ERNDIM DPT Centre Basel

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Metabolic Unit

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Diagnostic Proficiency Testing

Survey 2010

Annual Report

prepared by
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1. Geographical distribution of participants

In 2010, 21 laboratories from 10 countries participated in the scheme and all participants submitted results in at least one of the two surveys.

Country	Number of participants
Austria	1
Canada	3
China	1
Estonia	1
Germany	5
Norway	1
Sweden	2
Switzerland	2
UK	1
USA	4

2. Samples and Shipment

The samples contain a small amount of thiomersal and have been heat-treated. They were pre-analysed in our institute after 3 days incubation at ambient temperature (to mimic possible changes that might arise during transport). In all six samples the typical metabolic profiles were preserved after this process.
The urine samples were distributed to participants on May 10 at ambient temperature using the courier TNT Swiss Post.
Delivery of samples took between one and two days according to the tracking by the courier, except for 4 parcels that we assume were blocked at US customs for 8 days.

3. Tests

Analyses of amino acids, organic acids, mucopolysaccharides, oligosaccharides and purines/ pyrimidines were required in 2010.

4. Schedule of the scheme in 2010

Sample distribution	May 10, Monday
Start of analysis of Survey 2010/1	May 17, Monday
Survey 2010/1 ABC - Results submission	June 7, Monday
Survey 2010/1 - Reports	June 28, Monday
Start of analysis of Survey 2010/2	June 28, Monday
Survey 2010/2 DEF - Results submission	July 19, Monday
Survey 2010/2 - Reports	August 13, Friday
Annual meeting of participants	August 31, Istanbul
Annual Report 2010	December

5. Receipt of samples and results

Receipt of samples (sent on May 10, 2010)

Receipt (days after shipment)	Receipt (reported by participants)	Delivery (by TNT Swiss Post)
1 day	9	13
2 – 3 days	4	4
7 days	2	
8 days	1	4
10 days	2	

Date of reporting of results

A,B,C: twenty participants returned their results by the deadline. One participant failed to return results and was excluded from the evaluation of results.
D,E,F: Eighteen labs returned results by the deadline and the remaining three by three days later. All results were included in the evaluation.

Eleven laboratories also **submitted results by the website**. This will help us greatly in fine tuning the website system.

6. Scoring system

Three criteria are evaluated: analytical performance, interpretative proficiency and recommendations for further investigations. Due to the large variability in reporting results in various countries, recommendations pertaining to treatment are not evaluated in proficiency testing. However, they are still reported and summarised by the scheme organisers.

A	Analytical performance	Correct results of the appropriate tests	2	max 2
		Partially correct or non-standard methods	1	
		Unsatisfactory or misleading	0	
I	Interpretative proficiency	Good (diagnosis was established)	2	max 2
		Helpful but incomplete	1	
		Misleading or wrong diagnosis	0	
R	Recommendations	Helpful	1	max 1
		Unsatisfactory or misleading	0	

The **total score** is calculated as a sum of these three criteria. The maximum to be achieved is 5 points per sample. The scores were calculated only for laboratories submitting results.

7. Results of samples and evaluation of reporting

Sample A: MNGIE / Thymidine phosphorylase deficiency

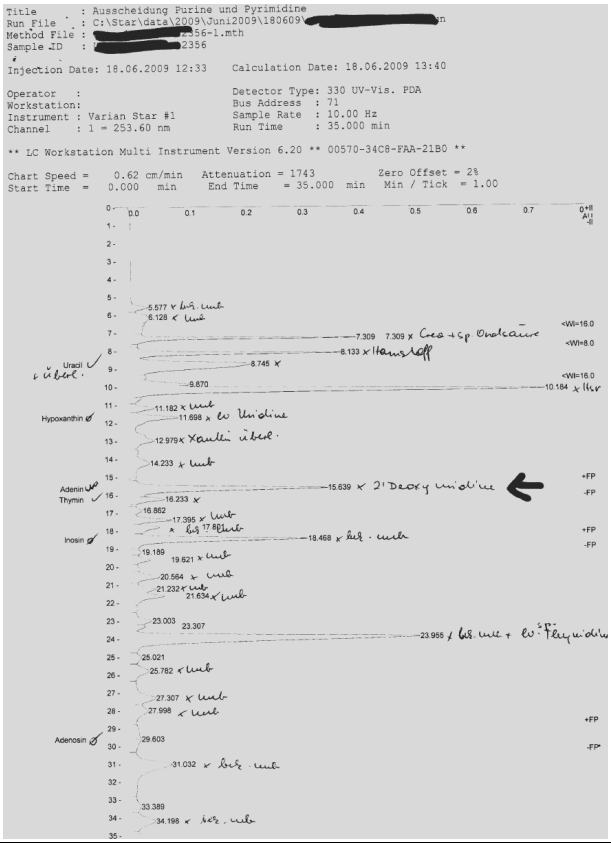
Patient: The urine was obtained from a 33 year old male patient with thymidine phosphorylase deficiency (MNGIE syndrome). The diagnosis was confirmed by mutation analysis. The sample was provided by Dr. S. Scholl-Bürgi from Innsbruck.

Analytical performance: The finding of thymidine and/or 2'-deoxyuridine was considered key to the diagnosis, with or without thymine/uracil. The finding of only thymine/uracil was considered as only partially correct. 14 labs reported on purine/pyrimidine analysis but only 8 labs reported the key metabolites. Overall performance was sub-optimal at 50%
Interpretative proficiency: The correct diagnosis was considered to be thymidine phosphorylase deficiency. Interpretation for this sample in labs that identified thymidine/deoxymidine was good. Overall proficiency was low at 50%.
Recommendations: Confirmation of the diagnosis by enzyme assay and/or mutation analysis was considered helpful as was the recommendation to perform purine/pyrimidine analysis when this had not been performed.

Overall impression: This was a difficult case correctly diagnosed by about half of labs. The finding of increased thymine/uracil should be followed up by purine/pyrimidine analysis.

