders. So if a lab misses for example methylmalonic aciduria, this represents a critical error. We have to agree what determines a *"critical error"* or a *"non-critical error" (non-prosecutable).* It is likely that a *"critical error"* could be viewed as an error which has the potential to attract a successful claim for negligence if it occurred in a patient sample. But this has to be discussed further. Perhaps this could be trialed in 2011 and introduced in 2012.

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 2011**

It will take place during the SSIEM meeting in Geneva **Tuesday 30 August 2011**, 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** |
| • **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** |