Reported critical metabolite values
Uracil: 79, 33, 63.7, 81 mmol/mol Creat.
Thymine: 14, 14, 13.3, 17.5 mmol/mol Creat.
Thymidine:4.2, 5.1, 13 mmol/mol Creat., 0.223 mmol/L
Deoxyuridine: 25 mmol/mol Creat., 0.108 mmol/L

HPLC analysis of Purines and Pyrimidines



Source:
Sabine Scholl-Bürgi
Innsbruck Austria

Sample B: MPS type 2 (Hunter)

Patient: this sample was obtained from a 4.5 year old boy suffering from mucopolysaccharidosis type II (Hunter). This urine was obtained in our hospital, Basel, Switzerland. The diagnosis had been confirmed by the finding of severe iduronate sulphate sulphatase deficiency in serum and leucocytes (Prof. B. Steinmann, Zurich, Switzerland).

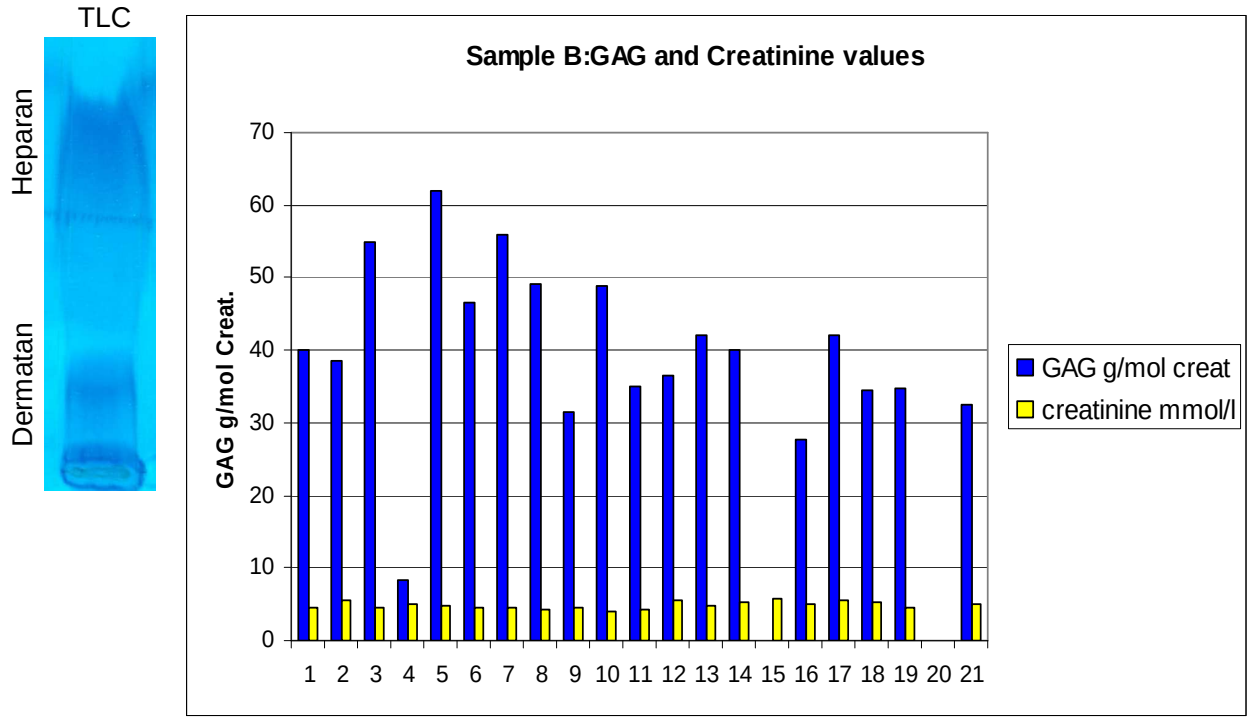
Analytical performance*:* mucopolysaccharide analysis was considered essential. The finding of increased GAG and dermatan sulphate with or without heparin sulphate was considered correct. 19 laboratories performed mucopolysaccharide analysis. 19 found increased GAG which received 1 point. 1 additional point was given for GAG differentiation with identification of dermatan sulphate. The analytical performance of this sample was 80%.

Interpretative proficiency: a diagnosis of MPS in general received 1 point, and 1 point was given for mention of MPS type II. The interpretative proficiency for this sample was 82.5%.

Recommendations*:* confirmation of diagnosis by enzyme assay (iduronate-2-sulfatase, α-iduronidase), mutation analysis (*ARSB* gene), GAG quantitation and differentiation were considered helpful.

Overall impression
This sample was also distributed in 2009 with very similar overall performance (2009, overall proficiency 84%). Thus no overall improvement occured although GAG levels varied much less between laboratories

Sample B: Total GAG & TLC



Sample C: Citrullinemia

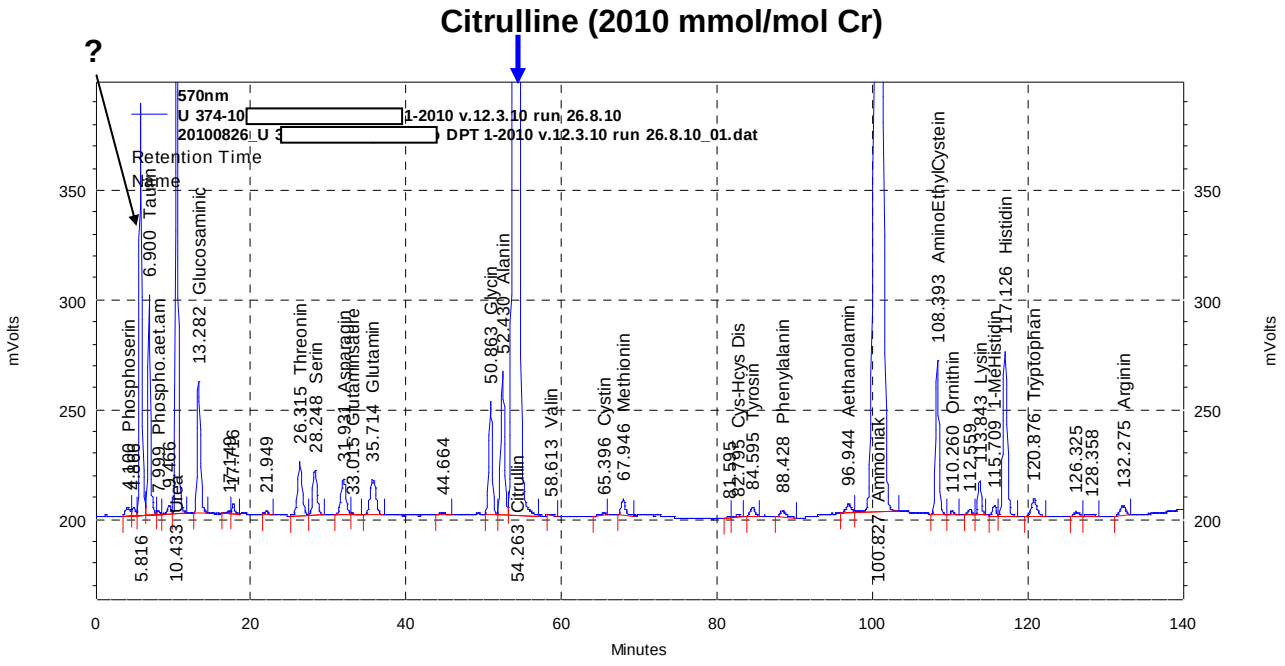
Patient: This sample was obtained from a 24 year old male, with citrullinaemia, treated with benzoate. The diagnosis was confirmed by mutation analysis. The urine was obtained in our hospital, Basel, Switzerland.

Analytical performance: Amino acid analysis was considered essential for the diagnosis in this case and was performed by 19 laboratories and received 2 points. Increased hippuric acid, benzoic acid and small increases of orotic acid were noted but not considered essential for the diagnosis. Analytical performance was high at 95%.

Interpretative proficiency: Citrullinaemia due to argininosuccinate synthetase deficiency was considered correct. 1 point was given for indication of another urea cycle disorder. Proficiency score was 95%.

Recommendations: follow-up by plasma amino acid and ammonia analysis was important. Confirmations of diagnosis by enzyme assay and mutation analysis were considered helpful.

Ion-Exchange Amino Acid Analysis (Biochrom)



Reported values of citrulline showed a very marked variation**.**

>282.5; 1505; 1764; 1773; 1781; 2515; 2706; 3553; 3816; >4000; 4474
4800; 5283; 5414; 5618; 5757; 6656; 7555; 32900 mmol/mol Creat.

The ion exchange amino acid chromatogram (Biochrom) shows a large peak which elutes immediately before Taurine. A search of the literature reveals a paper describing a cylic derivative of citrulline which may well be this substance. (Wilson RW, Gates SC, Kohrman AF, Biochem Med. 1978 Feb;19(1):90-4).

Sample D: 4-hydroxybutyric aciduria

Patient: The urine was obtained from a 7 months old patient with confirmed 4-hydroxybutyric aciduria (succinate-semialdehyde dehydrogenase deficiency).
The sample was provided by Dr. Jurgita Songailiene from Vilnius, Lithuania.

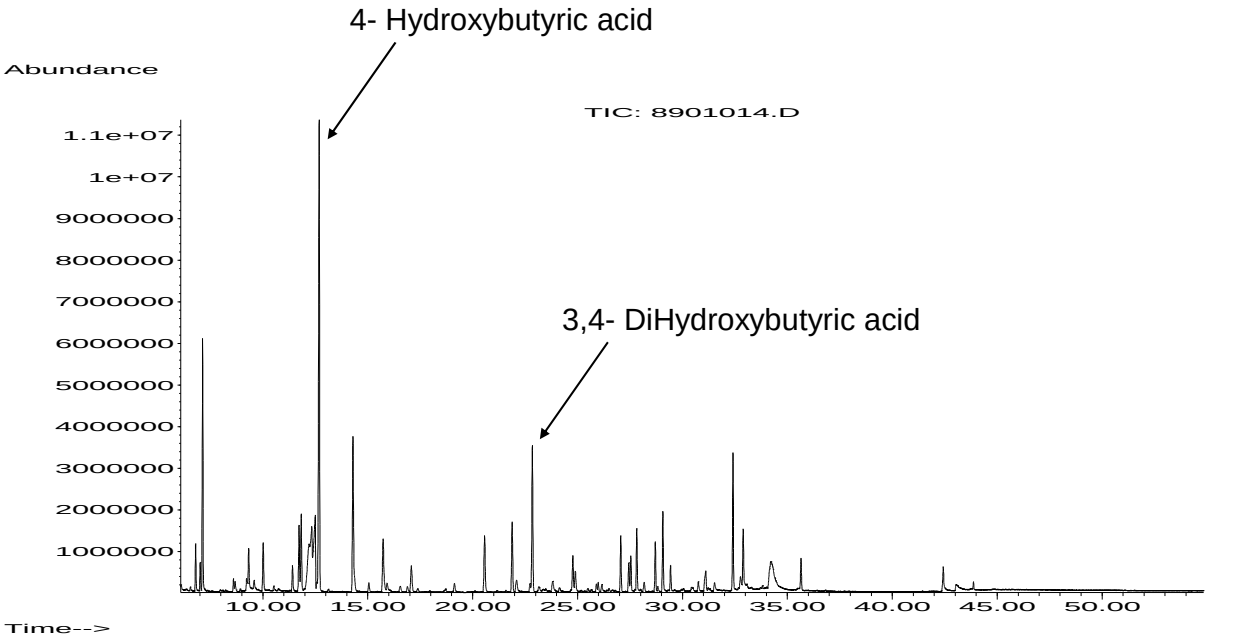
Analytical performance: The finding of 4-hydroxybutyric acid with or without other key metabolites such as 3,4-dihydroxybutyric acid was considered to be correct. 20 labs reported organic acid analysis. Overall performance was high at 95%.

Interpretative proficiency: The correct diagnosis was considered to be 4-hydroxybutyric aciduria due to SSADH deficiency. Proficiency for interpretation for this sample was also good at 90%.

Recommendations: Confirmation of the diagnosis by enzyme assay and/or mutation analysis was considered helpful. Proficiency was similar to that for the other aspects at 92%

Overall impression: This was a straightforward case correctly diagnosed by all but one lab with overall proficiency of 90%.

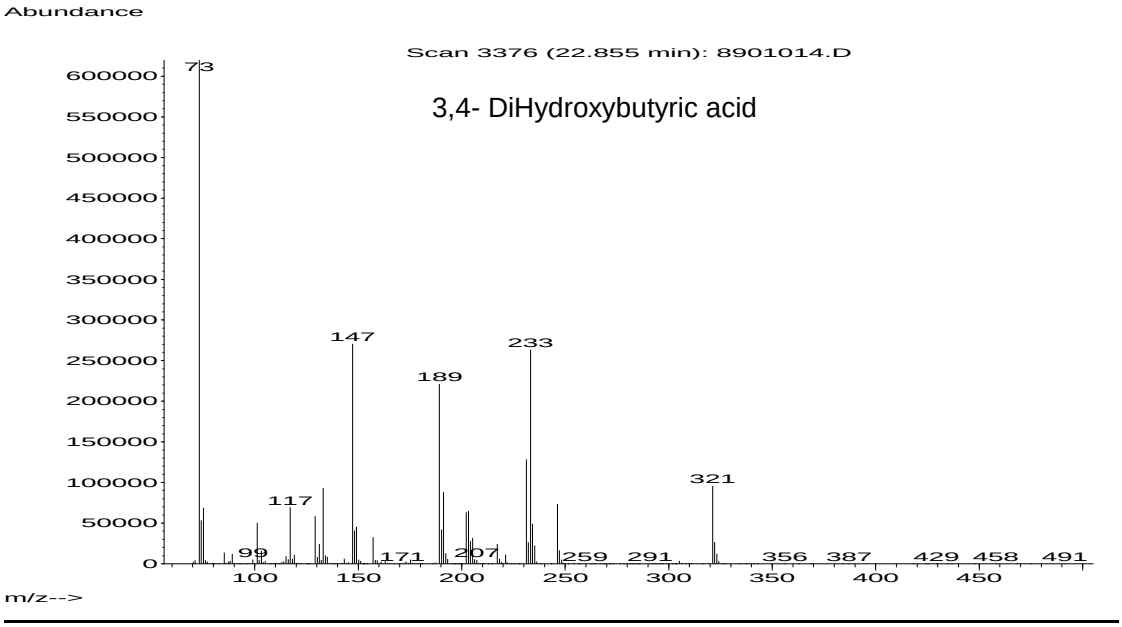
Sample D:
Total Ion Chromatogram



Reported values of 4-hydroxybutyric acid showed a wide variation as follows:

71, 160, 173, 510, 600, 670, 681, 796 mmol/mol Creat.

Sample D:Spectrum of 3,4-dihydroxybutyric acid



Sample E: Formiminoglutamic aciduria / formiminotransferase deficiency MIM229100

Patient: This sample was obtained from a 5 year old boy with delayed development and mild epilepsy in whom metabolic screening revealed clearly increased excretion of formimino-glutamic acid. Although the diagnosis has not been confirmed by molecular genetic analysis the finding of FIGLU-uria was confirmed in several urine samples and folate deficiency has been excluded. This urine was obtained in our hospital in Basel, Switzerland.

Analytical performance*:* Amino acid analysis and organic acid analysis were considered necessary. FIGLU can be detected by chromatography / electrophoresis or as a diffuse peak on ion-exchange chromatography or in acylcarnitine analysis. The cyclical derivative hydantoin-5-propionic acid is detectable by organic acid analysis. The finding of either FIGLU or hydantoin-5-propionate leading to th ecorrect diagnosis received 2 points. The analytical performance of this sample was low at 33%.

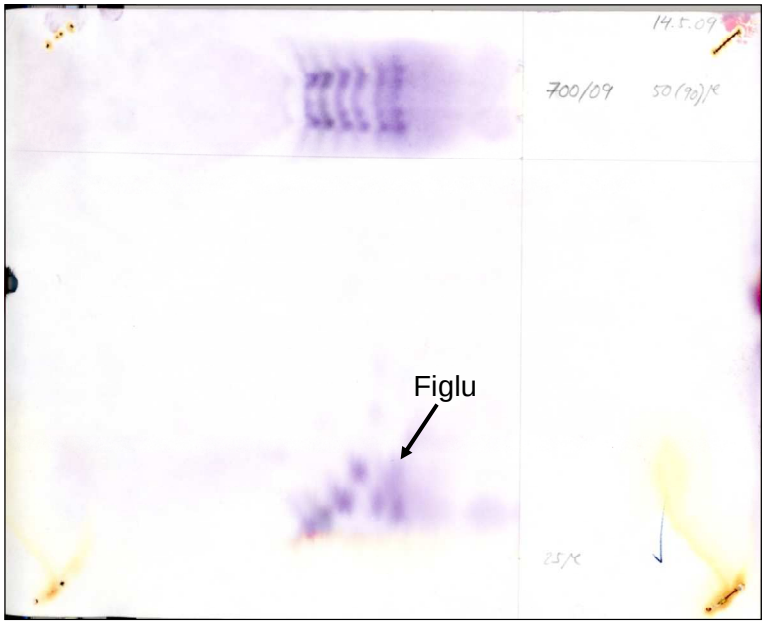
Interpretative proficiency: a diagnosis of glutamate-formiminotransferase deficiency was considered correct. The interpretative proficiency for this sample was low at 33%.

Recommendations*:* ruling out folate deficiency, histidine loading and mutation analysis of the FTCD gene were considered helpful. The appropriateness of an invasive liver biopsy for enzyme assay is questionable.

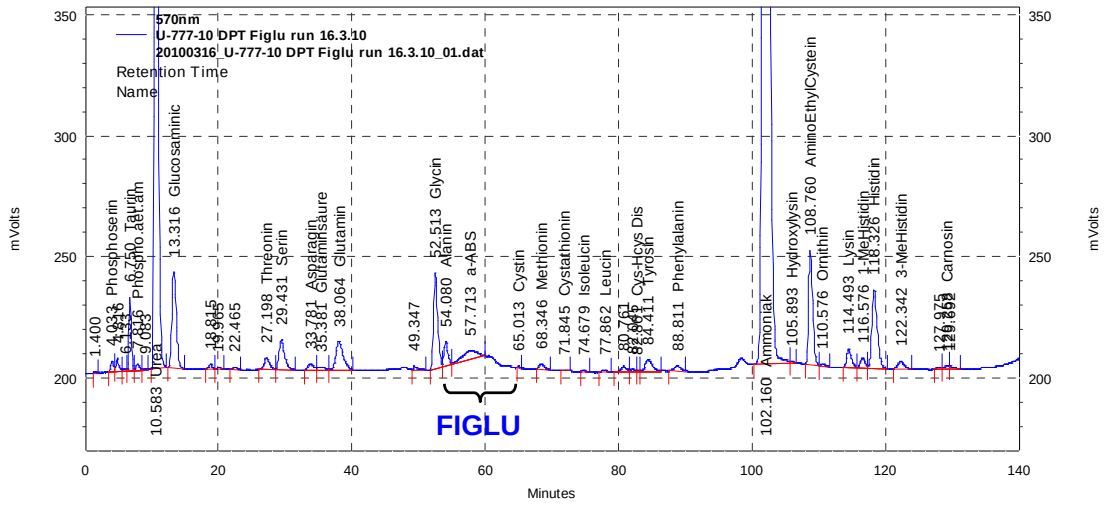
Overall impression: This sample turned out to be very difficult although standard methods should pick up the key metabolites. Low overall proficiency of 30%.

Details of detection of Figlu.

Amino acid analysis: 1-D High Voltage Electrophoresis & 2-D electrophoresis/ chromatography

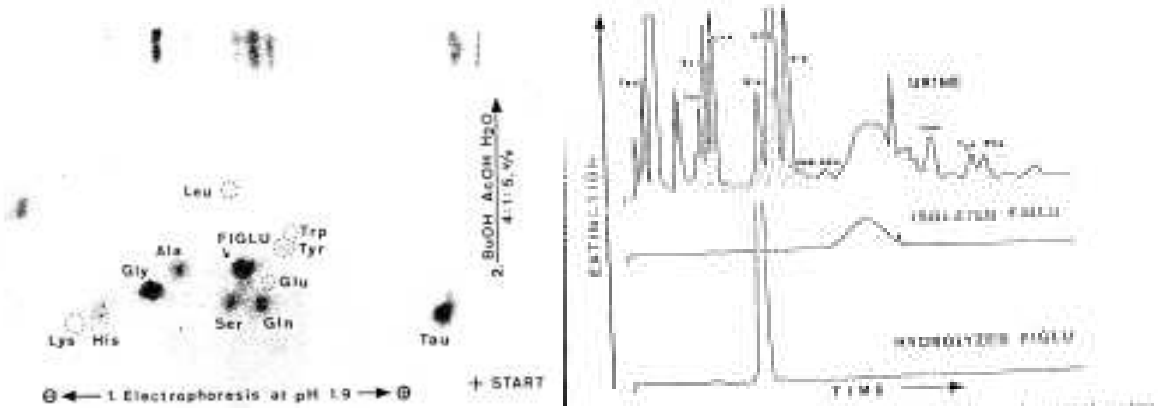


Amino acid analysis: ion exchange chromatography (Biochrom)

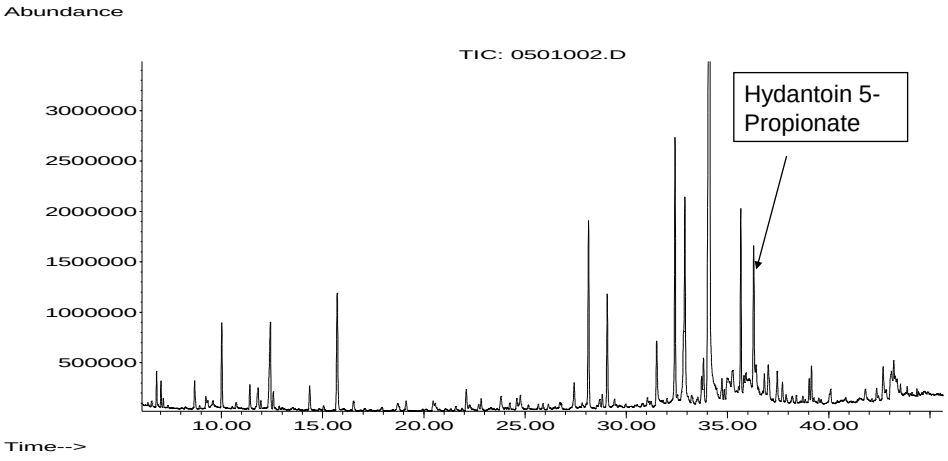


These findings are very similar to those reported by Niederweiser et al in an earlier description of the condition

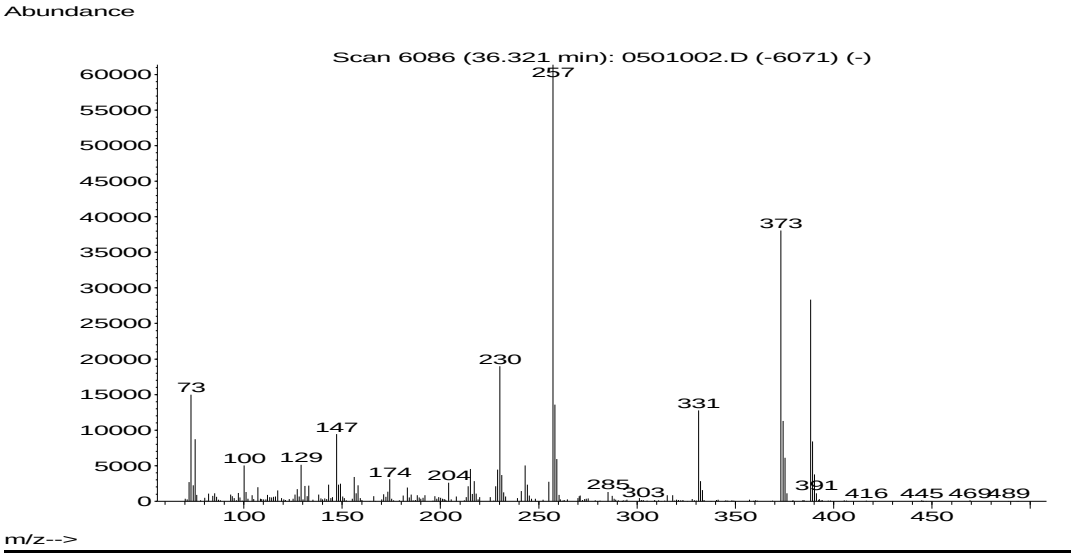
Niederwieser et al. Clin Chim Acta 1974; 54:293-316



Organic acid analysis: Total Ion Chromatogram

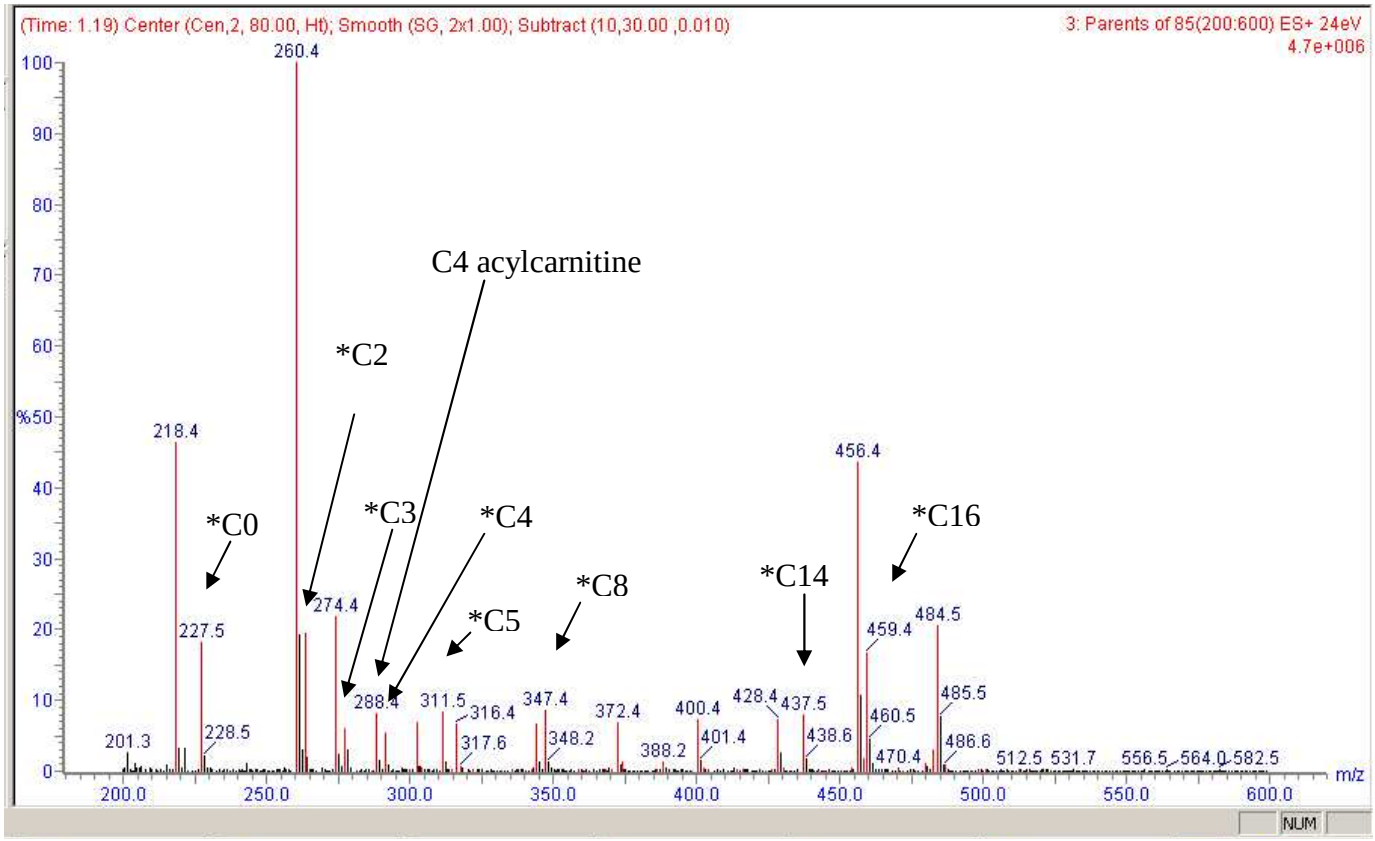


Hydantoin 5-Propionate: Spectrum

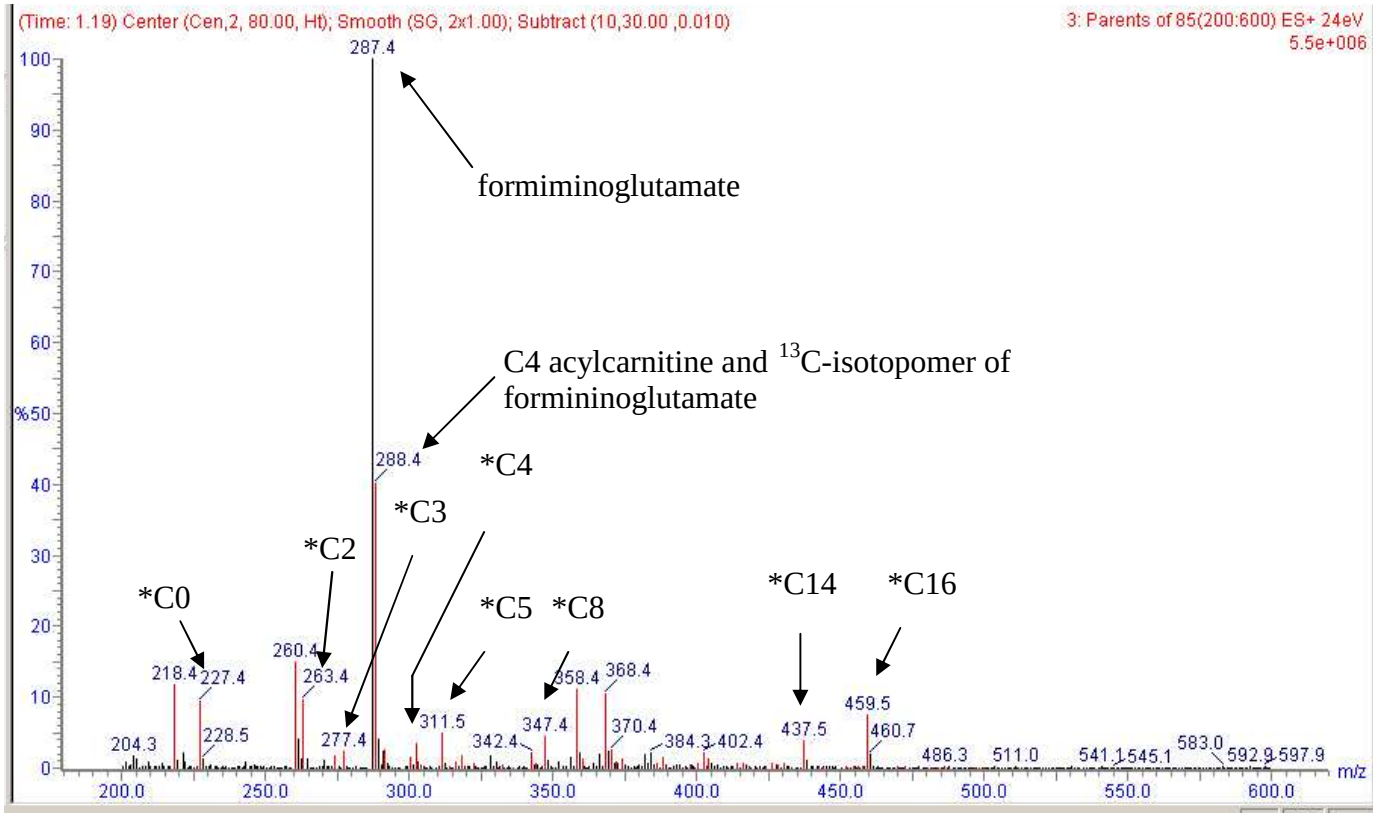


Three labs reported the finding of FIGLU by tandem MS. This is shown in the slides below kindly provided by Oliver Sass from Freiburg:

Chromsystems, Newborn Screening Dried blood spot control Level II, Lot 4409



DPT Basel sample 2010 E



Sample F: Sialidosis

Patient: The common sample was obtained from a 43 year old woman with Sialidosis/Neuraminidase deficiency. The diagnosis was confirmed enzyme assay in leukocytes and fibroblasts. The urine was provided by Dr. Christine Saban from Lyon.

Analytical performance: Oligosaccharide analysis was considered essential for the diagnosis in this case and was performed by 15 laboratories and received 2 points. The finding of increased bound sialic acid is helpful. Analytical performance was moderately good at 71%.

Interpretative proficiency: Sialidosis due to neuraminidase deficiency was considered correct. Proficiency score was 69%

Recommendations: Confirmation of diagnosis by neuraminidase assay with or without mutation analysis was considered helpful. Efficiency was 90%.

Full details of this sample can be found on the ERNDIM website under Meetings & Reports / ERNDIM Workshop, SSIEM Istanbul, 31.08.2010

8. Scores

Overall proficiency

Sample	Diagnosis	A (%)	I (%)	R (%)	total (%)
A	MNGIE / Thymidine phosphorylase deficiency	50	50	50	50
B	MPS type 2 (Hunter	80	82.5	90	83
C	Citrullinaemia	95	97.5	95	96
D	Succinate semialdehyde dehydrogenase deficiency	95	90	90	92
E	Formiminoglutamic aciduria	33	33	33	33
F	Sialidosis	71	78.5	90	78

Total scores

	Survey 1			Survey 2				
Lab No	**A**	**B**	**C**	**D**	**E**	**F**	**Total**	**Total without A & E***
1	0	5	5	5	0	5	20	**20**
2	0	5	5	5	0	5	20	**2**
3	0	5	5	5	5	5	25	**20**
4	2	3	2	5	0	2	14	**12**
5	0	4	5	5	0	5	19	**19**
6	5	4	5	5	5	5	29	**19**
7	4	5	5	5	0	2	21	**17**
8	5	3	5	5	0	5	23	**18**
9	0	3	5	2	0	0	10	**10**
10	0	3	5	5	0	5	18	**18**
11	5	5	5	5	5	5	30	**20**
12	5	5	5	5	5	5	30	**20**
13	5	5	5	5	5	5	30	**20**
14	4	5	5	5	5	5	29	**20**
15	0	1	5	5	0	1	12	**12**
16	3	5	5	5	0	2	20	**17**
17	0	5	5	5	0	5	20	**20**
18	2	3	4	5	0	0	14	**12**
19	5	4	5	0	0	5	19	**14**
20	-	-	-	5	0	5	10	**10**
21	5	5	5	5	5	5	30	**20**

*This year the scores proposed by us were evaluated by a second advisor and although this led to minor changes in scores these changes did not affect achievement or not of the cut off for satisfactory performance. Also after discussion with Scientific Advisory Board it was decided to treat samples A and E as purely educational and not to include their scores in the overall assessment of performance. Thus the cut off point for satisfactory performance is set at 12 points. Labs failing to reach this mark will receive a performance advice letter.

Detailed Scores: A,B,C

Lab no	Sample A MNGIE / Thymidine phosphorylase deficiency				Sample B MPS type 2 (Hunter)				Sample C Citrullinaemia				
	A	**I**	**R**	**Total**	**A***	**I**	**R**	**Total**	**A**	**I**	**R**	**Total**	**Total**
1	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**	**10**
2	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**	**10**
3	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**	**10**
4	1	1	0	**2**	1	1	1	**3**	0	1	1	**2**	**7**
5	0	0	0	**0**	1	2	1	**4**	2	2	1	**5**	**9**
6	2	2	1	**5**	2	1	1	**4**	2	2	1	**5**	**14**
7	2	1	1	**4**	2	2	1	**5**	2	2	1	**5**	**14**
8	2	2	1	**5**	1	1	1	**3**	2	2	1	**5**	**13**
9	0	0	0	**0**	1	1	1	**3**	2	2	1	**5**	**8**
10	0	0	0	**0**	1	1	1	**3**	2	2	1	**5**	**8**
11	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
13	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
14	1	2	1	**4**	2	2	1	**5**	2	2	1	**5**	**14**
15	0	0	0	**0**	0	0	1	**1**	2	2	1	**5**	**6**
16	1	1	1	**3**	2	2	1	**5**	2	2	1	**5**	**13**
17	0	0	0	**0**	2	2	1	**5**	2	2	1	**5**	**10**
18	1	1	0	**2**	1	2	0	**3**	2	2	0	**4**	**9**
19	2	2	1	**5**	2	2	0	**4**	2	2	1	**5**	**14**
20	-	-	-	**-**	-	-	-	**-**	-	-	-	**-**	**-**
21	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
ratio	20/40	20/40	10/20	**50/100**	32/40	33/40	18/20	**83/100**	38/40	39/40	19/20	**96/100**	
%	**50**	**50**	**50**	**50**	**80**	**82.5**	**90**	**83**	**95**	**97.5**	**95**	**96**	

Detailed Scores: D,E,F

Lab no	Sample D Succinate semialdehyde dehydrogenase deficiency				Sample E Formiminoglutamic aciduria				Sample F Sialidosis				
	A	I	R	Total	A*	I	R	Total	A	I	R	Total	Total
1	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**	**10**
2	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**	**10**
3	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
4	2	2	1	**5**	0	0	0	**0**	0	1	1	**2**	**7**
5	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**	**10**
6	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
7	2	2	1	**5**	0	0	0	**0**	0	1	1	**2**	**7**
8	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**	**10**
9	2	0	0	**2**	0	0	0	**0**	0	0	0	**0**	**2**
10	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**	**10**
11	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
12	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
13	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
14	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
15	2	2	1	**5**	0	0	0	**0**	0	0	1	**1**	**6**
16	2	2	1	**5**	0	0	0	**0**	0	1	1	**2**	**7**
17	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**	**10**
18	2	2	1	**5**	0	0	0	**0**	0	0	0	**0**	**5**
19	0	0	0	**0**	0	0	0	**0**	2	2	1	**5**	**5**
20	2	2	1	**5**	0	0	0	**0**	2	2	1	**5**	**10**
21	2	2	1	**5**	2	2	1	**5**	2	2	1	**5**	**15**
ratio	40/42	38/42	19/21	**97/105**	14/42	14/42	7/21	**35/105**	30/42	33/42	19/21	**82/105**	
%	**95**	**90**	**90**	**92**	**33**	**33**	**33**	**33**	**71**	**78.5**	**90**	**78**	

9. Assessment of performance

Steps have been taken within the Scientific Advisory Board of ERNDIM to set the level of good performance within a proficiency scheme. Letters of support to those laboratories with clear poor performance will be issued. This year 18 points or more has been set as the level for satisfactory performance. See however the comment to the scores above. After removing scores for the very difficult educational samples two laboratories failed to reach this level.

10. Annual meeting

The annual meeting of participants of the 5 DPT centres took place during the SSIEM symposium in Istanbul on Tuesday, August 31, 2010 at 9.00 Twenty four participants and/or guests attended the meeting of the Basel centre. General Agreement was received for the allocated points.

11. Changes planned for 2011

We plan important changes for 2011.
First you will be required to submit results online to our website. You will be contacted soon with more details and you will be given the opportunity to practice using the website with 2009 data.
Second the samples for the Basel scheme will be distributed by the CSCQ but we will remain responsible for the scientific and evaluation aspects of the scheme.

12. Tentative schedule and fee in 2011

Sample distribution	May 03, 2011, Tuesday
Start of analysis of Survey 2011/1	May 09, 2011, Monday
Survey 2011/1 - Results submission	May 30, 2011, Monday
Survey 201/1 - Reports	June 13, 2011, Monday
Start of analysis of Survey 2011/2	June 20, 2011, Monday
Survey 2011/2 – Results submission	July 11, 2011, Monday
Survey 2011/2 - Reports	August 05, 2011, Friday
Annual meeting of participants	August 30, 2011, in Geneva at SSIEM
Annual Report 2011	December

The next annual meeting of DPT participants will take place on August 31 in Istanbul Turkey at the SSIEM meeting.

The Executive Board of ERNDIM determined the fee for 2011 in the amount of 320 €.

13. ERNDIM certificate of participation

A combined certificate of participation covering all EQA schemes will be provided to all participants who take part in any ERNDIM scheme. For the DPT scheme this certificate will indicate if results were submitted and whether satisfactory performance was achieved in the scheme.

Basel, August 2011

Brian Fowler	Katharina Honegger	Marianne Zaugg
Scientific advisor	Scheme organiser	Scheme organiser